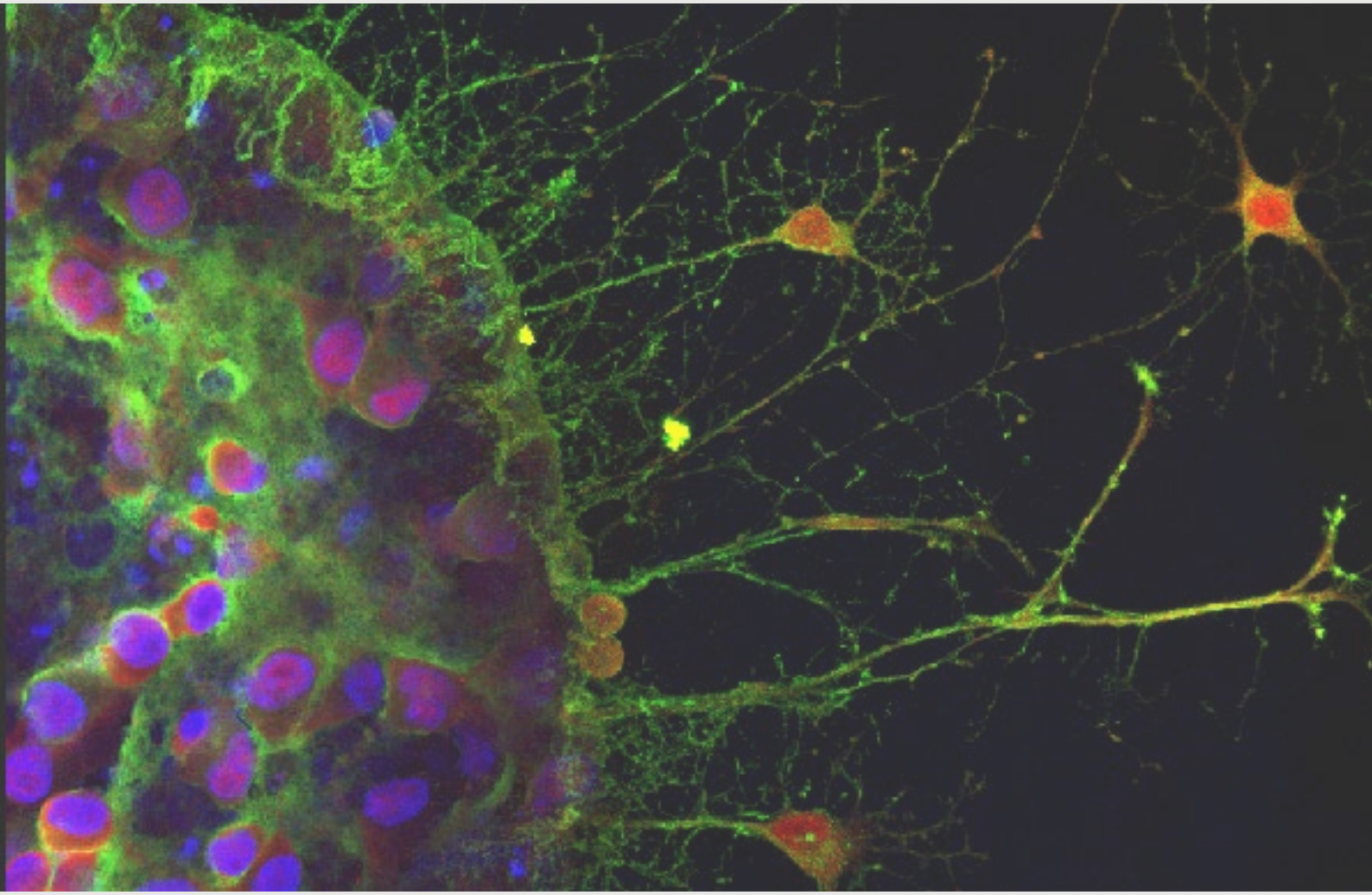
Príncipe Felipe Research Centre

Annual Report 2013



PRINCIPE FELIPE
CENTRO DE INVESTIGACION

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Príncipe Felipe Research Centre

Annual Report 2013

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INTRODUCTION

The Prince Felipe Research Foundation originated 50 years ago under the name of Cytological Research Institute and has since evolved into a center of international research focused on the prevention, diagnosis and treatment of diseases through more personalized medicine.

Today, the Prince Felipe Research Centre bases its development on scientific excellence, and has revised its priorities and resources to optimize its impact on improving lives

.

It is a Centre focused on responding to real problems that affect, disturb and distress millions of people
I was honored to be invited to join the Foundation as director in February 2013, and I know first-hand the Centre´s tradition of scientific excellence, the quality of its work and the day to day commitment of its staff.

These are difficult years for research, but they also present a stimulating challenge. Not surprisingly, one of the aspects that struck me when I arrived at the center was the youth of the people who work here. They displayed a positive, bold attitude - as it should be in science when addressing these and other challenges - which makes me trust in the future of this institution.
Thus, during the first half of the year we developed a strategic plan for the period 2013-2016, the priorities of which are to consolidate the structure and purpose of the Centre, to create an appropriately stimulating and working environment, to ensure the translation of research results, to bring biomedical research to the public domain and to ensure financial sustainability for the Foundation´s future.

Our mission is to support the research and development of scientists working in our Centre, to provide for them a suitable environment, laboratories and facilities, and a creative and challenging atmosphere that will encourage collaboration and interdisciplinary approaches, so that they can develop innovative research projects of scientific and social relevance

.

Our vision is to improve the health of people through science. We want that to constitute the true milestone of biomedical knowledge.

These have been the considerations that have guided our work through 2013. When, after a year, we evaluate the work done, which is included in this scientific report, we cannot fail to be pleased with the results and, at the same time, be aware of the Centre´s and its people potential.

However, before proceeding to the presentation of these results, I would like to remind us all that this year was marked by the death of one of the Centre´s most respected investigators, **Dr. Quique Perez-Paya**, head of the Laboratory of Peptides and Proteins. I hadn´t the opportunity to get to know him in depth, but his spirit is present among his colleagues and all the scientists he trained because he was, without any doubt, an excellent scientist and valued friend. If Quique, wherever he is, can see what his group is now doing to continue his work, in particular Dr. Mar Orzáez who continues his lines of investigation, he will be very proud of them.

As the result of the strategic plan the research groups of the Centre have been structured in five scientific programs:

The Computational Genomics program, led by Dr. Joaquín Dopazo, is investigating new models for rational diagnosis and treatment of diseases such as cancer and various inherited diseases using approaches based on biological systems and the management and processing of large volumes of genomic data.

The Advanced Therapies program, led by Dr. Antonio Pineda, is developing new diagnostic and therapeutic approaches to diseases of high social impact such as cancer, diabetes, spinal cord injuries, musculoskeletal injuries and neurodegenerative diseases, among others.

The program of Molecular Mechanisms of Disease, led by Dr. Susana Rodríguez, is investigating the molecular and cellular mechanisms underlying essential cellular processes and their changes in human diseases, with the aim of postulating new targets and therapeutic strategies based upon the information obtained.

The Neurological Deterioration program, led by Dr. Vi-

cente Felipo, is conducting basic and translational research on cognitive decline, motor, sleep and circadian rhythms in different pathological situations such as clinical and minimal hepatic encephalopathy, acute liver failure, hiperamonemias and perinatal exposure to environmental contaminants and food.

The program of Genetic and Rare Diseases is led by Dr. Francesc Palau who joined the center in 2013 to study the genetic, genomic, molecular and cellular basis of neuromuscular diseases and disorders of the brain, both neurological and mental. This program is also developing clinical translation services, such as translational genetics, which provide diagnosis and counseling in genetic diseases. The coordination of this program with the Rare Diseases at the Biomedical Research Network Centre (CIBERER), which is also run by Dr. Francesc Palau, places CIPF as a national and international point of reference in this field.

The scientific programs of the Centre cover the entire value chain of biomedical research and innovation, from the most basic aspects and the earliest stages to those with a strong translational and applied nature, both in the diagnosis and the treatment of various human diseases.

During 2013 the scientific production of the research groups of the Centre has been reported in 107 articles, of which 76% in magazines in the first quartile. These are good results, both quantitatively and qualitatively, given the current size of the Centre. Though good, they will undoubtedly improve in the coming years, the result of work being done and with the restructuring of the Centre that will inevitably affect the productivity of research groups.

The Foundation has continued through 2013 to provide support for the creation of technology-based firms as one of the most effective mechanisms for technology transfer in order to get research results to society and to the market. The 3 **spin off** in which the Foundation participates this year consolidated its business and strengthened its position in the market. The center hosted in November a meeting of the National Congress of Business Angels, when CIPF researchers had the opportunity to present their business ideas to potential investors.

The research activities of the center´s groups have resulted in obtaining two new **patents** which comprise new therapeutic approaches for the treatment of diseases such as AIDS or amyloidosis and in which development and marketing is working **successfully**.

The Foundation's vocation is to foster **collaboration** with other agencies, and proofs of this are the arrangements for the establishment of joint units or joint research labs with universities and foundations. During 2013 seven new agreements were formalized for the establishment of joint units: the assignment of Neuroendocrinology and Laboratory of Molecular Neurobiology Laboratory (CIPF joint the Institution for Research at the Clinical Hospital of Valencia); the Research Laboratory in Cardiovascular Repair (CIPF Joint Institute La Fe); the Node proteomics (CIPF Joint University of Valencia); the Laboratory of Biomedical Imaging (CIPF - Joint FISABIO); the Molecular Pathology Laboratory and Research translational Oncology (CIPF joint Catholic University of Valencia) and the Laboratory of Organic Molecules (CIPF Joint University of Valencia).

The strategic plan of the Foundation includes **training and teaching** as a priority and as one of the pillars to ensure the projection of the research conducted at the center. To this end, the Foundation has collaborations with prestigious national and international universities for predoctoral training, with whom we work to attract the best students. Proof of the potential of research groups in the center are the 12 doctoral theses in 2013 and more than another 40 currently underway.

Already, in **financial terms**, the Foundation has significantly lower maintenance costs. This allowed full investment in the recruitment of research personnel and the implementation of scientific projects, with total revenues greater than 7,800,000 euros.
We thank the Generalitat Valenciana for its commitment to the Foundation and its annual contribution of 4,400,000 euros, in accord with the provision contained in the charter that expressly states "the Generalitat Valenciana includes in their budgets sufficient provision to meet the economic needs of the Foundation ". We also thank the General Director of health Research from the Generalitat Valenciana, Dra. Teresa de Rojas, who is doing a wonderful work in promoting Biomedical research in this region.

The Foundation has received a major donation from **Dr. Francisco Pi**, a renowned physician from Oliva, who bequeathed to the Foundation his house in Oliva. To him, posthumously, and to the Pi- Aparicio family we express our gratitude.

In this regard, I note that in 2013 the Foundation launched its donation program under the theme **TOGETHER WE INVESTIGATE, TOGETHER WE ADVANCE.ORG.**

The present and future of the Foundation seeks the involvement of the whole of society in the achievements and progress of investigation, by providing the means to share passion for knowledge and advancement of science in the interests of human and economic development.

We are convinced that, as Louis Pasteur (1822-1895) said, "Science is the soul of the prosperity of nations and the life source of all progress" We firmly believe that investigation has been to sustain the economy of the future, that the results and findings of scientific research not only prolong and save human life, but promote a country's competitiveness through innovation.
Today´s Prince Felipe Research Center Foundation, whose activity during 2013 is included in this scientific report, is the result of half a century of progress and growth of research in the service of society.

To maintain and overcome this research tradition in Valencia is, no doubt, a challenge. But we have the will and the ability to place our center among the elite of biomedical research centers worldwide, to be a center to attract international talent that will establish the Valencian Community in the field of investigation as an engine for the generation of knowledge and wealth.
And again, this alone is possible because the people who make the center are struggling every day to maintain excellence in their work through a culture of effort.

To all of them, with these words, I express my most sincere appreciation.

Isabel Muñoz Criado
Director

Organization

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Organization chart

Director
Isabel Muñoz Criado

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Joaquín Dopazo Blázquez (until February 2013)

Scientific Deputy Directors
Antonio Pineda Lucena (until February 2013)
Susana Rodríguez-Navarro (until February 2013)

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Joaquín Dopazo Blázquez

Advances Therapies Programme
Antonio Pineda Lucena

Neurological Impairment Programme
Vicente Felipo Orts

Molecular Mechanism Of Disease Programme
Susana Rodríguez-Navarro

Rare And Genetic Disease Programme
Francesc Palau

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D. Ángel Villanueva Pareja
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CIPF Research in 2013

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Advanced Therapies Programme

Programme Coordinator: Pineda-Lucena, Antonio

Neuronal and Tissue Regeneration, Lead by Moreno-Manzano, Victoria

Organic Molecules, lead by Fustero, Santos

Peptide and Protein Chemitsry, lead by Pérez-Payá, Enrique and Orzáez, Mar

Polymer Therapeutics, lead by Vicent, Mª Jesus

Structural Biochemistry, lead by Pineda-Lucena, Antonio

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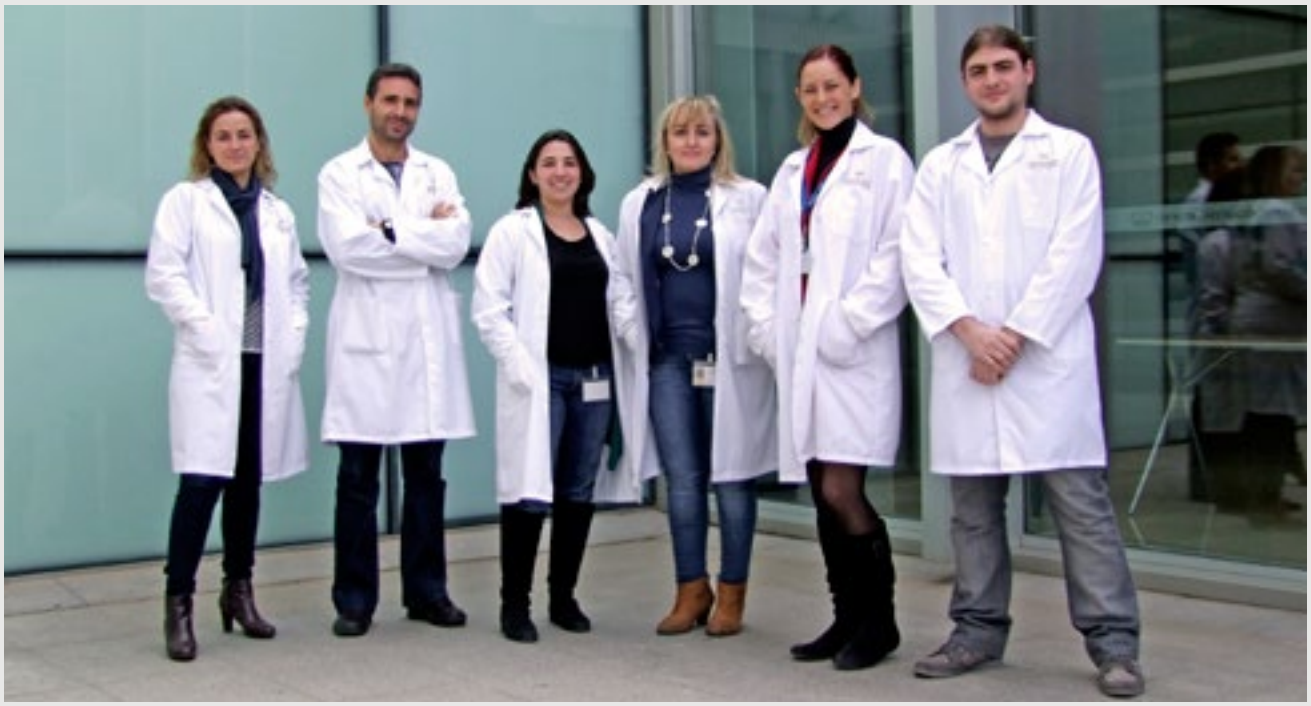
Neuronal & Tissue Regeneration



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Mireia García Roselló
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Vicente Monje López (until July 2013)
Laura Villalba

www.cipf.es/regeneracion-neuronal

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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 focus on the adult stem cells characterization and therapeutic applications into much challenged associated-pathologies: **Spinal cord Injury and Osteoarticular pathologies**

Spinal cord injury (SCI) results in an irreversible paralysis of the hind limb with no currently curable treatment. We showed that acute transplantation of activated ependymal stem/progenitor cell (epSPC) derivated from adult spinal cords can rescue lost neurological function after SCI in rodents (Stem Cells. 2009 Mar;27(3):733-43). The characterization of the "activation" process of the epSPC by the injury and its influence on its regenerative properties constitute an important experimental aim in our group. We aim to improve knowledge about the molecular and cellular process developed along the central nervous system injuries for a better understanding and search for pharmacological tools favoring the applied cell-based therapeutic strategies and look for synergistic effects within the pharmacological arsenal getting advantages on the application 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 much challenged option as a cell-based therapeutic approach. Because osteoarticular complications are very often occurring in dogs, it results as an ideal model for our studies with direct translational perspectives for the human application. 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.

Research results

Intrathecal Spinal Cord-derived neural Cells (epSPC) in traumatic SCI:

Considerable research has been performed in the last 20 years using experimental models to detail the SCI process and to apply potential therapeutic tools, in many cases with therapeutic success. Nevertheless, neuroscientists agree that often there is a problem of reproducibility of results from different labs, largely due to technical difficulties. Although regenerative medicine related research has been performed for the last few decades with therapeutic success, a large gap still exists between experimental data and clinical practice. Recent studies demonstrate the potential of certain cell therapies to incorporate new neural cells into the milieu of traumatic spinal cord injury, regenerate or remyelinate axons providing new oligodendrocytes or simply to reconnect injured tissue with newly generated neurons.
The question of “how” and “when” to perform cell transplantation is a critical one. Acute approaches have shown more promising results than treatment of chronic lesions marked by the potential effect of neuroprotective versus regenerative mechanisms. Subacute transplantation allows for better cell survival and avoids the initial inflammatory response which is highly toxic to transplanted cells. Acute intramedullar transplantation represents a more accepted proof of concept approach in order to minimize the surgical procedure. However, intrathecal cell administration is becoming more popular due to its suitability with regards to clinical translation, less invasive nature and reduced trauma for the spinal cord tissue surrounding the injured area.

Each SCI in humans features a different lesion, while lesions in the laboratory have to be made as reproducible as possible for consistent data. We extensively detailed an experimental model of SCI with different cell therapy approaches in a contusive traumatic spinal cord injury, more similar to that seen in a clinical scenario, suitable for examining acute, sub-acute or chronic cell transplantation and to investigate neural and functional repair of the damaged tissue by the exogenous transplanted cells with no additional damage due to cell transplantation.

Methacrylate-endcapped caprolactone and FM19G11 provide a proper niche for Spinal Cord-derived neural Cells (epSPC)

In the chronic scenario with axon repulsive reactive scar, pharmacological treatment or cell transplantation alone will not be sufficient to bridge a spinal cord lesion, therefore a combinatorial approach would be necessary to fill gaps within the damaged tissue with, for instance, biocompatible materials that would supports cell replacement and good tissue integration, including

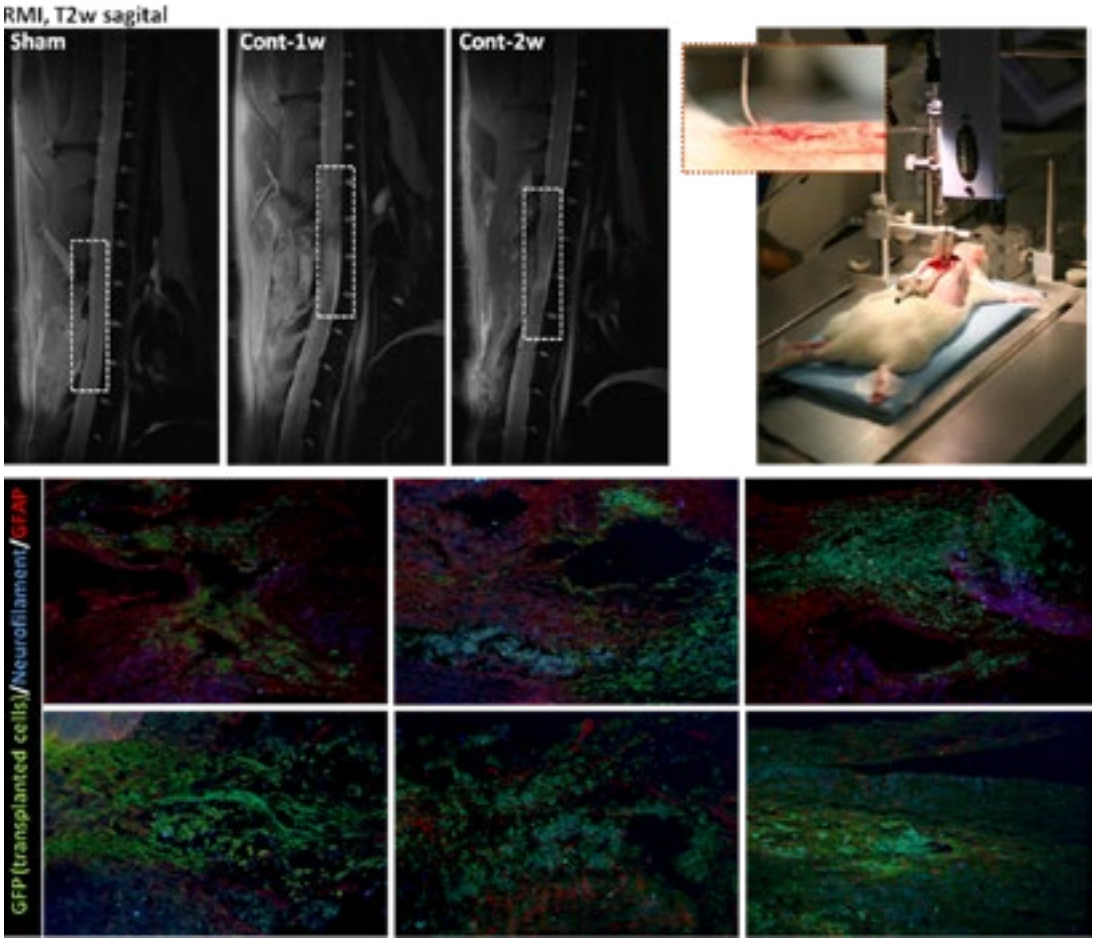

Figure 1. Contussion and Acute intrathecal transplation of epNSC

the ectopic transplanted stem cells and ensure that a better neural intrinsic integration and survival occurs. Caprolactone 2-(methacryloyloxy) ethyl ester (CLMA) is a porous nontoxic 3D scaffold that in our hands shows good biocompatibility with epSPC cultures. epSPC adhere to the scaffolds and maintain the ability to expand the culture through the biomaterial. However, a significant reduction of cell viability of epSPC after 6 days in vitro was detected.

FM19G11 that we have previously shown to enhance self-renewal properties rescues cell viability at 6 days with increased intracellular ATP content. Moreover, treatment with FM19G11 enhances the survival rates of mature neurons from the dorsal root ganglia when cultured with epSPC on CLMA scaffolds (Figure 2). Overall, CLMA porous scaffolds constitute a good niche to support neural cells for cell transplantation approaches that in combination with FM19G11 offer a new framework for further trials in spinal cord regeneration.

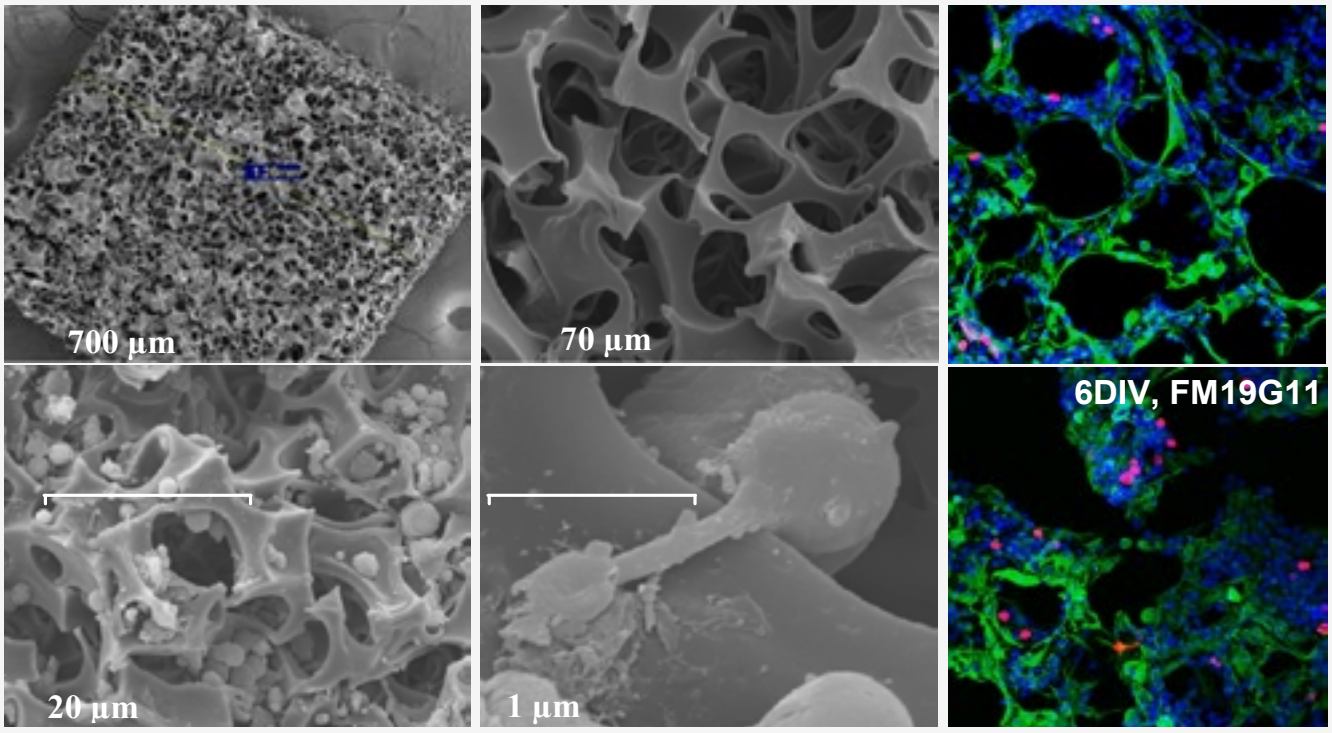

Figure 2: Methacrylate-endcapped caprolactone and FM19G11 provide a proper niche for epSPC

Publications

- 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.
  Stem cells and development,297-303.2013

- Lukovic D, Valdés-Sanchez L, Sanchez-Vera I, Moreno-Manzano V, Stojkovic M, Bhattacharya SS, Erceg S.
  Astrogliosis promotes functional recovery of completely transected spinal cord following transplantation of hESC-derived oligodendrocyte and motoneuron progenitors.
  Stem cells (Dayton, Ohio),131-6, 138, 140.2013

- Valdes-Sánchez T, Rodriguez-Jimenez FJ, García-Cruz DM, Escobar-Ivirico JL, Alastrue-Agudo A, Erceg S, Monleón M, Moreno-Manzano V.
  Methacrylate-endcapped caprolactone and FM19G11 provide a proper niche for spinal cord-derived neural cells.
  Journal of tissue engineering and regenerative medicine,84-7.2013

- Ana Alastrue-Agudo1§, Slaven Erceg2§, Marta Cases-Villar1, Viviana Bisbal-Velasco1, Richard Griffeth1, Francisco Javier Rodriguez-Jiménez1 and Victoria Moreno-Manzano1*.
  Experimental Cell Transplantation for Traumatic Spinal Cord Injury Regeneration: Intramedullar or Intrathecal administration.
  Methods in Stem
  Cell and Tissue Repair.2013

Conferences and Meetings

- Oral Communication, Congreso anual Aspaym,Valencia, Spain.

- Steering committe,Red Glial Española 2013,Oviedo, Spain.

- Oral Communication & Poster, RECI-Reunion Consolider 2013,Cuenca,Spain.

- Oral Communication & Poster, 2nd SCI Repair Meeting,Barcelona,Spain.

Organic Molecules

Group Leader

Santos Fustero Lardiés

→ Researchers

Natalia Mateu Sanchís
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→ Graduate students

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(until Dec. 2013)
Ignacio Ibañez Sánchez

→ Collaborators

Lidia Herrera Muñoz
Cristina Mulet Charro

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

Design and synthesis of new peptidomimetics with therphenylic structure able to interfere in the vital cycle of HIV-1.

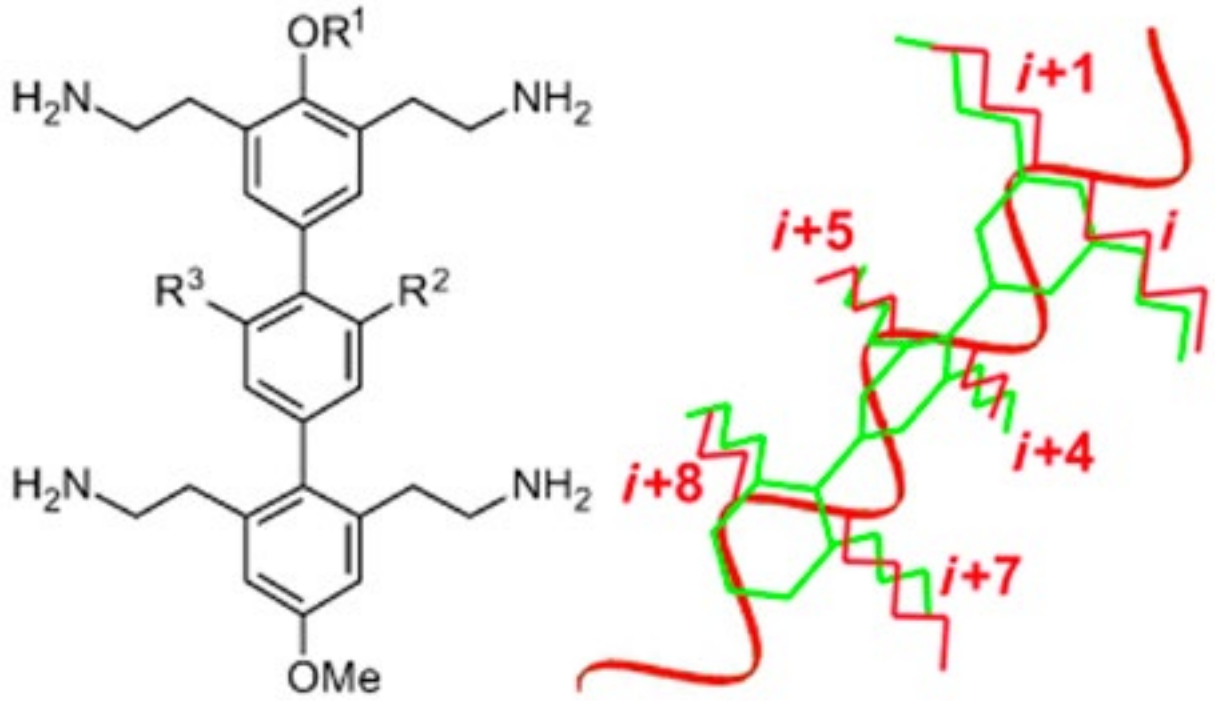
One of the methods for the design and development of new pharmaceuticals is based on the so-called target-oriented synthesis (TOS). This concept focuses on the preparation of a given kind of molecular structures, which are capable of interacting, for instance, with a pre-selected active enzyme center or protein. This project is embedded in this context: a series of bi- and terphenyl aromatic ligands which mimic the structure of Rev (HIV-1 virus protein) complexed to RRE (Rev binding region of the virus RNA) have been designed with the aid of molecular modelling techniques and docking studies. With this information in hand, a synthetic strategy based upon successive carbon-carbon bond formation by palladium catalyzed Suzuki couplings between aryl halides and pseudo-halides with boronic esters has been developed. Superficial Plasmon Resonance (SPR), NMR and fluorescence anisotropy experiments have shown that several of the synthesized compounds bind the RRE region specifically and inhibit the formation of the RRE-$Rev_{34\text{-}50}$ complex in vitro. These terphenyls can mimic one α-helix of the HIV-1 protein Rev and inhibit Rev function and HIV-1 replication in cells. The general structure of the synthesized compounds is represented in Figure 1. These are bilaterally substituted p-terphenylene scaffolds (green) that project their substituents in a broad spatial angle and reproduce the interactions of a protein α-helix (red) embedded in its RNA receptor. This study was carried out in collaboration with Dr. Jose Gallego's group of Catholic University of Valencia and led to a patent ("Nuevos p-terfenilos hexakis-sustituidos con grupos bilaterales para el tratamiento de la infección por el virus de la inmunodeficiencia humana tipo 1 (VIH-1) y otras enfermedades") and a publication ("*Structure-Based Design of an RNA-Binding p-Terphenylene Scaffold that Inhibits HIV-1 Rev Protein Function". Angew. Chem. Int. Ed. 2013, 52, 13405-13409*).

Stereoselective synthesis of new fluorinated building blocks with potential activity as BACE 1 inhibitors.

In January 2012, our group started a collaborative project with the pharmaceutical company Janssen-Cilag. The main goal in this project was the development of a methodology for the stereoselective synthesis of 1,2-difunctionalized fluorinated building blocks as well as new fluorinated heterocycles with potential biological activity as BACE 1 inhibitors (an enzyme involved in Alzheimer´s disease). 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 bioavailability or molecular recognition. In fact, nowadays fluorinated organic compounds are common in the field of medicinal chemistry since many of them have become 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 and this expertise is now being placed at the company´s service. Very recently, and related to this topic, we have published a review article: "Fluorine in Pharmaceutical Industry: Fluorine-Containing Drugs Introduced to the Market in the Last Decade (2001-2011)" (*Chemical Reviews 2014, 114, 2432-2506*).

Asymmetric synthesis of cyclic alcohols and amines by means of organocatalyst /transition metal binary systems (relay catalysis).

The importance of optically pure compounds in the pharmaceutical industry has prompted a great scientific effort towards the development of new strategies for their preparation. Among the existing asymmetric synthesis methodologies, so-called asymmetric organocatalysis has powerfully emerged during the last decade. On the other hand, chemical transformations involving



PICTURE 1

carbon-carbon or carbon-heteroatom bond-forming reactions in a sequence without intermediate purifications (tandem, cascade or domino processes) constitute one of the main goals in organic synthesis. Within the context of tandem processes, so-called relay catalysis in which two or more catalyst present in the same reaction medium are able to act consecutively in several discrete catalytic cycles without interfering with each other, has received remarkable interest. Recently, we have designed a tandem process including a first Brønsted acid catalyzed enantioselective allyl(crotyl)boration step, followed by a ring closing metathesis, thus generating six and seven membered benzo-fused cyclic homoallylic alcohols (“*Relay Catalysis: Enantioselective Synthesis of Cyclic Benzo-Fused Homoallylic Alcohols by Chiral Brønsted Acid-Catalyzed Allylboration/ Ring Closing Metathesis”. Adv. Synth. Catal. 2013, 355, 1058-1064*).

Synthesis of fluorinated nitrogen heterocycles by means of gold salt-catalyzed processes.

The quest for new molecular structures constitutes one of the major challenges for the discovery of new pharmaceuticals nowadays. For that purpose, the use of gold complexes in homogeneous organic reactions has gained great interest in last years. In this context, our research group has been involved in several projects. In one of them, the reactivity of homopropargyl fluorinated amino esters was studied. A tandem process hydroamination/ aza-Diels Alder reaction was observed, yielding tetracyclic structures with excellent diastereoselectivity. The scope of the process, its extension to non-fluorinated amino esters and the proposal of a plausible reaction mechanism were published in 2013 (*“Gold catalyzed stereoselective tandem hydroamination-formal aza-Diels-Alder reaction of propargylic amino esters”. Chem. Commun. 2013, 49, 1336-1338*).

In a second project within the area of gold chemistry, we carried out the synthesis of fluorinated isoindolines and dihydroisoquinollines by means of a diastereoselective addition of fluorinated nucleophiles to chiral sulfinylimines derived from 1-halobenzaldehydes, followed by Sonogahira coupling and cycloisomerization catalyzed by gold(I) salts. The heterocycles thus obtained, in enantiomerically pure form, are considered “privileged structures” in medicinal chemistry since they are present in many natural products with a variety of biological properties. (*“Gold-Catalyzed Intramolecular Hydroamination of o-Alkynylbenzyl Carbamates: A Route to Chiral Fluorinated Isoindoline and Isoquinoline Derivatives”. Org. Lett. 2013, 15, 832-835*).

Synthesis of ß-substituted cyclopentenones through the Pauson-Khand reaction in the presence of the trifluoromethyl moiety.

In collaboration with Professor Antoni Riera of the University of Barcelona we carried out an interesting study of the intermolecular Pauson-Khand reaction of terminal alkynes containing a trifluoromethyl group. The presence of this group is crucial for the inversion of the usual regiochemistry in this type of reaction, affording new ß-substituted cyclopentenones (*“Synthesis and Application of ß-Substituted Pauson–Khand Adducts: Trifluoromethyl as a Removable Steering Group”. Angew. Chem. Int. Ed. 2013, 52, 5355-5359*).

Publications

→ Aiguabella N, del Pozo C, Verdaguer X, Fustero S, Riera A.
Synthesis and application of ß-substituted Pauson-Khand adducts: trifluoromethyl as a removable steering group.
Angewandte Chemie (International ed. in English),5355-9.2013

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Asymmetric allylation/ring closing metathesis: one-pot synthesis of benzo-fused cyclic homoallylic amines. Application to the formal synthesis of Sertraline derivatives.
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Efficacy and activity prediction by molecular topology of new drugs against the Tetranychus urticae plague.
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Gold catalyzed stereoselective tandem hydroamination-formal aza-Diels-Alder reaction of propargylic amino esters.
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Conferences and meetings

- Keynote Speaker,"Stereoselective Synthesis of Fluorinated and Non-Fluorinated Building Blocks : New Strategies". University Southern California (USC),Los Angeles,USA
- Keynote Speaker,"Transition Metals in Fluorine Chemistry: New Strategies". University of Lousville, Kentucky,USA
- Keynote Speaker,"Transition Metals in Fluorine Chemistry: New Strategies". University of Münster, Germany.
- Keynote Speaker,"Stereoselective Synthesis of Fluorinated and Non-Fluorinated Building Blocks : New Strategies and Applications". University of Münster, Germany.
- Keynote Speaker,"Transition Metals in Fluorine Chemistry: New Strategies". Jacobs University, Bremen, Germany.
- Keynote Speaker,"Síntesis estereoselectivas de compuestos fluorados y no fluorados: Nuevas estrategias y aplicaciones". Ciclo de conferencias en CIPF,Valencia, Spain.
- Keynote Speaker,"Stereoselective Synthesis of Fluorinated and Non-Fluorinated Building Blocks: New Synthetic Strategies". Chinese Academy of Sciences, Shanghai, China .
- Keynote Speaker,"New Perspectives of Transition Metals in Fluorine Chemistry". International Swiss Chemical Society ,Syngenta Symposium , Frontiers in Fluorine Chemistry,Stein,Switzerland.
- Keynote Speaker,"Transition Metals in Fluorine Chemistry: New Strategies". 21st Winter Fluorine Conference (ACS), St Pete, Florida,USA.
- Plenary Keynote,"Transition Metals in Fluorine Chemistry: New Strategies". 4th International Fluorine Workshop, Tokyo, Japan.
- Plenary Keynote,"Transition Metals in Fluorine Chemistry: New Strategies". Fluor als Schlüsselelement, Berlin, Germany.
- Plenary Keynote,"New Perspectives of Transition Metals in Fluorine Chemistry". International Workshop on Organofluorine Chemistry 2013,Nanjing,China.

Peptide & Protein Chemistry

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→ Technicians
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→ Collaborators
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Overview

The world population is ageing and, as a consequence, there is a rapid increase in the total number of people affected by diseases that appear predominantly at the elderly. Therefore, there is a clear necessity to build new therapeutic strategies for the treatment of these pathologies.

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 the equilibrium in de-regulated apoptosis and inflammation processes responsible of pathological situations, such as cancer, neurodegenerative or ischemia-re-perfusion associated damages.

To this aim, our experimental approach includes the development of in vitro assays to mimic the PPI of interest, the screening of different chemical libraries and the 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 of disease.

Research results

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 the 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-re-perfusion processes are characterized by the appearance of 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.

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We have also initiated a collaboration with Dr. Ramón Martínez-Mañez to study the controlled delivery of pro-apoptotic drugs in cellular systems.
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INDEX

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 transmembrane regions (TM). We are studying the network of interactions established among these TM regions and their relevance in the control of the apoptotic process. Moreover, our group has analyzed the different cell death pathways used by cancer cells depending on the proapoptotic stimulus and on the availability of death-related cellular components. The study of the cell death molecular pathways activated has provided valuable information to improve the design of appropriate anticancer treatments.

Orzaez, M., et al., 2012. Intrinsic caspase-8 activation mediates sensitization of erlotinib-resistant tumor cells to erlotinib/cell-cycle inhibitors combination treatment. Cell Death Dis, 3. e415
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Development of inflammasome inhibitors.
The inflammasomes are macromolecular complexes from the innate immune system that activate immune and inflammatory pathways in response to invading pathogenic microbes and nonmicrobial danger signals. Inflammasome 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 the proteolytic activation of procaspase-1, the cleavage of the proinflammatory cytokines IL-1 beta and IL-18 and pyroptosis. 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 Crohns disease, cancer or diabetes.
Current therapies for treatment of inflammasomopathies target IL-1 beta signaling but do not address the other components of proinflamatory signalling. To overcome these limitations, our objective has been the development of new modulators of the inflammasome, targeting the ASC-mediated caspase-1 activation (iAC1s).
Our group has developed an in vitro assay, performed a high throughput screening and identified 14 new lead molecules as inhibitors of the ASC-mediated Caspase-1 activation that have been validated (iAC1) in in vitro and cellular models of inflammation.

Colaborations

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Centro de Investigación Príncipe Felipe. Genómica Computacional
20th Euroconference on Apoptosis (Poster). 2012 Rome(Italy)

PICTURE 1 - Dr. Pérez Paya, Group Leader.

Publications

→ Orzáez M, Guevara T, Sancho M, Pérez-Payá E..
Intrinsic caspase-8 activation mediates sensitization of erlotinib-resistant tumor cells to erlotinib/cell-cycle inhibitors combination treatment.
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Conferences and Meetings

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→ Oral communication . XI Aporeunión. Malaga, Spain.

→ Oral communication, XIII International congress of the Spanish Biophysical Society., Valencia, Spain.

→ Oral communication, "IL-1 mediated inflammation and diabetes: from basic science to clinical applications". Nijmegen Holland.

→ Oral communication, "Mechanisms of cell death: The Command to Die". International cell death society congress. Málaga, Spain.

Polymer Therapeutics

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Overview

Clinical proof of concept for **Polymer Therapeutics** has already been achieved, even 2 Polymer Therapeutics are within the US 10Top 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 2013 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.
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.

We have achieved in vivo proof in metastatic breast cancer models that the rational design of a combination conjugate bearing in the same mainchain a cocktail of bioactive agents could trigger anticancer synergistic effect. The combination of two drugs in the adequate ratio and with a controlled drug release kinetics could even change the molecular mechanism of action of the bioactive agent(s) enhancing their antitumour effect. Several conjugates bearing endocrine + chemotherapeutics agents have demonstrated their promising antitumor behavior after an adequate rational design (linkers, drug ratio, solution conformation) in orthotopic metastatic breast cancer tumor models (Deladriere sub). Following this successful strategy in breast cancer, pH responsive combination conjugates for the treatment of advanced prostate cancer have been also developed. Inspired by the bi-hydroxyl functionality Diethylethylstilbestrol (DES), a synthetic non-steroidal estrogen, that can be easily incorporated into a pH responsive polyacetalic mainchain (Giménez 2012; England 2012) and combined with curcumin (Plyduang in prep) or paclitaxel (Giménez in prep) provided with nanoconjugates showing an important synergistic effects in prostate cancer models and the capability to release the drug selectively under acidic environment such as that found in endosomes and lysosomes; in addition, they were shown to be stable at pH 7.4 mimicking blood plasma, being an ideal profile for a lysosomotropic drug delivery route. The prostate cancer projects are run in collaboration with Dr Lopez (IVO, Valencia) looking for some biomarkers of this heterogeneous diseases in patient samples (Casanovas 2013) and Dr. Schwartz Jr's group (VHIR, Barcelona) with the orthotopic LNCaP animal model.

Previously in our group, we have developed synthetic pathways to reach functional polyglutamates with well-defined structure, adjustable molecular weight and low dispersity applying a controlled ring opening polymerization of NCAs with novel initiators (*Conejos-Sanchez, 2013; Vicent, 2012*). 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 sites (*Barz, 2013*) suitable for further bioconjugations. This newly described methodology of NCA polymerization, based on the use of BF4s was the foundation of our Spin Off company Polypeptide Therapeutic Solution SL and has also been recently successfully applied for the synthesis of three-arm star shaped polyglutamates. 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 biodegradability in physiological fluids, cytotoxicity, cellular uptake and internalization mechanism (confocal microscopy, flow cytometry) followed by biodistribution and PK studies in vivo (figure 1). 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*).

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. Metabolic profile was studied in an in vitro and in vivo breast cancer model corroborating the greater anti-tumour effect observed after the treatment with selected polymer drug conjugates. Metabolic data was confirmed with proteomic and cytomic studies (*Armiñan, Palomino in prep*).

Non-invasive visualization of unique molecular processes behind human pathology would provide highly specific and potentially early indicators of ongoing diseases. These molecular processes are, i.e, up regulation or activation of specific disease related factors, in particular proteases like metalloproteases (MMPs) or cathepsins. To date, activatable protease agents for optical imaging have already been successfully applied. However, the most critical gap in molecular imaging approaches is the availability of target-specific and tissue-specific imaging probes. In this context and as part of a collaborative FP7 European project, Livimode (Light –based and functional in vivo monitoring of diseases related enzymes), we use our polyglutamates as carriers of highly specific smart probes for specific proteases to enhance site-specific accumulation maintaining probe specificity and catalytic efficiency. In vivo proof for a MMP13 selective probe has been achieve in an osteoarthritis mouse model (in collaboration with Prof Dive (CEA, France) and Prof Nagase (ICL, UK)) (*Duro-Castaño in prep*).

A collaborative funding scheme was performed in collaboration with the Univ. Nottingham (UK) and Univ. Padova (Italy) for a European NanoSci ERA-net transnational project. A novel pH sensitive targeted polymeric vesicular system for the delivery of oligonucleotides to cancer cells was developed and physicochemically and biologically characterized (*Matini 2014; Gallon, Arminan, in prep*). In the same line a research collaboration was established with Prof Wagner (Univ. Munich) a leading researcher in the field.

Finally is worth mentioning the collaborative INNPACTO project with Nanoimmunotech, Oryzon, NanoScale Biomagnetics and the University of Zaragoza with the aim of developing a nanosystem capable to target tumors and once there to ablate them by the combination of magnetic hyperthermia and thermo-responsive drug release.

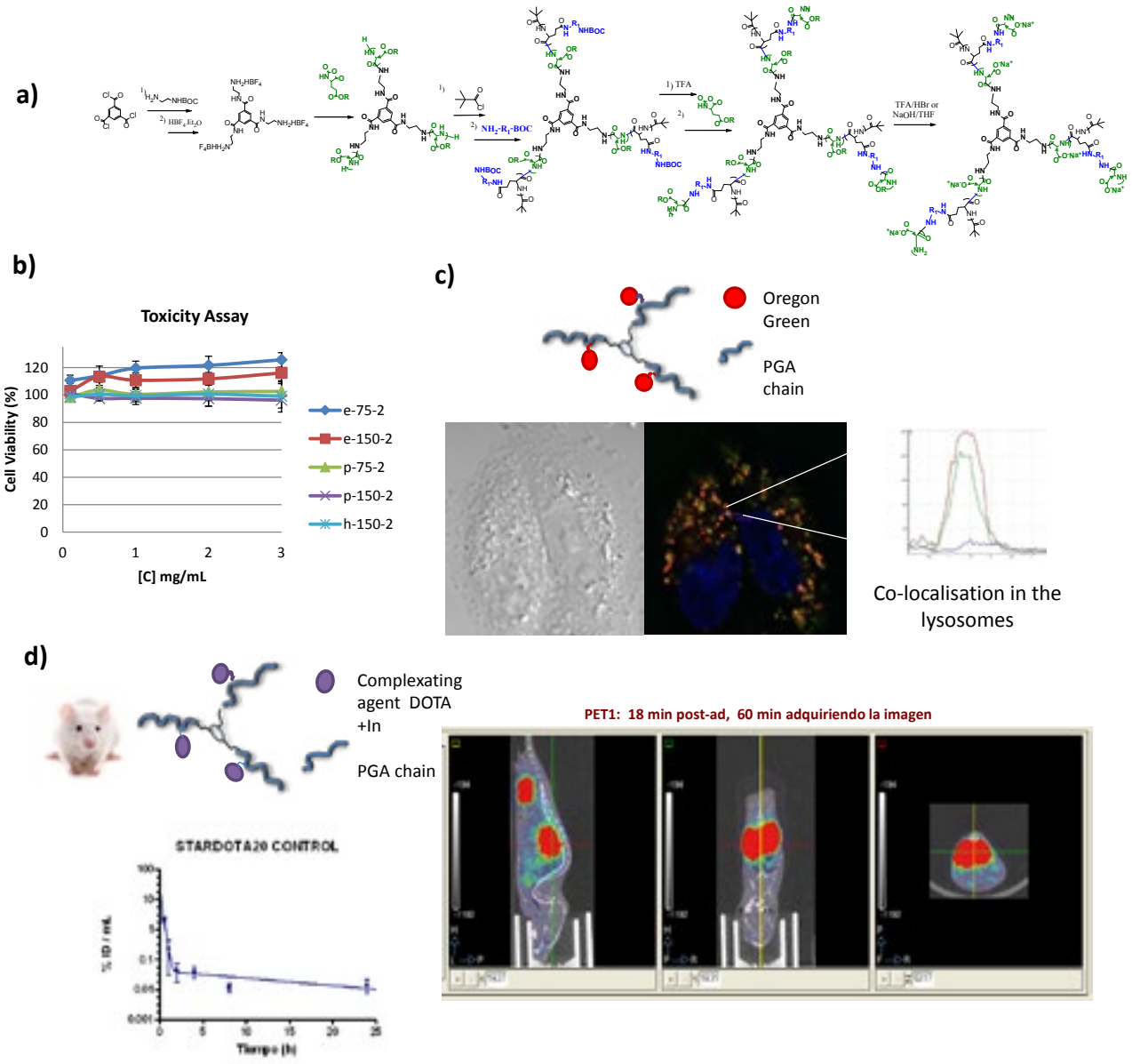

Figure 1. A) Synthetic route to Star-shaped polyglutamates. B) Toxicity assay in HUVEC of star-shaped PGAs by MTS assay at 72 hours after treatments. C) Scheme of a star-shaped PGA fluorescently labelled OG488, uptake study by flow cytometry in SHSY5Y cell line and confocal image of the uptake at 2 hours after treatment. D) Graphic example of star-shaped PGA labelled with DOTA-111In, biodistribution profile of a star-shaped PGA by PET and PET images (Collaboration with MA Morcillo, CIEMAT).

Publications

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- Ucero AC, Berzal S, Ocaña-Salceda C, Sancho M, Orzáez M, Messeguer A, Ruiz-Ortega M, Egido J, Vicent MJ, Ortiz A, Ramos AM. A polymeric nanomedicine diminishes inflammatory events in renal tubular cells. PloS one,e51992.2013
- 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 entryPolymer Chemistry,DOI: 10.1039/C3PY00744H .2013
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- Inmaculada Conejos Sánchez; Isabel Cardoso; Maria J. Saraiva; María J. Vicent Docón. Targeting a rare amyloidotic disease through rationally designed polymer conjugatesJournal of controlled release : official journal of the Controlled Release Society,.2013
- Irene Casanovas Salas; Jose Rubio Briones; Ana Calatrava; Caterina Mancarella; Esther Masià; Juan Casanova, Antonio Fernández Serra; Luis Rubio; Miguel Ramírez; Ana Armiñán; José Domínguez Escrig; Francisco, Martínez; Zaida García Casado; Catia Scotland; María J. Vicent; José A. López Guerrero. Identification of miR-187 and miR-182 as biomarkers for early diagnosis and prognosis in prostate cancer patients treated with radical prostatectomyJournal of Urology,.2013

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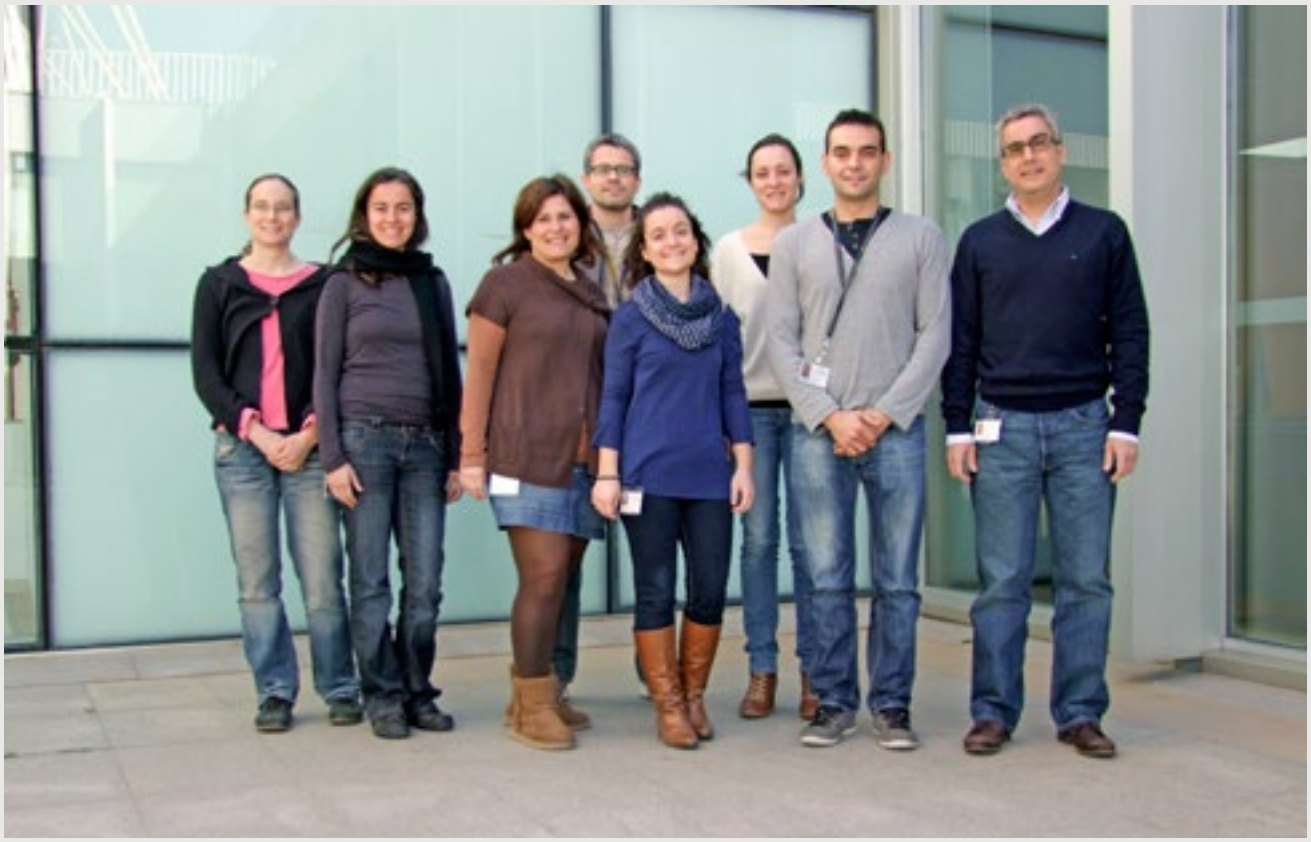
- Biomaterial Science RSC Publishing

Congress

- Plenary Keynote,"Polymer Conjugates as Nano-sized Medicines". Jornadas Anuales del CIBER-BBN 2013,Torremolinos, Spain.
- Plenary Keynote,"Polymer Conjugates as Nano-sized Medicines".4th International NanoMedicine Conference,Sydney,Australia
- Keynote speaker,"Polymer Conjugates as Nano-sized Medicines". 25th European Conference on Biomaterials and 10th Young Scientific Forum (ESB2013).,Madrid, Spain.
- Keynote Speaker,"Polymer Conjugates as Nano-sized Medicines". V Spanish Drug Discovery Network Meeting,Valencia,Spain.
- Keynote Speaker,"Polymer Conjugates as Nano-sized Medicines". XXXVII Congreso Nacional de Inmunología,Salamanca,Spain.
- Keynote Speaker,"Vesicles as drug delivery systems. Extracellular Vesicles implications in Biomedicine". UIMP,Valencia,Spain.
- Keynote Speaker,"Polymer Conjugates as Nano-sized Medicines". 2nd NanoFar School,CIMUS. Santiago de Compostela,Spain.
- Steering committe, V Spanish Drug Discovery Network Meeting, Valencia,Spain.
- Steering committe, 10th SPLC-CRS Conference on Controlled Drug Release,Valencia,Spain.
- Steering committe, 3rd International Symposium Frontiers in Polymer Science,Sitges, Barcelona, Spain.

Structural Biochemistry



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Overview

The **Structural Biochemistry Laboratory** is interested in the development of novel molecularly targeted agents (MTAs) that could be useful in cancer and metastatic progression using a combination of structure-based drug design and metabolomics by NMR. Whereas localized tumors could be treated by focal therapy, extensive or metastatic tumors and hematological malignancies require the development of systemic anticancer therapies. To date there are still far too few examples of therapies leading to cure; and cancer remains as one of the largest causes of death worldwide. In this context, the suppression of metastatic tumor growth, using targeted approaches, would have a major impact on the outcome of many solid tumors and would improve the duration and quality of life for many patients with cancer. Also, there is a strong need to identify robust biomarkers for predicting the therapeutic response to a given therapy and stratify patients for the treatment.

Our group focuses on these two aspects and is working on the development of novel MTAs against a number of targets involved in cancer and cell invasion. Furthermore, clinically relevant biomarkers for the management of multiple myeloma, lung and prostate cancer are being sought using metabolomics by NMR approaches. 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

During 2013, we have conducted several studies that could contribute to the development of small-molecule inhibitors against several pharmacologically relevant targets. In this context, Nuclear Magnetic Resonance (NMR) has being used in combination with other biochemical, biophysical and computational screening of libraries of fragments and the characterization of high-resolution structures. On the other hand, NMR metabolomics has also being 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. Moreover, metabolomics allows the characterization of the metabolic disturbances caused by the antineoplastic agents and thereby provides a means to evaluate the efficacy (clinical validation) and selectivity/toxicity (mechanism of action) of the drugs.

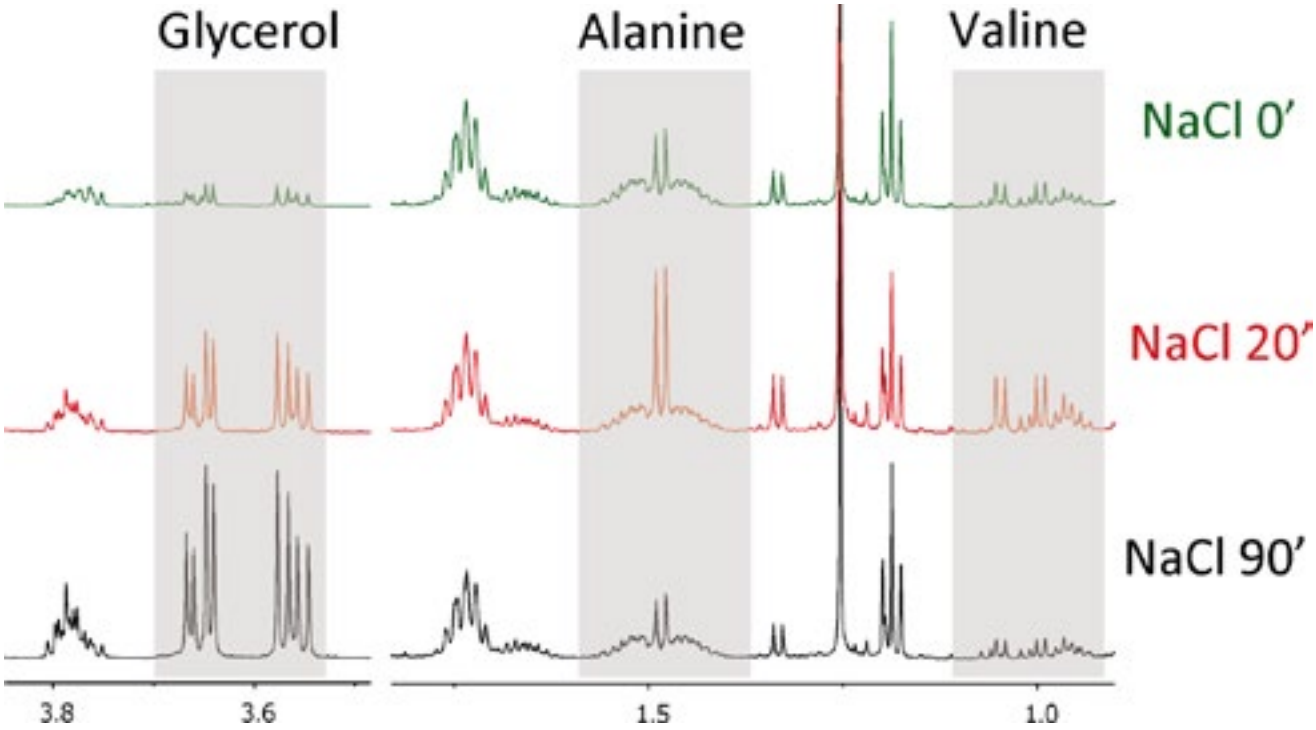
Structure-based drug design: Most of our efforts have focused on heparanase. This enzyme is an endo-ß-D-glucosidase involved in the specific degradation of heparan sulfate (HS), the polysaccharide component of heparan sulfate proteoglycans (HSPGs) which are present on the cell surface and extracellular matrix (ECM). Increased levels of heparanase are found in numerous cancer processes such as melanomas, breast, colon, lung, prostate, liver, bladder, intestine, ovarian and pancreatic tumors, and there seems to be a direct correlation between the overexpression of heparanase and the invasiveness of tumor cells.

The implication of heparanase in cancer progression makes it a very attractive target in antineoplastic strategies, emphasized by the fact it appears to be a unique enzyme, in contrast to the multiple proteases involved in the same phenomena. The utility of heparanase, in relation to the design of inhibitors for tumor treatment, has been always limited by the absence of the 3D structure of this enzyme. Throughout 2013, we have used a combination of in silico techniques and experimental methods to identify new potential inhibitors against this target. A 3D model of heparanase was built from sequence homology and applied to the virtual screening of a library composed of known heparanase inhibitors and a commercial collection of drugs and drug-like compounds. The docking re-

sults from this campaign were combined with those obtained from a pharmacophore model we recently published based on the same set of chemicals. Compounds were then ranked according to their theoretical binding affinity, and the top-rated commercial drugs were selected for further experimental evaluation. Biophysical methods (NMR and SPR) were applied to assess experimentally the interaction of the selected compounds with heparanase. This strategy has led us to the discovery of novel inhibitors that are now being characterized from a biochemical and biological point of view.

Metabolomics: Metabolomics, an analytical method used in conjunction with pattern recognition approaches and bioinformatics to detect metabolites and follow their changes in biofluids and tissues, is increasingly recognized as a very powerful tool to identify disease biomarkers in a non-invasive manner. Because cancer cells are known to possess a highly unique metabolite phenotype, it would be possible to develop specific biomarkers in oncology that might be used for identifying fingerprints, profiles, or signatures to detect the presence of cancer, determine prognosis, and/or assess the pharmacodynamics effects of therapOur group has developed, over the last few years, a demonstrated capability in the application of metabolomics to the identification of biomarkers for Chronic Lymphocytic Leukemia, Minimal Hepatic Encephalopathy, as well as in the characterization of human embryonic stem cells conditioning media.

Last year, we carried out a number of clinical and methodological studies that could contribute to the characterization of different pathologies and facilitate the analysis of relevant biological matrices. Among them, in collaboration with the Grupo Español de Mieloma Múltiple, we have conducted an analysis of serum samples from patients with MM collected at the time of diagnosis and after complete remission.
In this study, we also included a set of samples from healthy individuals with a similar distribution of age and sex to that of patients. A number of important changes have been observed in the different comparisons performed (MM at the time of diagnosis vs healthy individuals, MM at the time of diagnosis vs MM at complete remission, and MM at complete remission vs healthy individuals) that will facilitate the clinical monitoring of these patients. Also, through the analysis of umbilical vein blood plasma of newborns, we have been trying to better understand the molecular mechanisms associated to different clinical forms of intrauterine growth restriction (IUGR).The results have revealed that IUGR is not associated with a unique metabolic profile, but important changes are present in different clinical subsets used in research and clinical practice.



Título del Resultado de Investigación 1
1H NMR spectra of aqueous extracts of S. cerevisiae 0, 20 and 90 minutes after induction of osmotic stress with NaCl.

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Publications

- Puchades-Carrasco L, Lecumberri R, Martínez-López J, Lahuerta JJ, Mateos MV, Prósper F, San-Miguel JF, Pineda-Lucena A. Multiple myeloma patients have a specific serum metabolomic profile that changes after achieving complete remission. Clinical cancer research : an official journal of the American Association for Cancer Research,4770-9. 2013

- Fabre B, Filipiak K, Zapico JM, Díaz N, Carbajo RJ, Schott AK, Martínez-Alcázar MP, Suárez D, Pineda-Lucena A, Ramos A, de Pascual-Teresa B. Progress towards water-soluble triazole-based selective MMP-2 inhibitors. Organic & biomolecular chemistry,6623-41. 2013

- Rodríguez-Díaz J, Carbajo RJ, Pineda-Lucena A, Monedero V, Yebra MJ. Synthesis of fucosyl-N-acetylglucosamine disaccharides by transfucosylation using a-L-fucosidases from Lactobacillus casei. Applied and environmental microbiology,3847-50. 2013

- Palomino-Schätzlein M, Molina-Navarro MM, Tormos-Pérez M, Rodríguez-Navarro S, Pineda-Lucena A. Optimised protocols for the metabolic profiling of S. cerevisiae by 1H-NMR and HRMAS spectroscopy. Analytical and bioanalytical chemistry,4770-9.2013

- Pérez-Benavente B, García JL, Rodríguez MS, Pineda-Lucena A, Piechaczyk M, Font de Mora J, Farràs R. GSK3-SCF(FBXW7) targets JunB for degradation in G2 to preserve chromatid cohesion before anaphase. Oncogene,2189-99. 2013

- Gozalbes R, Mosulén S, Ortí L, Rodríguez-Díaz J, Carbajo RJ, Melnyk P, Pineda-Lucena A.Hit identification of novel heparanase inhibitors by structure- and ligand-based approaches. Bioorganic & medicinal chemistry,1944-51. 2013

- Sanz-Cortés, M., Carbajo, R.J., Crispi, F., Figueras, F., Pineda-Lucena*, A., Gratacós*, E. (*: corresponding authors) Metabolomic profile of umbilical cord blood plasma from early and late intrauterine growth restricted (IUGR) neonates with and without signs of brain vasodilation. PLoS ONE,e80121 -80121.2013

- Puchades-Carrasco, L., Jantus-Lewintre, E., Lucas, R., Blasco, A., Pineda-Lucena, A., Camps, C. Metabolomic profile of lung cancer patients. Lung Cancer,S11-S11.2013

Conferences and meetings

- Oral Communication, "From Biomarkers to Lead Finding". Pre-SMASH Bruker Meeting,Santiago de Compostela,Spain.

- Oral Communication, "Metabolomics applications in oncology: Disease diagnosis, prognosis and treatment monitoring". URBAM-13,Málaga,Spain.

- Poster, "Metabolomic profile of lung cancer patients". European Multidisciplinary Conference in Thoracic Oncology (EMCTO-2013),Lugano,Switzerland.

- Oral Communication, "Mycobacterium tuberculosis: bioinformatic and structural strategies towards treatment". EU-India STI Cooperation Days,París,France

- Oral Communication, "Hacia una medicina personalizada". IV Curso de Metodología en Gestión Farmacoterapéutica,Denia, Alicante,Spain.

- Oral Communication, "Herramientas al Servicio de la Medicina Personalizada". Open Innovation en Investigación Biomédica,Valencia, Spain.

- Oral Communication, "Plataformas tecnológicas en Metabolómica: RMN y EM". Jornada Metabolómica,Valencia,Spain..

- Oral Communication, "Metabolomics: The metabolic pathway to target identification". Invited Seminar (GSK),Madrid,Spain..

- Oral Communication, "Metabolomics application in disease diagnosis, prognosis and treatment monitoring". Seminarios de Investigación (Hospital Dr Peset),Valencia,Spain.

- Oral Communication, "New approaches in Anticancer Drug Discovery: From target characterization to drug evaluation". Seminarios de Investigación (BIONAND),Málaga,Spain..

- Oral Communication, "New approaches in Anticancer Drug Discovery: From target characterization to drug evaluation". Seminarios de Investigación (IMIBIC),Cordoba,Spain..

- Steering committe,Plataformas tecnológicas en Metabolómica: RMN y EM,Valencia,Spain.

- Steering committe, XVII Congreso de la Sociedad Española de Química Terapéutica.

- Steering committe, Open Innovation en Investigación Biomédica,Valencia,Spain..

Computational Genomics Programme

Programme Coordinator: Dopazo, Joaquín

Genomics Of Gene Expresion, lead by Conesa-Cegarra, Ana

Systems Biology, lead by Dopazo, Joaquín

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Genomics of Gene Expression

Group Leader

Ana Conesa Cegarra

→ Researchers
Rodrigo Lomas
Enrique Vidal
Miguel Angel Pardo Cea
(until April 2013)

→ Graduate Students
Sonia Tarazona
Pedro Furió
Rafael Hernández
Lorena de la Fuente

→ Technicians
Cristina Martí
Claudio Navarre

→ Project Manager
Eugenia Flores

→ Collaborators
Diego Nicolas de Panis
(until may 2013)
Henk Jan Van Hasselar
(until may 2013)
Claudia Oliveira
(until July 2013)
Carlos Company Nevado
(until Sept. 2013)

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 it 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, most recently making use of Next Generation Sequencing technologies. The current areas of research are:

Patho-transcriptomics: genome architecture and gene expression regulation in pathogenic bacteria
Development of molecular biomarkers for diagnosis and prognosis
Systems Biology in the immune system and its association to leukemia
Functional role long-non coding RNA and their association to disease.

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), and NOIseq (RNA-seq analysis).

The laboratory is currently coordinator of two FP7 research projects: STATegra, on the development of statistical tools for integration of heterogeneous omics datasets, and DEANN, a Marie Curie IRSES for developing a Europe-LatinAmerica NGS analysis network

Research results

Major lab results during 2013

Molecular Biomarkers

1.Biomarkers in serrated colon carcinoma.
Previously, we identified new biomarkers of colorectal serrated adenocarcinoma by gene expression profiling and immunohistochemistry. Both fascin1 and hippocalcin were detected as significantly associated to the serrate state of colon carcinomas and can be used to predict the progression of primary adenomas. Results were published in *Int J Cancer. 2013 Jan 15;132(2):297-307*. We have complemented this research with the analysis of epigenomic makers of serrated carcinomas and compared this to the normal phenotype in a Spanish and Finnish population. Our results indicate over a hundred of genes with differential methylation pattern between the two carcinoma types, involving functions related to cytoskeleton organization, signaling, ion channel, endothelial growth factors and several metabolic functions. These results shed new light into the molecular mechanisms behind the serrated phenotype.

2.Biomarkers for Systemic Lupus Erythematosus (SLE).
We have analyzed the gene expression and methylation patterns in discordant twins for SLE and of patients with Rheumatoid Arthritis (RA). Our results indicate a differential methylation in affected individuals of transcription factors related with the differentiation and activation of the immune system, as well as several genes involved in the regulation of transcription. These results suggest that epigenetic modifications of key regulators of the immune system may underlay the development of the disease in affected individuals.

3.Development of a molecular signature for totipotency and pluripotency in early development.
In collaboration with Dr. Simón from IVI, we used gene expression profiling of 3-days blastomers and pluripotent inner cell masses (ICM), compared to derived human embryonic stem cells (hESCs) to identify a genomic signature that establish the pluripotency character of hESCs. We characterized the underlying gene interaction network of this signature. *Published in PLoS One 2013 Apr 17;8(4):e62135.*

Pathogenomics

4.Identification of operon structures in pathogenics bacteria.
Within the Pathomics ERAnet project we have characterized the operon structure of pathogenic bacteria (Pseudomonas aeruginosa) and analyzed gene and operon expression under different nutritional conditions that tiger the expression of different bacterial membrane transport systems (T3SS and T6SS) associated to pathogenicity. We obtained the operon composition of different clinic isolates and identified operon structure differences associated to the growth conditions. This comparison has allowed us to identify Pseudomonas specific cellular functions of the PsedoCAP database, which are regulated by changes in operon composition, such as the Protein secretion-export apparatus, the Two-component regulatiory systems, and Cell wall / LPS / capsule (Fig. 1). This project is a collaboration with Dr. Romé Voulhoux from Marseille University.
Manuscript in preparation.

Computational Biology and Bioinformatics

5.The STATegra project. STATegra is a FP7 project, coordinated by our group, which aims at the development of statistical tools for the integration of omics and NGS data. The project has 11 partners, including geneticist, biostatisticians, bioinformaticians and two software companies. During 2013 we have generated RNA-seq, miRNA-seq, Methyl-seq, DNase-seq, ChIP-seq, metab-

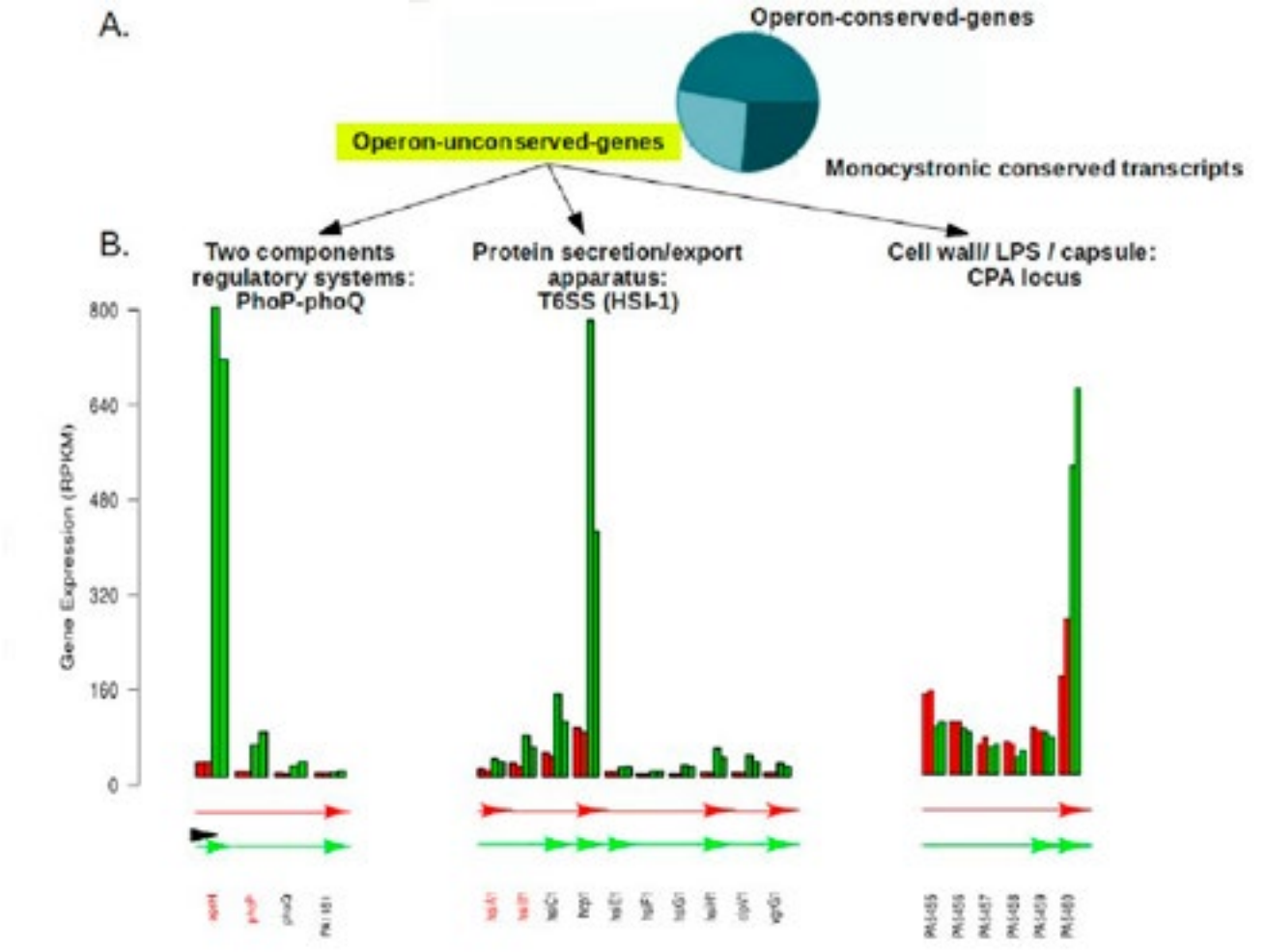

FIGURE 1: Operon distribution variability in Pseudomonas aeruginosa PAO1 grown using two different conditions. In red, the Calcium-lacked medium (T3SS) and in green the LB suplemented medium by EGTA-Mg (T6SS). A. Pie chart with operon variability. B. Variability in three different locus containing genes from three representative enriched functions.

olomics and proteomics data on the course of differentiation of B-cells under the control of the Ikaros transcription factor. One of the first results of this project is the development of the STATegraEMS, an Experiment Management System to annotate, store and query complex omics experiments and their analysis pipelines (Figure 2). The STATegraEMS is a freely available Java application that can be downloaded from *http://bioinfo.cipf.es/stategraems_app/*. The software has been published as:
STATegra EMS: An Experiment Management System for complex next-generation omics experiments. Hernández R, Boix-Chova N, Gómez-Cabrero D, Tegner J, Abugessaisa I, Conesa A. BMC Systems Biology 2014, 8(Suppl 2):S9.

6.Pathway network analysis (PANA).
We have developed new computational approaches to investigate functional interconnection between cellular pathways using serial gene expression data. Our approach, called PANA, infers the network of active pathways under a given developmental or physiological condition and identifies key connecting genes. This is a systems biology approach relevant to investigate how cellular functionality is orchestrated and identify side-effects of cellular dysfunction. This approach has been published recently as: *Pathway network inference from gene expression data. Ponzoni I, Tarazona S, Götz* **S, Montaner D, Dussaut JS, Dopazo J, Conesa A. BMC Systems Biology 2014, 8(Suppl 2):S7**

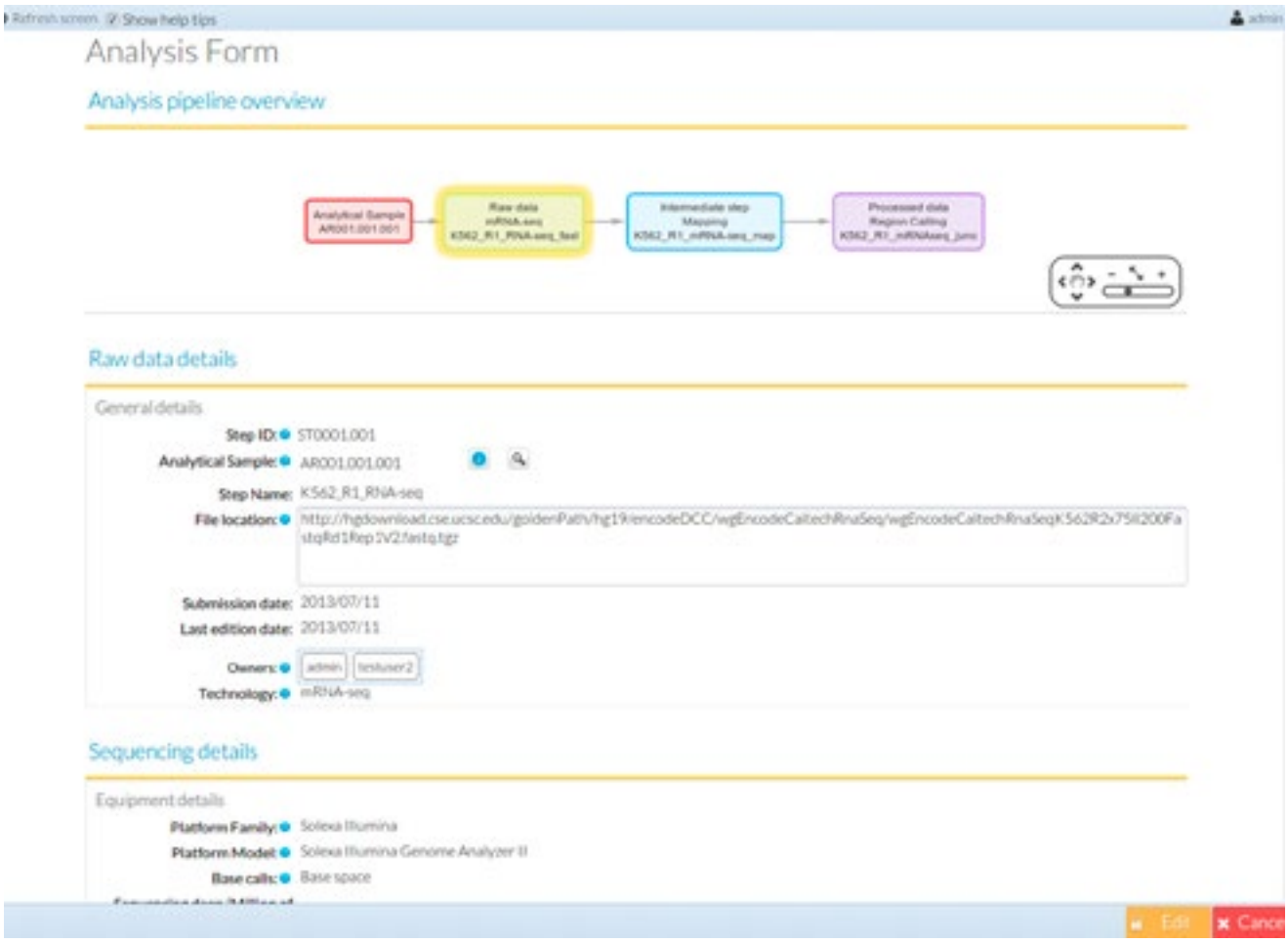

Figure 2 Screenshot of the STATegraEMS application

7.Functional characterization of long-non coding RNAs.

We are currently developing computational approaches to predict the functional involvement of lncRNAs from the analysis of their gene expression patterns along a wide variety of tissues and cell-lines. We have identified the tissue specificity of these genes and developed association metrics to identify possible target coding genes in statistically sound manner (Figure 3). This approaches has allowed us to propose cellular functions where these lncRNAs might be involved. At present we are validating these results and developing new approaches to study lncRNA-miRNA interactions.

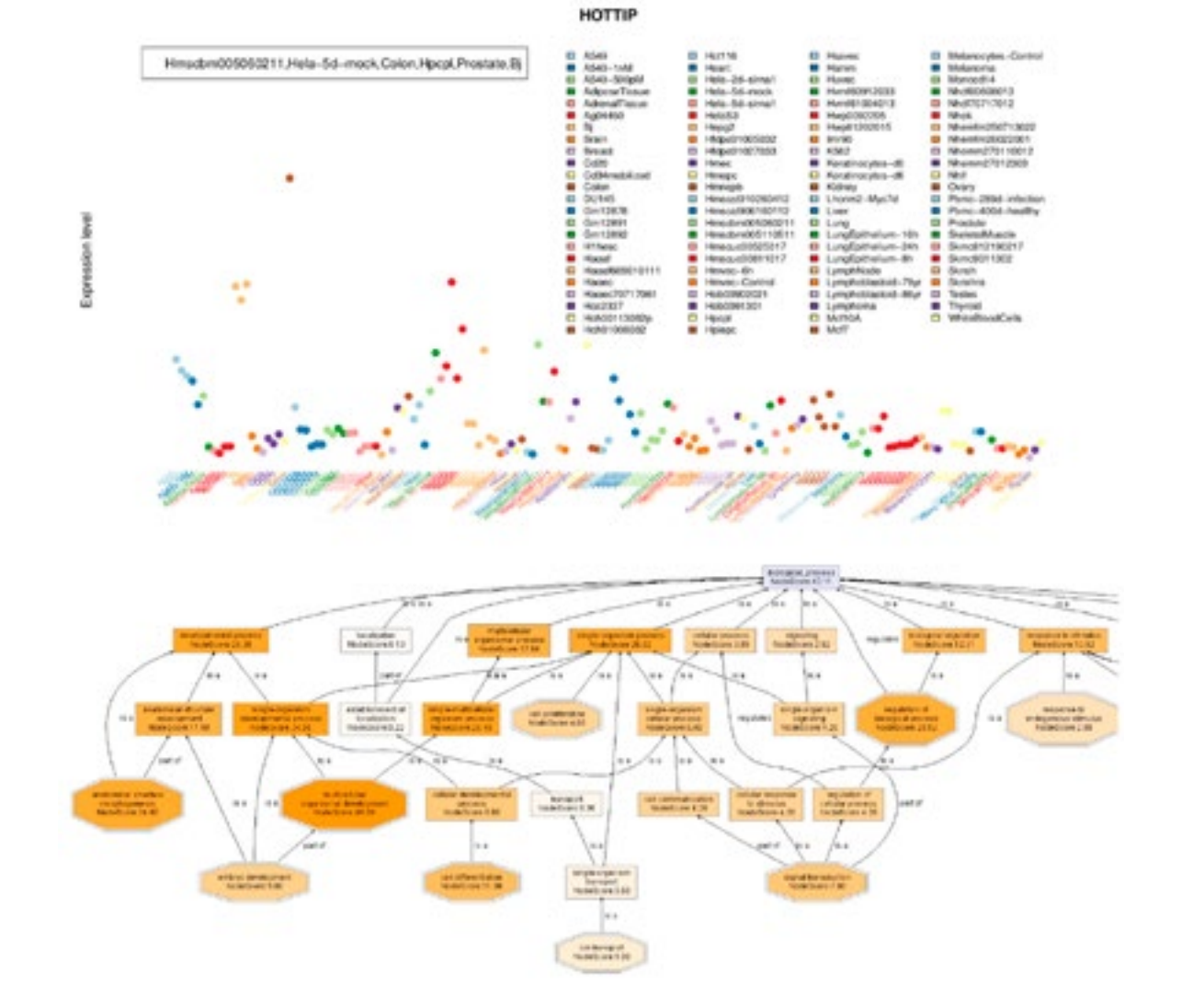

Figure 3 Tissue-specific expression of the lncRNA HOTTIP and Gene Ontoloy graph of associated functions.

Publications

- Conesa-Zamora P, García-Solano J, García-García F, Turpin Mdel C, Trujillo-Santos J, Torres-Moreno D, Oviedo-Ramírez I, Carbonell-Muñoz R, Muñoz-Delgado E, Rodriguez-Braun E, Conesa A, Pérez-Guillermo M.
  Expression profiling shows differential molecular pathways and provides potential new diagnostic biomarkers for colorectal serrated adenocarcinoma.
  Journal international du cancer,297-307 2013

- Galan A, Diaz-Gimeno P, Poo ME, Valbuena D, Sanchez E, Ruiz V, Dopazo J, Montaner D, Conesa A, Simon C.
  Defining the genomic signature of totipotency and pluripotency during early human development.
  PloS one,e62135. 2013

Conferences and meetings

- Keynote Speaker,"Analysis of Fungal Transcriptomics with CLC Genomics". Workbench and Blast2GO.,XXI Plant and Animal Genome Conference ,San Diego,USA

- Keynote Speaker, Multidisciplinary multi-institutional PhDs,ISCB/GOBLET Meeting,Berlín, Germany.

- Invited Professor, Curso H3ABioNet Introduction to Bioinformatics en International Center for Insect Physiology and Ecology (ICIPE),Nairobi,Kenya.

- Invited conference,"Transcriptome analysis with RNAseq: promises and biases". Department of Genetics, Stanford University,Stanford,USA.

- Invited seminar, "The STATegra project: new statistical methods and tools for integrative omics data analysis". Workshop in Translational Genomics,Barcelona, Spain.

- Invited conference,"Pathway-based linear models".SeqAhead/STATegra High throuput and data integration workshop,Barcelona, Spain.

- Host organizer of the Confernece the Next NGS Challgenlle, May 2013, Valencia, Spain.

Member of the Editorial Board of

- BMC System Biology Special Issue in Data Integration

Systems Biology

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Rosa Hernansaiz

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Roberto Alonso
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Lorena Garrido Lluna

→ Collaborators

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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 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 .

Algorithms for genomic data

We 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 produced. Therefore, we have been developing different tools for facilitating these tasks, such as sequence mappers that accelerates the process up to a 20x (*Gonzalez el al., 2013, Lecture Notes in Computer Science*). Probably, the most successful development is the genomic viewer Genome Maps (*Medina et al., 2013, NAR*). This tool enables the representation of different genomic features in the context of the genome using an intelligent technology based on Google Maps, which can cope with huge data transfers interactively. 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 is used by thousands of researchers around the world (see Figure 1).
http://www.fda.gov/downloads/ScienceResearch/BioinformaticsTools/MicroarrayQualityControlProject/ucm122981.pdf

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 whole exome sequencing (WES) experiments of a large number of cases, some of which started to be published during 2013. For example, our analyses discovered new mutations with clear diagnostic potential in metabolic diseases (Tort et al., 2013, Mol Genet Metab), degenerative retinal dystrophies (Méndez-Vidal et al., 2013 Mol Vis) or gut neurocristopathies (Fernandez et al., 2013 Orphanet J Rare Dis.) 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 2013 we have published several studies on cancer metabolism, from a systems biology viewpoint (Sánchez-Tena et al., 2013, PLoS One; Sánchez-Tena et al., 2013, Carcinogenesis), breast cancer development (Iglesias et al., PLoS One) or skin cancer prevention (Puig-Butillé et al., Exp Dermatol.). We have also addressed a number of collaborative works with other

Figure 1 - The Genome Maps (Medina et al., 2013 NAR) implementation at the data analysis portal of the ICGC (http://dcc.icgc.org/) showing the genomic context of the BRAF gene..

laboratories in the topic of genomic analysis in different fields such as coronary diseases, were new disease genes were found *(Silbiger et al., 2013 Clin Chim Acta)*, cardial growth restriction (*Gonzalez-Tendero et al., Am. J. Physiol. Heart Circ. Physiol*), allergies (*Aguerri et al., J Biol Regul Homeost Agents*) or stem cells (*Galan et al., PLoS One*).

Systems biology

The most strategic aspect of our work in genomics is the interpretation of the 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., NAR). Figure 2 shows an example of the application of pathway analysis to discover apoptosis inhibition in colorectal cancer in an experiment in which the observation of gene expression does not help very much.

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.

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 15 papers published in international journals during 2013.

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 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.

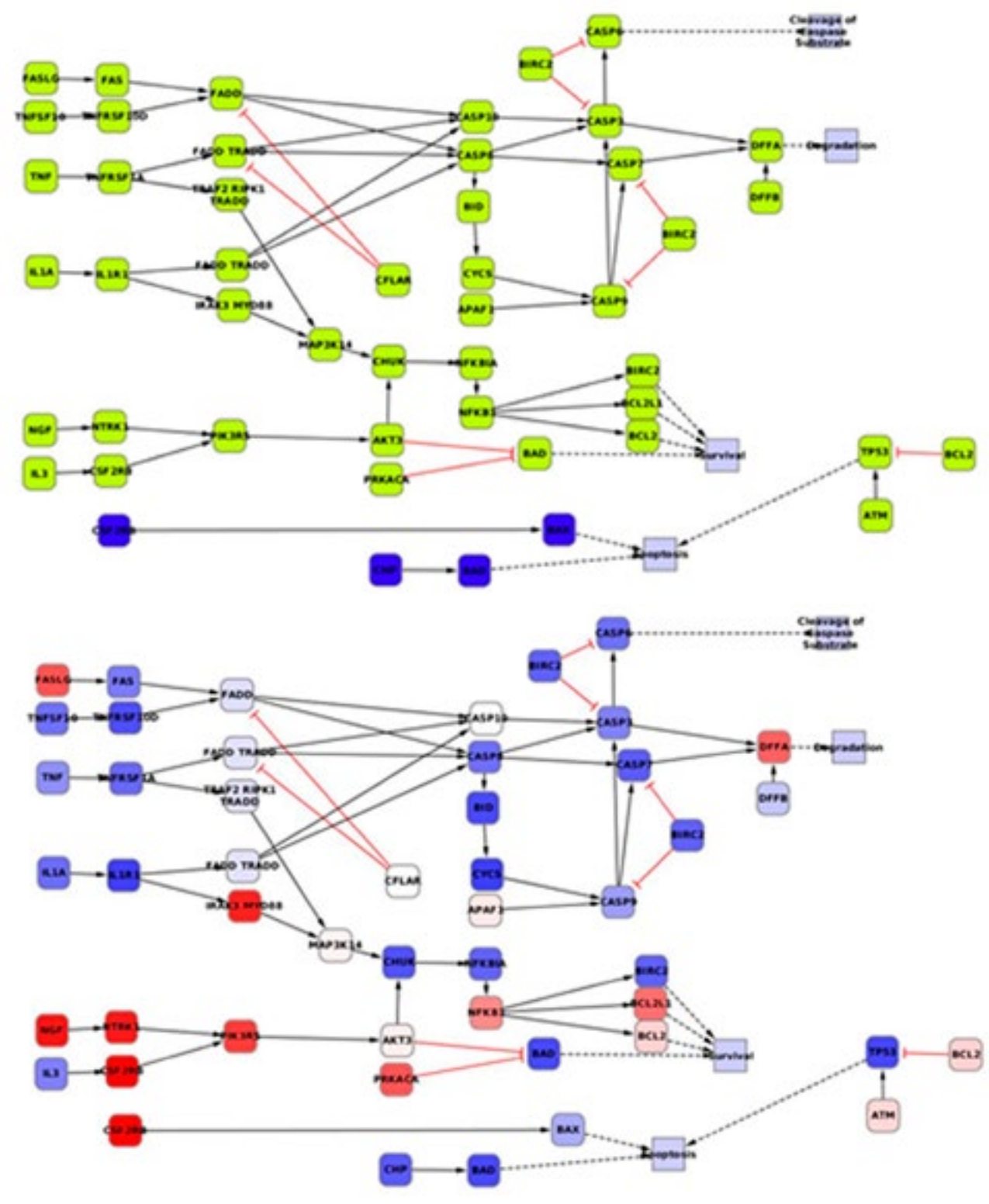

Figure 2 - Use of pathway analysis to discover Apoptosis inhibition, a fact that is not obvious from the sole direct analysis of gene expression. Two of the three possible sub-pathways leading to apoptosis are inhibited in colorectal cancer. Upper panel shows the inhibited sub-pathways in blue. Lower panel shows the actual gene up-regulations (red) and down-regulations (blue) that justify this change in the activity of the sub-pathways

Publications

→ Tort F, García-Silva MT, Ferrer-Cortès X, Navarro-Sastre A, Garcia-Villoria J, Coll MJ, Vidal E, Jiménez-Almazán J, Dopazo J, Briones P, Elpeleg O, Ribes A.
Exome sequencing identifies a new mutation in SERAC1 in a patient with 3-methylglutaconic aciduria.
Molecular genetics and metabolism,73-7

→ Manuel Iglesias J, Beloqui I, Garcia-Garcia F, Leis O, Vazquez-Martin A, Eguiara A, Cufi S, Pavon A, Menendez JA, Dopazo J, Martin AG.
Mammosphere Formation in Breast Carcinoma Cell Lines Depends upon Expression of E-cadherin.
PloS one,e77281

→ Gonzalez-Tendero A, Torre I, Garcia-Canadilla P, Crispi F, Garcia-Garcia F, Dopazo J, Bijnens B, Gratacos E.
Intrauterine growth restriction is associated with cardiac ultrastructural and gene expression changes related to the energetic metabolism in a rabbit model.
American journal of physiology. Heart and circulatory physiology,W213-7

→ Silbiger VN, Luchessi AD, Hirata RD, Lima-Neto LG, Cavichioli D, Carracedo A, Brión M, Dopazo J, García-García F, dos Santos ES, Ramos RF, Sampaio MF, Armaganijan D, Sousa AG, Hirata MH.
Novel genes detected by transcriptional profiling from whole-blood cells in patients with early onset of acute coronary syndrome.
Clinica chimica acta: international journal of clinical chemistry,184-90

→ Dopazo J.
Genomics and transcriptomics in drug discovery.
Drug discovery today,W41-6

→ Medina I, Salavert F, Sanchez R, de Maria A, Alonso R, Escobar P, Bleda M, Dopazo J;Genome Maps, a new generation genome browser.
Nucleic acids research,W41-6.2013

→ Sebastián-León P, Carbonell J, Salavert F, Sanchez R, Medina I, Dopazo J.
Inferring the functional effect of gene expression changes in signaling pathways.
Nucleic acids research,W213-7.2013

→ Puig-Butillé JA, Malvehy J, Potrony M, Trullas C, Garcia-García F, Dopazo J, Puig S.
Role of CPI-17 in restoring skin homoeostasis in cutaneous field of cancerization: effects of topical application of a film-forming medical device containing photolyase and UV filters.
→ Experimental dermatology,494-6.2013

Sánchez-Tena S, Lizárraga D, Miranda A, Vinardell MP, García-García F, Dopazo J, Torres JL, Saura-Calixto F, Capellà G, Cascante M.
Grape antioxidant dietary fiber inhibits intestinal polyposis in ApcMin/+ mice: relation to cell cycle and immune response.
Carcinogenesis,1881-8.2013

→ Aguerri M, Calzada D, Montaner D, Mata M, Florido F, Quiralte J, Dopazo J, Lahoz C, Cardaba B.
Differential gene-expression analysis defines a molecular pattern related to olive pollen allergy.
Journal of biological regulators and homeostatic agents,337-50. 2013

→ Sánchez-Tena S, Reyes-Zurita FJ, Díaz-Moralli S, Vinardell MP, Reed M, García-García F, Dopazo J, Lupiáñez JA, Günther U, Cascante M.
Maslinic acid-enriched diet decreases intestinal tumorigenesis in Apc(Min/+) mice through transcriptomic and metabolomic reprogramming.
PloS one,e59392.2013

→ Galan A, Diaz-Gimeno P, Poo ME, Valbuena D, Sanchez E, Ruiz V, Dopazo J, Montaner D, Conesa A, Simon C.
Defining the genomic signature of totipotency and pluripotency during early human development.
PloS one,e62135. 2013.

Member of the Editorial Board of

- → The Open Applied Informatics Journal Bentham Science open
- → The Open Chemical and Biomedical Methods Journal Bentham Science open
- → The Open proteomics Journal Bentham Science open
- → Recent Patents on Computer Science Bentham Science open
- → The Open Systems Biology Journal Bentham Science open
- → BMC Research notes Biomed Central
- → BMC Bioinformatics Biomed Central
- → BMC genomics Biomed Central
- → Journal of Data Mining in Genomics & Proteomics OMICS Publishing group
- → PLoS One Public Library of Sciences
- → Advances in Bioinformatics Springer
- → Clinical and Translational Oncology Springer

Conferences and meetings

- Invited conference,"Using mechanism-based biomarkers to predict complex traits".SBV Improver Symposium, Atenas, Greece
- Invited conference,"Management of genomic big data in a country-wide collaborative initiative for rare disease gene finding". Fifth International Clinical Genomics & Informatics Europe event, Lisboa ,Portugal
- Invited conference, "From Mendel to deep sequencing". 12th World Congress of Pediatric Dermatology, Madrid,Spain.
- Invited conference,"Next generation of challenges in bioinformatics". XXXVI SEBBM Congress , Madrid, Spain.
- Invited conference, "Integral management of a country-wide collaborative initiative for rare disease exome sequencing". NGS Congress ,Londres , United Kingdom.
- Invited conference,"Secuenciación de genomas y medicina personalizada,Salon de Grados". Facultad de Medicina de Valencia, Valencia, Spain.
- Invited conference,"Nuevas aproximaciones bioinformáticas para buscar genes de enfermedad: encontrando la aguja en el pajar genómico". XXVII Congreso AEGH,Madrid,Spain.
- Invited conference, "New solutions for Big Data Analysis and Visualization. From HPC to cloud-based solutions". BIOSTEC 2013 - 6th International Joint Conference on Biomedical Engineering Systems and Technologies,Barcelona,Spain.
- Invited conference,"Preparing the scenario for the use of patient's genome sequences in clinic". Arab Health 2013,Dubai,United Arab Emirates
- Invited seminar,"Interpretación biológica: desde los datos de secuenciación filtrados al informe". Secuenciación Genómica en la Práctica Clínica, Jornadas Instituto Roche. CIPF,Valencia,Spain.

Courses

- Invited conference, "La revolución de la secuenciación masiva y su impacto en la genómica humana". Cursos Universidad Internacional de Andalucía, Baeza-Jaen,Spain.

Membership

- Genometra. Empresa bioinformática de consultoría
- Centre of Excellence in Bioinformatics Ghent University – VIB. Belgium.
- FGED, Functional Genomics data society.

Mollecular Mechanism of Disease Programme

Programme Coordinator: Rodriguez-Navarro, Susana

Cell Pathology, lead by Guerri, Consuelo

Gene Expresion Coupled To Rna Transport, lead by Rodriguez-Navarro, Susana

Molecular Neuroendocrinology, lead by Burks, Deborah (until Sept. 2013)

Oncogenic Signalling, lead by Farrás, Rosa

Rho Signalling in Neurophatologies, lead by Guasch, Rosa

Rna Modification and Mitocondrial Diseases, lead by Armengod, MEugenia

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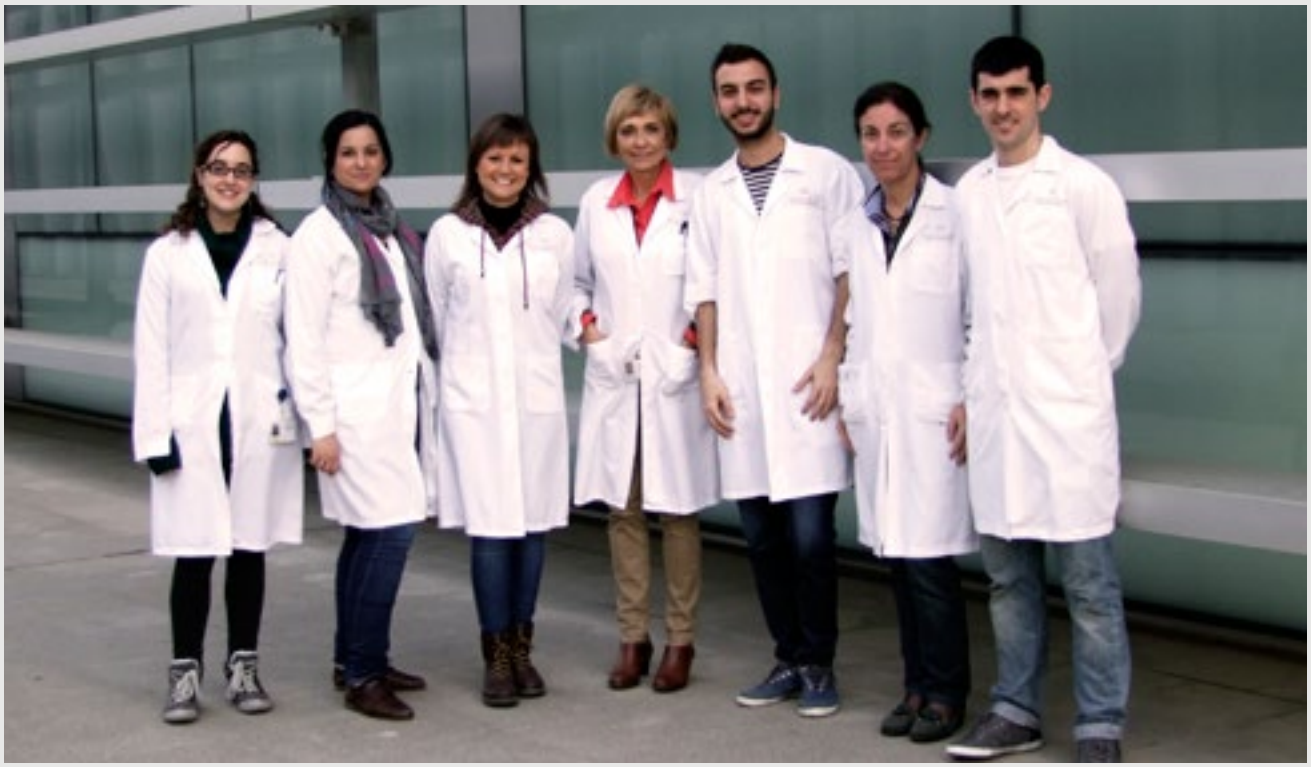
Cellular Pathology



Group Leader
Consuelo Guerri Cirera

→ Researchers
María Pascual Mora
Silvia Alfonso Loeches
Oliver de la Cruz
(until March 2013)
Ana Gil Tebar

→ Graduate Students
Antoni Pla Rodríguez
Juan Ramón Ureñ Peralta
Jorge Montesinos Selfa

→ Technicians
Mª José Morillo Bargues

→ Collaborators
Clara Mar Guarch Pérez
(until July 2013)
Sandra Monteagud
Guillermo Climent
(until July 2013)

www.cipf.es/patologia-celular

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. Alcohol abuse can also induce brain damage and neurodegeneration and we are interesting to investigate the molecular actions of ethanol in the 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 actions of ethanol of the immune receptors (TLRs, NLRs) in cortical glial cells in primary culture,
ii) Assess the activation of the innate immune system in the brain damage and behavioral dysfunction induced by alcohol abuse. In particular we are interesting on the role of TLRs and the inflammasome in alcohol-induced inflammation
iii) Investigate new biological markers, such as cytokine and chemokine, that could detect neuroinflammation and prevent further brain damage.

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

Ethanol induces TLR4/TLR2 association, triggering an inflammatory response in microglial cells.

Alcohol consumption can induce brain damage, demyelination, and neuronal death, although the mechanisms are poorly understood. Toll-like receptors are sensors of the innate immune system and their activation induces inflammatory processes. We have reported that ethanol activates and recruits Toll-like receptor 4 (TLR4) receptors within the lipid rafts of glial cells, triggering the production of inflammatory mediators and causing neuroinflammation. Since TLR2 can also participate in the glial response and in the neuroinflammation, in the present year we assess the effects of ethanol on TLR4/TLR2 responses. Our results demonstrate that ethanol up-regulates TLR4 and TLR2 expression in microglial cells, inducing the production of inflammatory mediators which triggers reactive oxygen species generation and neuronal apoptosis. Ethanol also promotes TLR4/TLR2 recruitment into lipid rafts-caveolae, mimicking their activation by their ligands, lipopolysaccharide, and lipoteichoic acid (LTA).

Immunoprecipitation and confocal microscopy studies (Figure 1) reveal that ethanol induces a physical association between TLR2 and TLR4 receptors, suggesting the formation of heterodimers. Using microglia from either TLR2 or TLR4 knockout mice, we show that TLR2 potentiates the effects of ethanol on the TLR4 response reflected by the activation of MAPKs and inducible NO synthase.

In summary, we provide evidence for a mechanism by which ethanol triggers TLR4/TLR2 association contributing to the neuroinflammation and neurodegeneration associated with alcohol abuse.

Ethanol or LPS triggers clathrin- and rafts/caveolae-dependent endocytosis of TLR4 in cortical astrocytes.

Toll-like receptor 4 (TLR4) activation and signalling in glial cells play critical roles in neurological disorders and in alcohol-induced brain damage. TLR4 endocytosis upon lipopolysaccharide (LPS) stimula-

tion regulates which signalling pathway is activated, the MyD88-dependent or the TIR-domain-containing adapter-inducing interferon-ß (TRIF)-dependent pathway. However, it remains elusive whether ethanol-induced TLR4 signalling is associated with receptor internalization and trafficking, and which endocytic pathway(s) are used in cortical astrocytes. Using the adenoviral over-expression of $TLR4^{GFP}$, confocal microscopy and the imagestream technique, we show that upon ethanol or LPS stimulation, TLR4 co-localizes with markers of the clathrin and caveolin endocytic pathways (Figure 2), and that this endocytosis is dependent on dynamin.

Using chlorpromazin and filipin as inhibitors of the clathrin and rafts/caveolae endocytic pathways, respectively, we demostrate that TRIF-dependent signalling relies on an intact clathrin pathway, whereas disruption of rafts/caveolae inhibits the MyD88- and TRIF-dependent signalling pathways. Immunofluorescence studies also suggest that lipid rafts and clathrin cooperate for appropriate TLR4 internalization. We also show that ethanol can trigger similar endocytic pathways as LPS does, although ethanol delays clathrin internalization and alters TLR4 vesicular trafficking.

Our results provide new insights into the effects of ethanol or LPS on TLR4 signalling in cortical astrocytes, events that may underlie neuroinflammation and brain damage. The results demonstrate that ethanol or lipopolysaccharide (LPS) triggers TLR4 endocytosis by caveolae and clathrin-dependent pathways in astrocytes. We proposed that while clathrin is the protein responsible for TLR4 internalization, caveolin-1/lipid rafts membrane microdomains are required for TLR4 signaling. The results provide new insights into the effects of ethanol on TLR4 signalling in astrocytes, events that may underlie neuroinflammation.

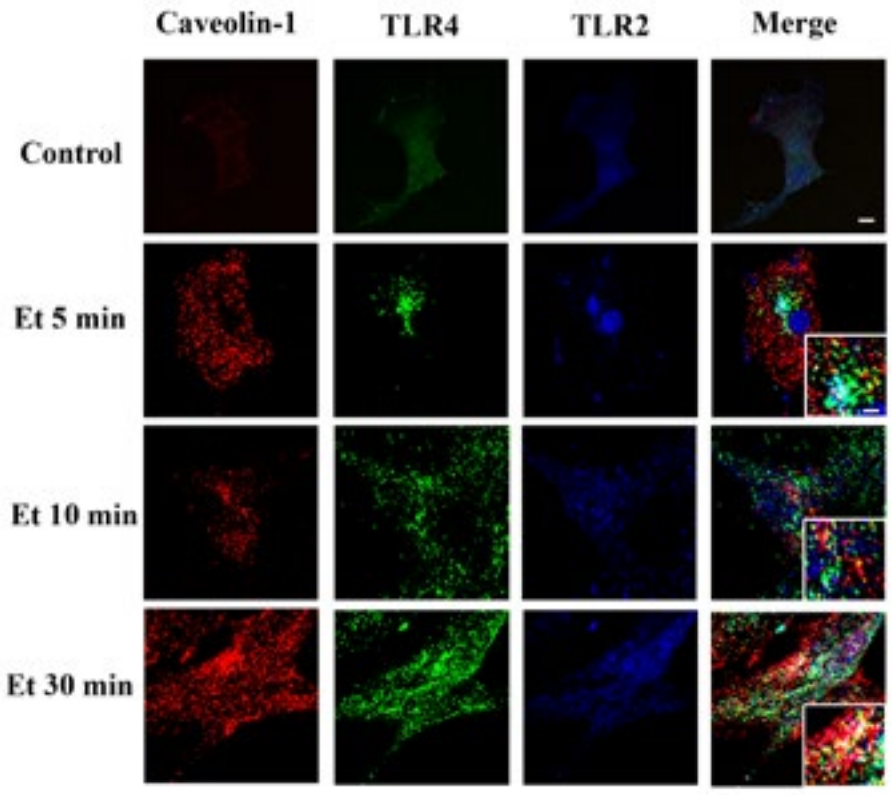


Figure 1: Role of ethanol in the co-localization of toll-like receptor (TLR)4 and TLR2 in caveosomes. Microglial cells were treated with 50 mM ethanol for 5, 10, or 30 min, and were then fixed and immunostained for the expressions of caveolin-1, TLR4, and TLR2. The co-localization of TLR4 and TLR2 with caveolin-1 generated by the treatments was quantified by a confocal microscope. A representative photomicrograph of four different experiments is shown. Scale bar = 10 µm, amplifications = 22 µm.

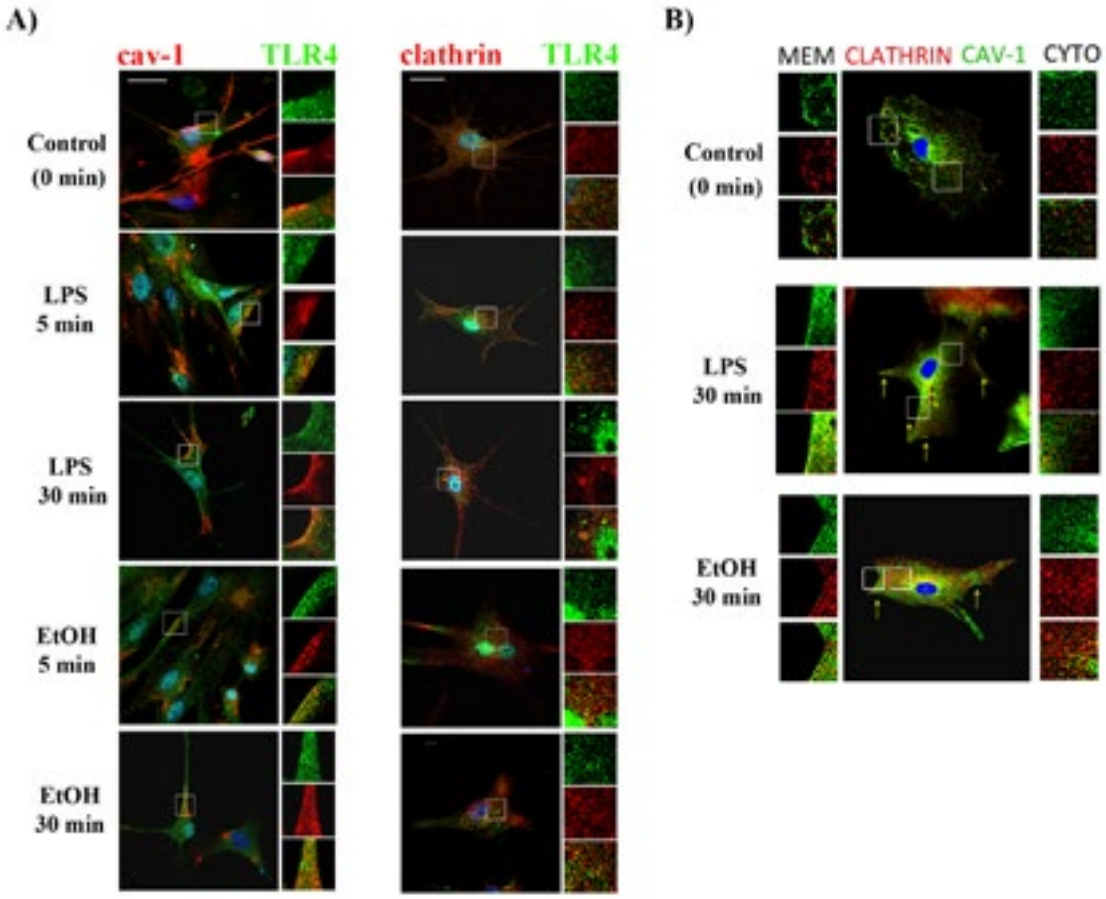


Figure 2 - Toll-like receptor 4 (TLR4) receptor is endocyted in a caveolin-and clathrin-dependent manner upon lipopolysaccharide (LPS) or ethanol (EtOH) treatment. (A) $TLR4^{GFP}$-expressing rat astrocytes were treated with LPS (50 ng/mL) or EtOH (50 mM) for 5 or 30 min,and then were fixed and stained for GFP (green), nuclei (blue) and specific markers for endocytosis (red): cav-1 or clathrin. (B) Clathrin/cav-1 co-localization within the plasma membrane (arrows) or cytosol was evaluated by confocal microscopy in the cells stimulated with LPS or EtOH. Cav-1 (green),clathrin (red) and nuclei were stained with Hoechst (blue). A representative photomicrograph of at least three different cultures is shown. Scale bar = 25 µm and 10x for amplifications.

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Publications

- Alfonso-Loeches S, Pascual M, Guerri C. Gender differences in alcohol-induced neurotoxicity and brain damage. Toxicology,27-34
- Fernandez-Lizarbe S, Montesinos J, Guerri C. Ethanol induces TLR4/TLR2 association, triggering an inflammatory response in microglial cells. Journal of neurochemistry,261-73
- Pascual-Lucas M, Fernandez-Lizarbe S, Montesinos J, Guerri C. LPS or Ethanol Triggers Clathrin- and Rafts/Caveolae-dependent endocytsis of TLR4 in cortical astrocytes. Journal of neurochemistry,
- Antoni Pla, María Pascual, Jaime Renau-Piqueras and Consuelo Guerri. TLR4 mediates the impairment of ubiquitin-proteasome and autophagy-lysosome pathways induced by ethanol treatment in brain. Cell death & disease,
- María Pascual, Antoni Pla, José Miñarro and Consuelo Guerri. Neuroimmune activation and myelin changes in adolescent rats exposed to high dose alcohol and associated cognitive dysfunction: a review with reference to human adolescent drinking. Alcohol and alcoholism (Oxford, Oxfordshire),

Conferences and meetings

- Keynote Speaker, Congreso ESBRA , Varsovia, Poland.
- Keynote Speaker, Congreso de Socidrogalcohol, Murcia, Spain.
- Keynote Speaker, Conferencia en Plasencia, Plasencia, Spain.
- Conferencia invitada, “Alcohol, embarazo y síndrome alcohólico Fetal” . Curso y conferencia en el Hospital de la Ribera, Valencia, Spain.
- Comunicación oral, Congreso SEBBM ,Madrid, Spain.
- Docente, Cursos de verano de la uCM-S.L del Escorial, Madrid, Spain.
- Docente, Abuso de alcohol y alcoholismo. Curso de verano Univ. Complutense. ,Madrid, Spain.
- Docente: “Tabaco y Alcohol: Prevenir mejor que Curar". Cuando se enferma por el entorno”, Curso para residentes de pediatría, neurología, cardiología, obstetricia-ginecología y neumología , Valencia, Spain.
- Docente, “Biología del alcohol”, Curso IES Villena, Villena, Spain.
- Colaboración,Reunión con la Ministra de Sanidad, Madrid, Spain.
- Colaboración, Reunion de coordinadores de la RED-RTA, Instituto Carlos III,Madrid,Spain.

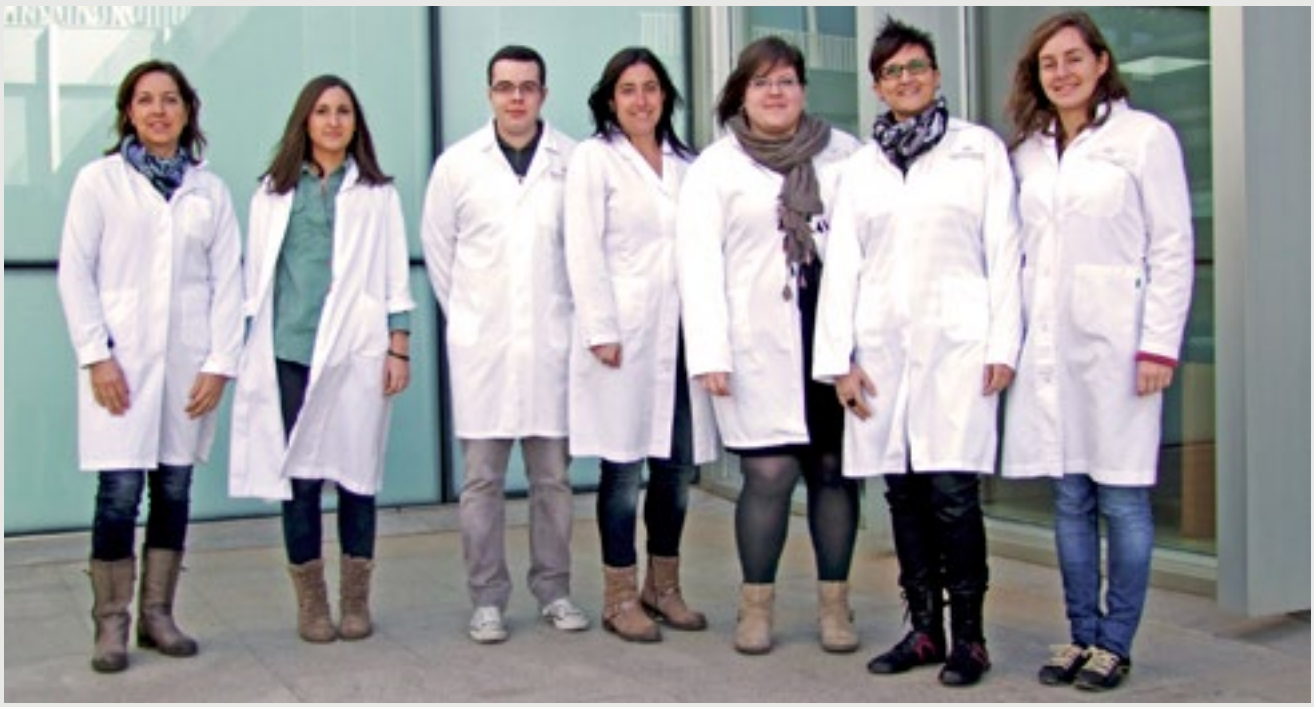
Member of the Editorial Board of

- Revista Española de Drogodependencias Asociación Española de Estudio en Drogodependencias, AESED
- Alcohol and Alcoholism Oxford Journals
- Alcohol (USA)
- Alcohol and Alcoholism (UK)
- Neurochemical Research (USA)
- Adiciones (Spain)
- Revista Española de Drogodependencia (Spain)

Memberships

- Comité Ético de bienestar animal en el CIPF Presidente

Gene Expression Coupled to RNA Transport



Group Leader

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Ali AbuQattam
Paula Oliete -Calvo
Adrían Ruíz-García
Encarnacion Garcia Oliver
(until Feb 2013)

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Joan Serrano
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Carmen Nuño
Francisco A. Aparicio
Antonio Bahilo Gomez
Mirianm Brines Julian
Ruben Fernandez
David Fuente Herraiz
Ana Montserrat
Tatiana Rodriguez
Elia Pérez Leon
David Sanjaime

→ Technicians

Eloisa Barber Cano

www.cipf.es/control-expresion-genica-metabolismo-rna

Overview

The main aim of the lab is to understand in a molecular and cellular level **gene expression and RNA metabolism**, a key biological process that allows the genetic code stored in the nucleus to be translated into the cells building blocks into proteins in the cytoplasm.

The different stages of gene expression are coupled in eukaryotes and we study the role of the different cellular machineries in charge of each step and how this impacts in human health. The understanding of gene expression control is fundamental to all aspects of biology. Dissection of this control mechanism has potential ramifications in our understanding of many aspects of biology from aging, stress response and disease development.

Our research will advance this understanding by providing novel factors and pathways that help us to understand the entire process. The evidences so far indicate that regulation of transcription, mRNA transport, stability, localization and translation is more than just the fine tuning of a transcriptionally controlled reaction, it is in fact a critical controller of gene activity and biological responses.

Notably, both the processes and the components of the machineries that we investigate are highly conserved in evolution. Thus, our findings would likely be of importance to address the physiological function of these factors in human cells. It is unsurprising that they are involved in the aetiology of distinct human diseases as for instance cancer and rare diseases.

Research results

1. A novel role for Sem1 and TREX-2 in transcription involves their impact on recruitment and H2B deubiquitylation activity of SAGA. *(Nucleic Acids Research Jun 2013,* **FIGURE 1)**

Transcription and mRNA export are linked processes. However, the molecular mechanisms of this coordination are not clear. Sus1 (hENY2) participates in this coordination as part of two protein complexes: SAGA, a transcriptional co-activator; TREX-2, which functions in mRNA biogenesis and export. Here, we investigate the coordinated action of SAGA and TREX-2 required for gene expression. We demonstrate that TREX-2 subunit Sem1 also participates in transcription activation. Like Sus1, Sem1 is required for the induction of ARG1 and GAL1, these being SAGA-regulated genes. Chromatin immunoprecipitations show that proper recruitment of certain SAGA subunits to the GAL1 promoter depends on Sem1. Notably, both in vivo and in vitro analyses reveal that Sem1 influences SAGA-dependent histone H2B deubiquitylation. Most of these phenotypes are also found to depend on another TREX-2 subunit, Thp1. These results unveil a new role for Sem1 in the activation of the SAGA-dependent gene GAL1 and influencing H2B deubiquitylation. Our work provides insights into a novel functional relationship between Sem1 and the SAGA complex. This work is part of the PhD of Encarna García-Oliver defended on May 2013.

2. Optimised protocols for the metabolic profiling of S. cerevisiae by 1H-NMR and HRMAS spectroscopy. *(Anal Bioanal Chem. Oct 2013*, **FIGURE 2)**

In colaboration with Dr Pineda-Lucena (Structural Biochemistry Lab) an optimised extraction protocol for the analysis of Saccharomyces cerevisiae aqueous and organic metabolites by nuclear magnetic resonance spectroscopy that allows the identification and quantification of up to 50 different compounds has been developed. In addition, the analysis of intact S. cerevisiae cells by HRMAS was implemented for the first time as a complementary method. The optimised protocols were applied to study the metabolic effect of glucose and galactose on S. cerevisiae growth. This tool will allow us to use it in studyin the molecular mechanisms of rare diseases in yeast.

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3. Insights into RNA polymerase II biogenesis

Through two collaborations we have studied different aspects of RNA Polymerase biogenesis.

3.1 The prefoldin bud27 mediates the assembly of the eukaryotic RNA polymerases in an rpb5-dependent manner. (*PLoS Genetics Feb 2013*)
The unconventional prefoldin URI/RMP, in humans, and its orthologue in yeast, Bud27, have been proposed to participate in the biogenesis of the RNA polymerases. In collaboration with with Dr F. Navarro (University of Jaen), we show evidence that Bud27 is the first example of a protein that participates in the biogenesis of the three eukaryotic RNA polymerases and the first example of a protein modulating their assembly instead of their nuclear transport. Notably, the role of URI seems to be conserved in humans, suggesting conserved mechanisms in RNA pols biogenesis.

3.2 Rtp1p is a karyopherin-like protein required for RNA polymerase II biogenesis. (*Mol Cell Biol May 2013*)
In collaboration with Dr F. Estruch (University of Valencia) we identified interactions between Rtp1p and members of the R2TP complex. Rtp1p also interacts, to a different extent, with several RNA pol II subunits. The pattern of interactions is compatible with a role for Rtp1p as an assembly factor that participates in the formation of the Rpb2/Rpb3 subassembly complex and its binding to the Rpb1p-containing subcomplex. Besides, Rtp1p has a molecular architecture characteristic of karyopherins, composed of HEAT repeats, and is able to interact with phenylalanine-glycine-containing nucleoporins. Our results define Rtp1p as a new component

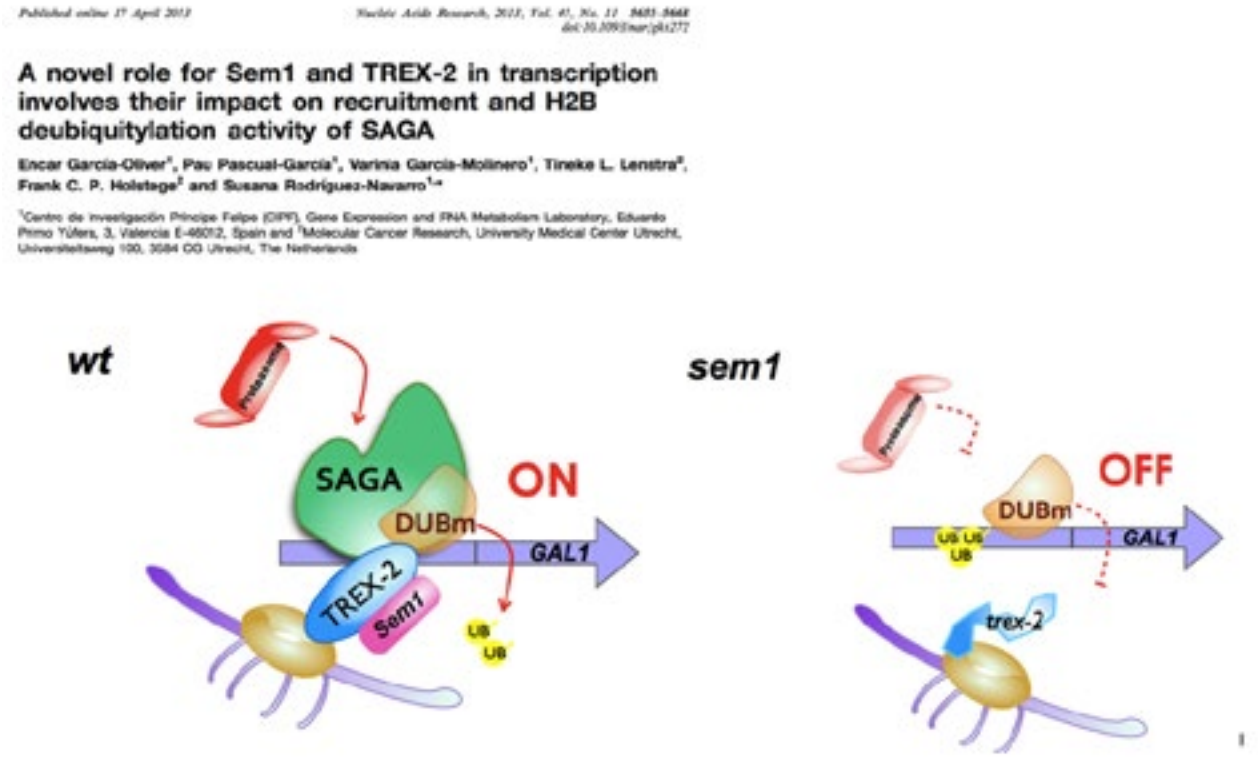
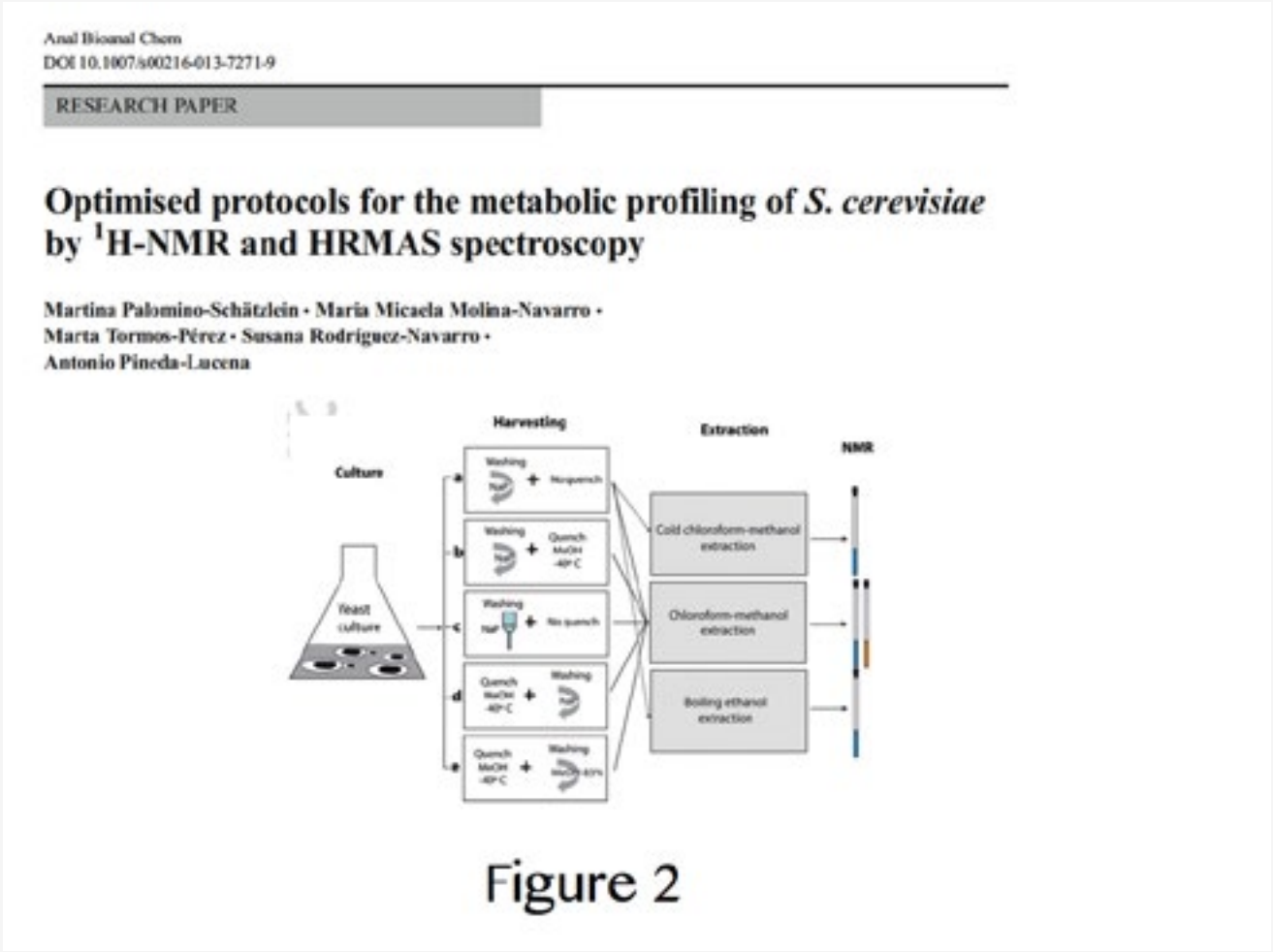


Figure 1

of the RNA pol II biogenesis machinery that plays roles in subunit assembly and likely in transport through the nuclear pore complex.

4. The Hog1 stress-activated protein kinase targets nucleoporins to control mRNA export upon stress. (*J Biol Chem Jun 2013*)

In collaboration with Dr F. Posas (Pompeu Fabra University, Barcelona) we study the functional link between the Hog1 SAPK, which plays a key role in reprogramming the gene expression pattern and the mRNA export machinery. Hog1 SAPK associates with nuclear pore complex components and directly phosphorylates the Nup1, Nup2, and Nup60 components of the inner nuclear basket. Mutation of those factors resulted in a deficient export of stress-responsive genes upon stress. Association of Nup1, Nup2, and Nup60 to stress-responsive promoters occurs upon stress depending on Hog1 activity. Accordingly, STL1 gene territory is maintained at the nuclear periphery upon osmostress in a Hog1-dependent manner. Cells containing non-phosphorylatable mutants in Nup1 or Nup2 display reduced expression of stress-responsive genes. Together, proper mRNA biogenesis of stress-responsive genes requires of the coordinate action of synthesis and export machineries by the Hog1 SAPK.


Anal Bioanal Chem
DOI 10.1007/s00216-013-7271-9

RESEARCH PAPER

Optimised protocols for the metabolic profiling of *S. cerevisiae* by ^{1}H-NMR and HRMAS spectroscopy

Martina Palomino-Schätzlein · María Micaela Molina-Navarro · Marta Tormos-Pérez · Susana Rodríguez-Navarro · Antonio Pineda-Lucena

Figure 2

Publications

- García-Oliver E, Pascual-García P, García-Molinero V, Lenstra TL, Holstege FC, Rodríguez-Navarro S.
  A novel role for Sem1 and TREX-2 in transcription involves their impact on recruitment and H2B deubiquitylation activity of SAGA.
  Nucleic acids research,2013 Jun.

- Andrés-Colás N, Perea-García A, Mayo de Andrés S, Garcia-Molina A, Dorcey E, Rodríguez-Navarro S, Pérez-Amador MA, Puig S, Peñarrubia L.
  Comparison of global responses to mild deficiency and excess copper levels in Arabidopsis seedlings.
  Metallomics : integrated biometal science,2013 Aug 21

- Palomino-Schätzlein M, Molina-Navarro MM, Tormos-Pérez M, Rodríguez-Navarro S, Pineda-Lucena A.
  Optimised protocols for the metabolic profiling of S. cerevisiae by H-1-NMR and HRMAS spectroscopy.
  Analytical and bioanalytical chemistry,2013 Aug 14

- Gómez-Navarro N, Peiró-Chova L, Rodriguez-Navarro S, Polaina J, Estruch F.
  Rtp1p is a karyopherin-like protein required for RNA polymerase II biogenesis.
  Molecular and cellular biology,2013 May

- Regot S, de Nadal E, Rodríguez-Navarro S, González-Novo A, Pérez-Fernandez J, Gadal O, Seisenbacher G, Ammerer G, Posas F.
  The Hog1 stress-activated protein kinase targets nucleoporins to control mRNA export upon stress. The Journal of biological chemistry,2013Jun14

- Mirón-García MC, Garrido-Godino AI, García-Molinero V, Hernández-Torres F, Rodríguez-Navarro S, Navarro F.
  The prefoldin bud27 mediates the assembly of the eukaryotic RNA polymerases in an rpb5-dependent manner.
  Plos Genetic, e1003297. 2013.

Conferences and meetings

- Invited conference, International Workshop Mechanisms of Nuclear Transport,Woods Hole, MA,USA.

- Invited conference, Congreso SEBBM,Madrid, Spain.

- Invited conference, International Workshop Transcription is a circular Process.
  UNIA,Baeza-Jaen, Spain.

Molecular Neuroendocrinology

Group Leader
Deborah Burks (until September 2013)

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(until July 2012)
Barbara Hammerle López
(until July 2013)

→ CIBERDEM Researchers
Carlos Acosta Umanzor
Luke Noon

→ Graduate Students
Richard Griffeth
(until March 2013)
Verónica Moreno Viedma
(CIBERDEM)

→ Technicians
Aranzazu Leal Tassias
Melisa Vera Abarca

→ Collaborators
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David Millan Esteban
(until August 2013)
Hanna Myllymaa
(until July 2013)

Overview

The incidence of diabetes and obesity is increasing at alarming rates throughout the world, creating a significant social and economic burden in industrialised countries. Defective expression or function of insulin signaling pathway components causes insulin resistance, which occurs with normal ageing but is also a hallmark of disease states such as diabetes. The overall aim of our research is to understand precisely how impaired insulin signaling contributes to metabolic diseases.

Throughout the lifetime of an individual, stem cells represent a mechanism for the maintenance and regeneration of tissues. The ability of stem cells to contribute to these processes depends on both the generation of new stem cells (self-renewal) as well as specialized cell types (differentiation). However, the effects of insulin resistance and metabolic disease on stem function are not known at present. Thus, one specific goal of our laboratory is to identify the molecular mechanisms by which insulin signaling modulates the proliferation and differentiation of progenitor cells of the adipose, pancreatic/hepatic, and neuronal lineages.

Another line of research is related with the role of cancer stem cells in the origin and progression of lung cancer. Thus, our current research will impact both basic research related with stem cell biology and clinical investigation of metabolic disorders and cancer. Given that it seeks to identify the molecular basis of obesity and diabetes, our investigation will promote the development of new and innovative strategies for the detection, treatment and prevention of metabolic disorders including lifestyle changes or drugs that promote IRS-2 expression or signaling.

Publications

→ Arias-González L, Moreno-Gimeno I, del Campo AR, Serrano-Oviedo L, Valero ML, Esparís-Ogando A, de la Cruz-Morcillo MÁ, Melgar-Rojas P, García-Cano J, Cimas FJ, Hidalgo MJ, Prado A, Callejas-Valera JL, Nam-Cha SH, Giménez-Bachs JM, Salinas-Sánchez AS, Pandiella A, del Peso L, Sánchez-Prieto R..ERK5/BMK1 is a novel target of the tumor suppressor VHL: implication in clear cell renal carcinoma.Neoplasia,649-59, 2013

→ Arroba AI, Revuelta-Cervantes J, Menes L, González-Rodríguez Á, Pardo V, de la Villa P, Burks DJ, Valverde ÁM.Loss of protein tyrosine phosphatase 1B increases IGF-I receptor tyrosine phosphorylation but does not rescue retinal defects in IRS2-deficient miceInvest Ophthalmol Vis Sci.,4215-25, 2013

→ Carretero J, Blanco EJ, Carretero M, Carretero-Hernández M, García-Barrado MJ, Iglesias-Osma MC,Burks DJ, Font de Mora J..The expression of AIB1 correlates with cellular proliferation in human prolactinomasAnn Anat. ,253-9, 2013

→ González-Navarro H, Vinué Á, Sanz MJ, Delgado M, Pozo MA, Serrano M, Burks DJ, Andrés V.Increased dosage of Ink4/Arf protects against glucose intolerance and insulin resistance associated with aging.Aging cell,102-11, 2013

→ Griffeth RJ, Carretero J, Burks DJ..Insulin receptor substrate 2 is required for testicular developmentPLoS One, 2013

→ Hämmerle B, Yañez Y, Palanca S, Cañete A, Burks DJ, Castel V, Font de Mora J..Targeting Neuroblastoma Stem Cells with Retinoic Acid and Proteasome InhibitorPLoS One, 2013

→ Roncero I, Alvarez E, Acosta C, Sanz C, Barrio P, Hurtado-Carneiro V, Burks D, Blázquez E..Insulin-receptor substrate-2 (irs-2) is required for maintaining glucokinase and glucokinase regulatory protein expression in mouse liver.PLoS One, 2013

→ Tilgner K, Neganova I, Moreno-Gimeno I, Al-Aama JY, Burks D, Yung S, Singhapol C, Saretzki G, Evans J, Gorbunova V, Gennery A, Przyborski S, Stojkovic M, Armstrong L, Jeggo P, Lako M..A human iPSC model of Ligase IV deficiency reveals an important role for NHEJ-mediated-DSB repair in the survival and genomic stability of induced pluripotent stem cells and emerging haematopoietic progenitors.Cell Death Differ.,1089-100, 2013

→ Yang G, Si-Tayeb K, Corbineau S, Vernet R, Gayon R, Dianat N, Martinet C, Clay D, Goulinet-Mainot S, Tachdjian G, Tachdjian G, Burks D, Vallier L, Bouillé P, Dubart-Kupperschmitt A, Weber A..Integration-deficient lentivectors: an effective strategy to purify and differentiate human embryonic stem cell-derived hepatic progenitors.BMC Biol,86, 2013

Oncogenic Signalling

Group Leader

Rosa Farrás

→ Collaborators

Beatriz Pérez Benavente
Sandra Tejedor Gascon
(until Sept. 2013)
Alejandro Herreros Ponares
(Until Sept. 2013)

→ Graduate Students

José Miguel Pardo

www.cipf.es/senalizacion-oncogenica

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Overview

Our laboratory is dedicated to the study of the **molecular biology of cancer**. On one hand, we investigate the signaling networks and post-translational modifications to control the abundance of cell cycle regulatory proteins. We study how dysregulation of these produces 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 small cell lung cancer (NSCLC). NSCLC accounts for almost 80% of all lung tumors and as a whole , the overall 5-year survival is less than 15% . 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 of therapeutic potential. Our goal is to analyze the transcriptome, the proteome and the phospho-oncogenic signaling pathways activated in these cells.

Research results

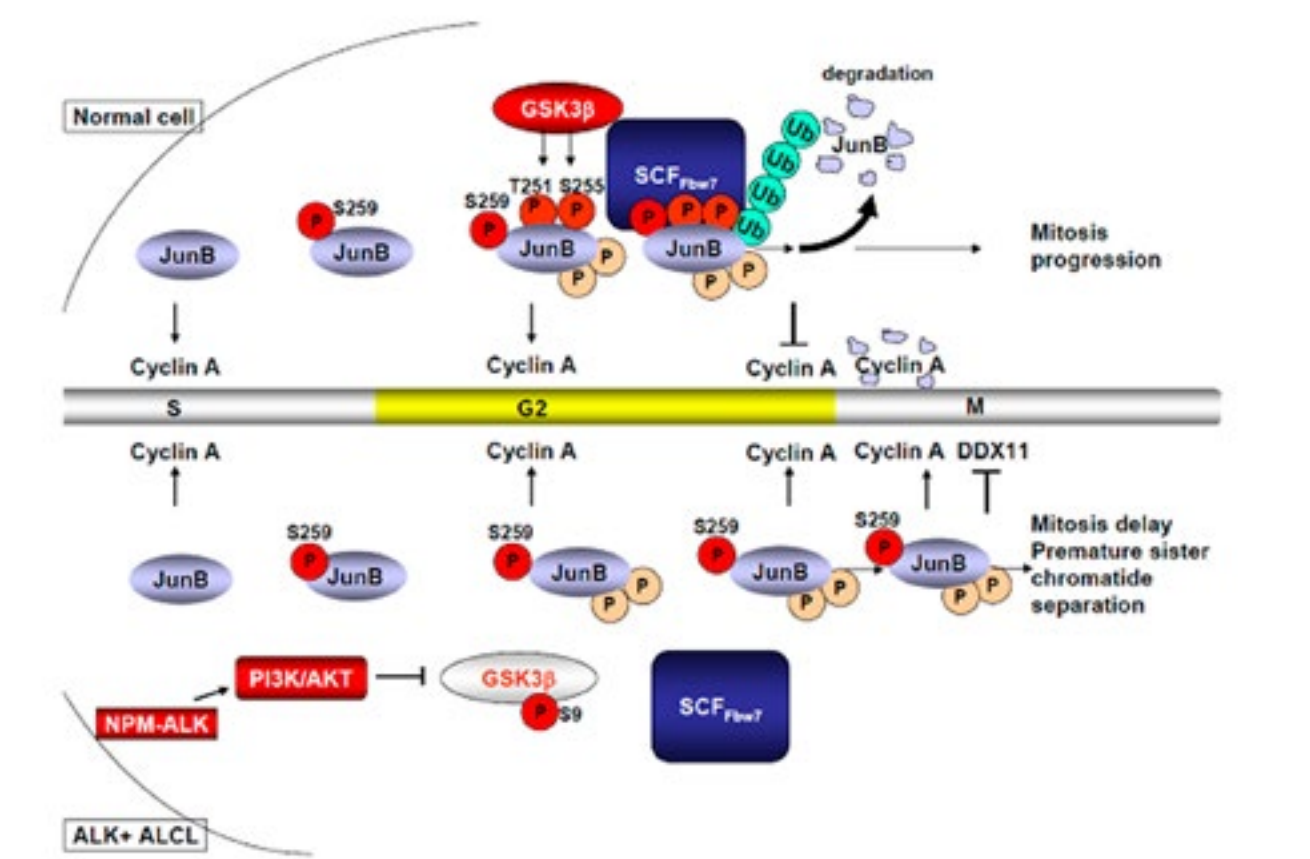
1. GSK3-SCFFBXW7 targets JunB for degradation in G2 to preserve chromatid cohesion before anaphase

In this work we described the mechanism regulating the abundance of JunB transcription factor during the cell cycle, and we show that this mechanism is altered in some cancers. Specifically , we show 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.
In the published study, we demonstrate that JunB normally undergoes a series of phosphorylations that leads to its recognition by the E3 ubiquitin ligase SCFFBXW7, targeting JunB for proteasomal degradation in a very precise time during cell cycle progression. However, in non-Hodgkin's lymphoma cells, which are characterized, for having a translocation between chromosome 2 and 5 that causes the formation of the oncogenic protein NPM-ALK, activation of a signaling cascade that inactivates GSK3ß kinase activity occurs, leading to inhibition of JunB phosphorylation. This prevents JunB to be recognized by the FBXW7 and degraded by the proteasome, therefore, aberrant JunB accumulation is produced. In addition, we show that overexpression of JunB results in premature separation of chromosomes, leading to chromosomal instability.
In conclusion , the article suggests that the same process could be involved in other types of malignancies because FBXW7 is mutated in many cancers. We also propose therapeutic strategies aimed at modulating the activated signaling cascade to allow JunB degradation correctly.

2. OCT4 and NANOG expression in tumor samples from patients with resectable NSCLC stages

This year we have initiated a line of research dedicated to the study of cancer stem cells derived from tumors of patients with NSCLC in collaboration with the group of Dr. Camps of the Research Foundation of the General Hospital University of Valencia. We have presented the following results in the conferences " *14th Interna-*

tional Congress ASEICA " and " *15th World Conference on Lung Cancer (WCLC 2013)"*: The epithelial-mesenchymal transition (EMT) is an important process in the mechanisms of invasion and metastasis of tumors and is associated with the properties of pluripotency and self-renewal of cancer cells. It is known that transcription factors OCT4 and NANOG are expressed in many tumors , but its role as biomarkers in NSCLC is not yet clearly established. We analyzed the expression of OCT4 and NANOG in NSCLC tumor samples. For this, RNA was isolated from frozen samples of tumor 114 and adjacent normal lung tissue of patients with resectable NSCLC stages. The expression of the OCT4 and NANOG genes was determined by RTqPCR .
The results show that the average age of patients was 64 years [37-85] , 91.2 % were male and 75.4 % had good performance status (PS = 0) . The predominant histology was squamous (48.2% of cases). In the analysis of correlation of analytical variables with clinicopathologic, we observed a trend towards increased expression of OCT4 and NANOG in samples with poor degree of differentiation. The Kaplan-Meier analysis revealed a significant correlation between a lower progression-free survival in the group of patients with higher levels of expression of OCT4 (median 46.64 vs. 19.23 months , p = 0.034). These results indicate that the transcription factor OCT4 is a potential prognostic biomarker in patients with resectable NSCLC stages .



Model to explain the negative effect of dysregulation of the GSK3-FBXW7-JunB axis in ALK-positive ALCL. Targeted degradation of JunB in mid/late G2 is dependent on GSK3ß phosphorylation and SCFFBXW7 ubiquitylation. Failure to degrade JunB causes dysregulation of the cell cycle and defects in chromosome segregation. This phenotype is due to dysregulation of specific molecular species leading to mitosis delay and chromatid cohesion defects. Hence, tight temporal and quantitative control of JunB levels is needed for proper cell-cycle regulation and chromatin stability.

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Publications

- Isabelle Jariel-Encontre and Rosa Farràs. Systeme ubiquitine-protéasome, proliferation cellulaire et cancer. Livre Protéasome, ubiquitine et protéines apparentées à l'ubiquitine. Ed. Lavoisier, 2013.
- Pérez-Benavente B, Farràs R. Regulation of GSK3ß-FBXW7-JUNB axis. Oncotarget,956-7. 2013
- Pérez-Benavente B, García JL, Rodríguez MS, Pineda-Lucena A, Piechaczyk M, Font de Mora J, Farràs R. GSK3-SCF(FBXW7) targets JunB for degradation in G2 to preserve chromatid cohesion before anaphase. Oncogene,2189-99. 2013

Congress and Meetings

- Poster, "OCT4 and NANOG expression in tumor samples from patients with resectable NSCLC stages". Congreso Nacional de SEOM Salamanca, Castilla y León, Spain.
- Poster, "Expression of stemness factors OCT4 and NANOG in resectable non-small cell lung cancer". 15th World Conference on Lung Cancer (WCLC 2013) Sidney, Australia.
- Poster, "Tumor expression levels of OCT4 and NANOG in resectable non-small cell lung cancer". 14th ASEICA International Congress Madrid, Spain.

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Rho Signaling in Neuropathologies

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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 specific processes of 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. In particular, our objectives are:

1) To investigate the role of RhoE during neuronal development, focusing on its involvement 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.

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 showing motor disorders, abnormal myelination and axon neuronal development.

Research results

1.- The absence of RhoE protein increases the proliferation of neural progenitors cells.

To perform these studies we have used genetically modified mice that do not express the protein RhoE. These mice exhibit several alterations in neurodevelopment. We dissect the subventricular zone, adjacent to the lateral ventricle of the brain, and neural progenitor cells were cultivated as neurospheres or spherical clusters that grow in flotation. Thus, we can study the amplification rate, reflecting proliferating progenitor cells. We have observed that progenitor cells lacking RhoE expression proliferate more rapidly than control cells. We have also investigated for changes in DNA synthesis by using DNA labeling techniques, which were performed by the incorporation of the nucleoside analog BrdU or Edu into the DNA. We have found that neural progenitor cells that do not express RhoE show an increase in DNA synthesis, suggesting an increase in cell proliferation. These results support those obtained with the cell counting, indicating that the lack of RhoE in neural progenitors from the subventricular zone correlates with an increase in DNA synthesis. We have also observed that the absence of the RhoE protein induces a decrease in the migration of neural progenitor cells. (These studies were performed in collaboration with the University UCH-CEU and the University of Valencia).

2.- Neural progenitor cells lacking RhoE expression show enhanced survival.

RhoE absence can alter the number of progenitor cells, not only by modifying their proliferation rate but also altering cell survival. By using the anexin binding method we have studied cell death by necrosis and apoptosis. Anexin-V binds to the phosphatidylserine exposed to the outer side of the cytoplasmic membrane when cells undergo early apoptosis. Neurospheres were treated with anexin in combination with a nuclear staining to investigate both necrotic and apoptotic progenitor population by flow cytometry analysis. We have observed that neurospheres lacking RhoE expression show a decrease in apoptosis, suggesting that RhoE absence induces an enhanced survival of the neural progenitor cells.

Based on these and previous results, our purpose is to analyze the molecular mechanisms involved in 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 process of neurogenesis, and therefore attenuate the alterations in neurodegenerative diseases.

3.- RhoE is degraded at the proteasome during cell cycle progression.

It has been previously shown that RhoE is involved in the regulation of cell proliferation, survival, and metastasis. We have examined RhoE expression levels during cell cycle and have investigated the mechanisms controlling them. We have shown that RhoE accumulates during G1, in contact-inhibited cells, and when the Akt pathway is inhibited. Conversely, RhoE levels rapidly decrease at the G1/S transition and remain low for most of the cell cycle. We have also shown that the half-life of RhoE is shorter than that of other Rho proteins and that its expression levels are regulated by proteasomal degradation.

The expression patterns of RhoE overlap with that of the cell cycle inhibitor p27. Consistently with an involvement of RhoE in cell cycle regulation, RhoE and p27 levels decrease after overexpression of the F-box protein Skp2. We have identified a region between amino acids 231 and 240 of RhoE as the Skp2-interacting domain and Lys (235) as the substrate for ubiquitylation. We propose that RhoE is a mediator of cell cycle arrest after DNA damage and following contact inhibition. RhoE has to be degraded, most likely by Skp2, for the cell cycle to proceed into the S phase. From a therapeutic perspective, increasing the stability of RhoE could be a novel mechanism to attenuate proliferation that otherwise may lead to the initiation and progression of cancer. (We have collaborated with the University UCH-CEU to perform these studies)

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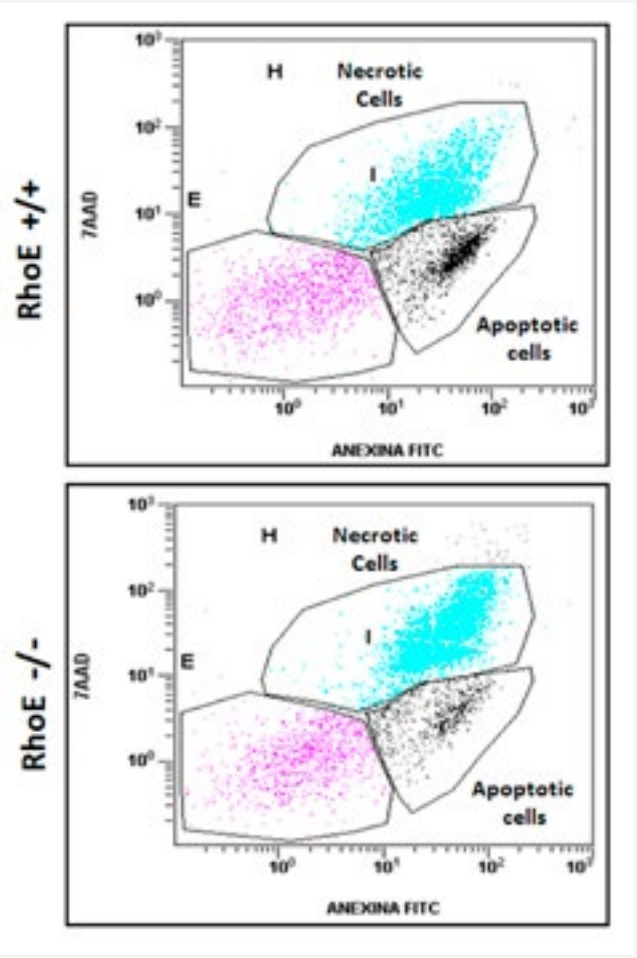

The absence of RhoE induces an increase in neural progenitor cells survival in vitro. Progenitor cells were cultured from the subventricular zone of mutant mice lacking RhoE expression. Cell survival was studied by annexin labeling (detecting a phospholipid of the plasma membrane) and a nuclear stain (7-AAD), and analyzed by flow cytometry. Neural progenitor cells lacking RhoE show a decrease in apoptosis, suggesting an increased survival.

Publications

→ Lonjedo M, Poch E, Mocholi E, Hernandez-Sanchez M, Ivorra C, Franke TF, Guasch RM, Perez-Roger I. The Rho family member RhoE interacts with Skp2 and is degraded at the proteasome during cell cycle progression. The Journal of biological chemistry,903-14. 2013

RNA Modification & Mitochondrial Diseases

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Overview

Transfer RNAs (tRNAs) are ancient molecules which play a central role in translating genomic information. Between 5 and 25% of nucleosides of a tRNA molecule are usually modified, and about 90 different tRNA modifications have been reported.

Modifications are post-transcriptionally introduced by enzymes that are highly specific for the tRNA species and target nucleoside. Recent data suggest that certain tRNA modifications are dynamics, changing in response to stress conditions, but how these changes are regulated is unknown. The tRNA modification status affects gene expression due to the decoding function of tRNAs, but also to their non-conventional functions, which appear to be involved in cell signaling. Our research in 2013 has aimed to understand how the activity of certain tRNA modifying enzymes is regulated. These enzymes are evolutionarily conserved from bacteria to humans and are responsible for the modification of the uridine located at the wobble position in a tRNA set. In humans, defects in the modification of the wobble uridine of mitochondrial tRNAs are associated with neuromuscular diseases due to mitochondrial dysfunction.

As a consequence, the capability of mitochondria to produce ATP, the major energy carrier molecule in cells, results importantly affected. Understanding how modification of tRNAs is regulated and how changes in the tRNA modification status affect cellular functions will help to uncover the molecular mechanisms of human diseases associated with tRNA modification defects, and to find specific treatments for them.

Research results

Line 1: tRNA modifying activity regulation of protein MnmE.

MnmE is a homodimeric multidomain GTPase, conserved between bacteria and eukarya, which participates in and regulates a tRNA modification pathway. MnmE, together with the conserved FAD-binding protein MnmG, is involved in the modification of the wobble uridine of a group of tRNAs. The Escherichia coli MnmE and MnmG proteins form a functional $\alpha 2 ß 2$ heterotetrameric complex (MnmEG) in which both proteins are interdependent. The eukaryotic homologues of MnmE and MnmG are targeted to mitochondria and have been related to the pathogenesis of certain mitochondrial diseases like the MERRF (myoclonus epilepsy associated with ragged-red fibers) and MELAS (mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes) syndromes. Moreover, mutations in the human MnmG homolog have been shown to cause infantile hypertrophic cardiomyopathy and lactic acidosis. Crystal structures of MnmE from several bacteria show a dimeric protein with each monomer consisting of three domains: an N-terminal domain responsible for constitutive dimerization and binding of tetrahydrofolate; a central helical domain; and a G-domain, which conserves the canonical Ras-like fold.

The X-ray structures show MnmE as a constitutive homodimer in which the highly mobile G-domains face each other. MnmE differs from Ras-like GTPases in its low affinity for guanine nucleotides and mechanism of activation, which occurs by a cis, nucleotide-, and potassium-dependent dimerization of its G-domains. Moreover, MnmE requires GTP hydrolysis to be functionally active. However, how GTP hydrolysis drives tRNA modification and how the MnmE GTPase cycle is regulated remain unresolved. In our work, the kinetics of the MnmE GTPase cycle was studied under single-turnover conditions using stopped- and quench-flow techniques (Figure 1). We found that the G-domain dedimerization is the rate-limiting step of the overall reaction. Mutational analysis and fast kinetics assays revealed that GTP hydrolysis, G-domain dedimerization, and Pi release can be uncoupled and that G-domain dedimerization is directly responsible for the “ON” state of MnmE. Thus, MnmE provides a new paradigm of how the ON/OFF cycling of GTPases may regulate a cellular process.

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We also demonstrated that the MnmE GTPase cycle is negatively controlled by the reaction products GDP and Pi. This feedback mechanism may prevent inefficacious GTP hydrolysis in vivo. We propose a biological model whereby a conformational change triggered by tRNA binding is required to remove product inhibition and initiate a new GTPase/tRNA-modification cycle.

Line 2: The output of the tRNA modification pathways controlled by the MnmEG complex depends on metabolic conditions and the tRNA species.
In E. coli, MnmEG catalyzes two different modification reactions, which add an aminomethyl (nm) or carboxymethylaminomethyl (cmnm) group to position 5 of the anticodon wobble uridine using ammonium or glycine, respectively. In $tRNA^{Gln}_{cmnm5s2UUG}$ and $tRNA^{Leu}_{cmnm5UmAA}$, however, $cmnm^5$ appears as the final modification, whereas in the remaining substrate tRNAs, the MnmEG products are converted into 5-methylaminomethyl (mnm^5) through the two-domain, bifunctional enzyme MnmC. MnmC(o) transforms $cmnm^5$ into nm^5, while MnmC(m) converts nm^5 into mnm^5, thus producing an atypical network of modification pathways (Figure 2). In our study, we investigated the activities and the tRNA specificity of MnmEG and the MnmC domains, and analyzed how the wobble-uridine modification status is influenced by genetic and physiological conditions. We demonstrated that the two MnmC domains function independently of each other and that $tRNA^{Gln}_{cmnm5s2UUG}$ and $tRNA^{Leu}_{cmnm5UmAA}$ are substrates for MnmC(m), but not MnmC(o).

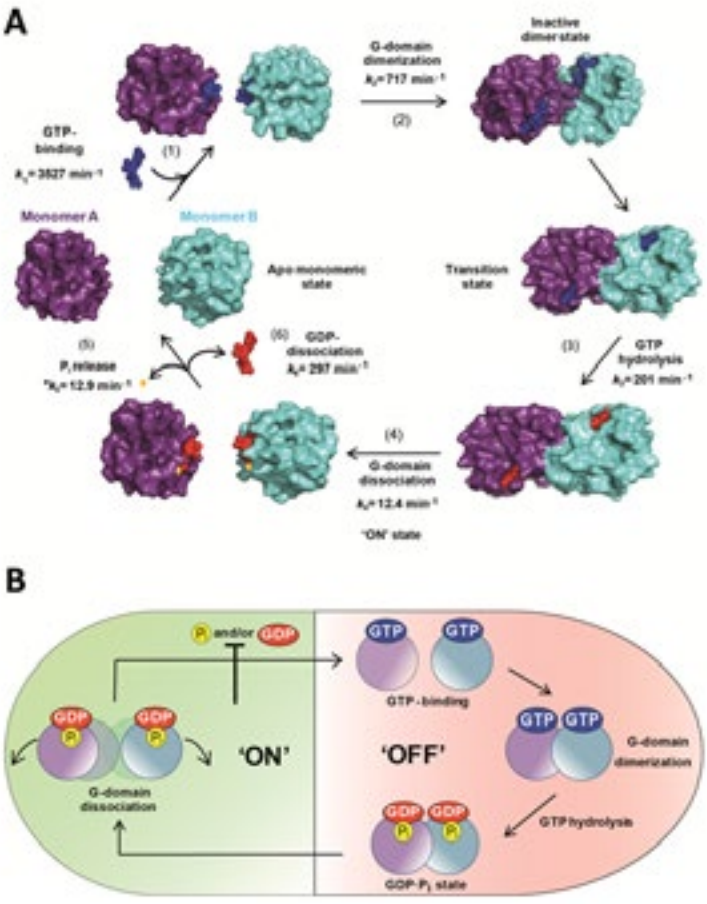

FIGURE 1 A-B

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Our data suggest that MnmEG and MnmC are kinetically tuned to produce only the fully modified nucleoside mnm^{5}U in tRNA$^{Lys}_{mnm5s2UUU}$. We demonstrate that all the tRNA substrates of MnmEG are modified in vitro through the ammonium pathway. However, the net output of the ammonium and glycine pathways of MnmEG in vivo depends on growth conditions and tRNA species. Despite the molecular basis of this behavior remains unclear, our data support the notion that the modifications at the wobble uridine are dynamic and that their syntheses respond to cellular stresses.

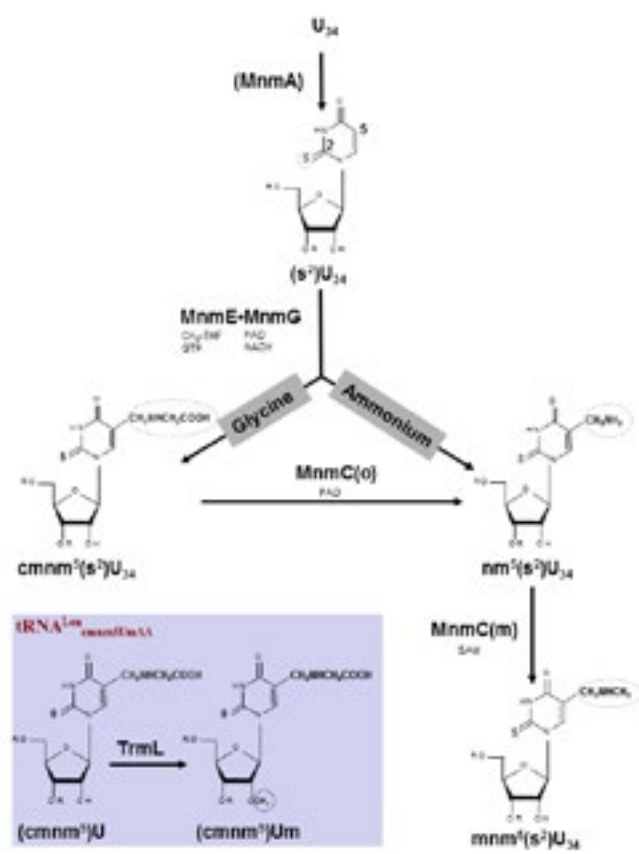

Figure 2

FIGURE 2

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The tRNA modifying function of MnmE is controlled by post-hydrolysis steps of its GTPase cycle.
Nucleic Acids Res.,6190-6208.2013

→ 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 Res.,10.1093/nar/gkt1228.2013

Congress

→ Keynote Speaker, "Gene Translation: fidelity and quality control",Barcelona,Spain.

Oral Communication, "The non-coding genome ".
EMBL Symposium,Heidelberg, Germany.

→ Poster, XIII International Congress of the Spanish Biophysical Society,Valencia,Spain.

→ Poster, "Mitochondria: from signaling to disease". Cell symposia, Lisboa,Portugal

→ Oral Communication, 4th Spanish Worm Meeting,Carmona,Spain.

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Neurological Impairment Programme

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Neurobiology, lead by Felipo, Vicente

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www.cipf.es/neurobiologia

Overview

The **Laboratory of Neurobiology** performs basic and translational research on the mechanisms, diagnosis and treatment of neurologic (cognitive, motor, in sleep and circadian rhythms) impairment in different pathological situations.

Using animal models we study the molecular mechanisms responsible for the neurological alterations in patients with hepatic encephalopathy (HE). Once identified the molecular alteration, we try to restore normal cerebral and neurological function through pharmacological treatments. These studies allow us to

- prevent death induced by acute ammonia intoxication;
- prevent or delay death in rats with acute liver failure;
- restore learning ability and,
- reverse hypokinesia in rats with chronic HE.

In parallel studies we assess the neurologic and cerebral alterations in patients with liver cirrhosis and minimal HE (MHE) and underlying mechanisms and look for new diagnostic procedures. We have identified 3-nitrotyrosine as the first good indicator for early MHE diagnosis. This will allow generalization of MHE diagnosis and treatment.

We also study the effects on brain development of environmental and food contaminants, (methylmercury, PCBs, pesticides). We found that ingestion of these contaminants in food by female rats leads to impaired cognitive function and altered motor activity and coordination of their pups. We are studying the underlying mechanisms

Research results

Hyperammonemia alters the modulation by different neurosteroids of the glutamate-nitric oxide-cyclic GMP pathway through NMDA- GABAA - or sigma receptors in cerebellum in vivo.

Several neurosteroids modulate the glutamate-nitric oxide (NO)-cGMP pathway in cerebellum through modulation of the ionotropic receptors NMDA- GABAA - or sigma receptors. Hyperammonemia alters the concentration of several neurosteroids and impair the glutamate-NO-cGMP pathway, leading to impaired learning ability. This work aimed to assess whether chronic hyperammonemia alters the modulation by different neurosteroids of GABAA, NMDA and/or sigma receptors and of the glutamate-NO-cGMP pathway in cerebellum. Neurosteroids were administered through microdialysis probes and the effects on the glutamate-NO-cGMP pathway activation were assessed.

Hyperammonemia completely modifies the effects of pregnanolone and pregnenolone. Pregnanolone acts as a GABAA receptor agonist in controls but as a NMDA receptor antagonist in hyperammonemic rats.

Pregnenolone does not induce any effect in controls but acts as a sigma receptor agonist in hyperammonemic rats. Hyperammonemia potentiates the actions of THDOC as GABAA receptor agonist, allopegnanolone as NMDA receptor antagonist and pregnenolone sulphate as NMDA receptor activation enhancer.

Neurosteroids that reduce the pathway (pregnanolone, THDOC, allopregnanolone, DHEAS) may contribute to cognitive impairment in hyperammonemia and hepatic encephalopathy. Pregnenolone would impair cognitive function in hyperammonemia. Neurosteroids that restore the pathway in hyperammonemia (pregnenolone-sulphate) could restore cognitive function in hyperammonemia and encephalopathy.

This has been published in: González-Usano, A., et al (2013) J Neurochem125(1):133-43

Pregnenolone Sulfate Restores the Glutamate-Nitric-Oxide-cGMP pathway and Extracellular GABA in Cerebellum and Learning and Motor coordination in Hyperammonemic Rats

Around 40% of cirrhotic patients show minimal hepatic encephalopathy (MHE), with mild cognitive impairment which reduces their quality of life. There are no specific treatments for neurological alterations in MHE.

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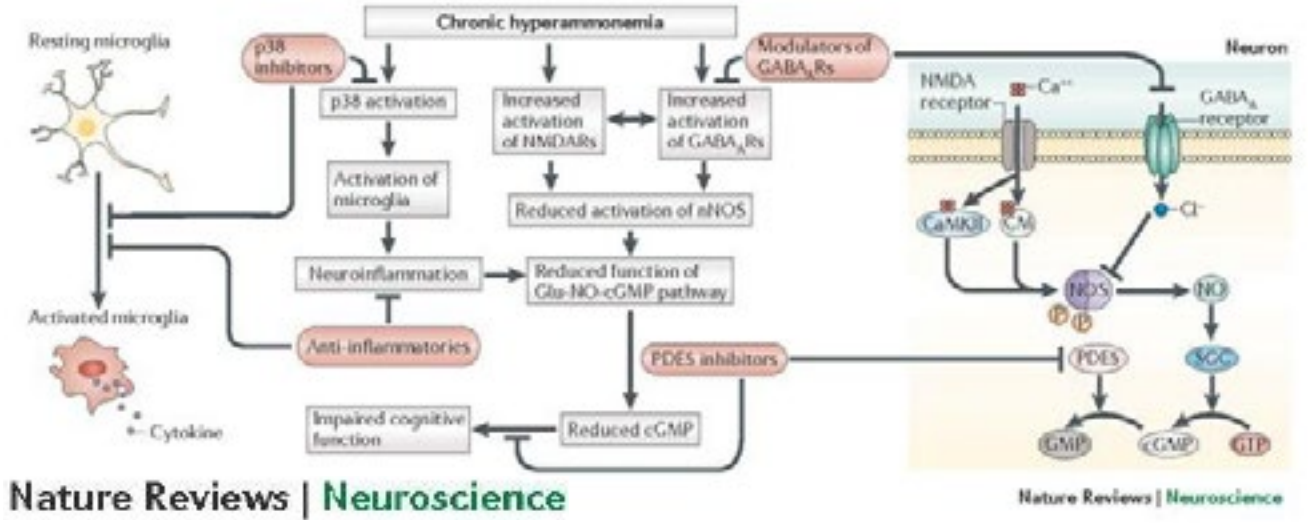
Hyperammonemia is the main contributor to neurological alterations in MHE. Chronic hyperammonemia impairs learning of a Y-maze task by impairing the glutamate-nitric-oxide (NO)-cGMP pathway in cerebellum, in part by enhancing GABAA receptor activation, which also induces motor in-coordination. Acute pregnenolone sulfate (PregS) restores the glutamate-NO-cGMP pathway in hyperammonemic rats.
This work aimed to assess whether chronic treatment of hyperammonemic rats with PregS restores (1) motor coordination; (2) extracellular GABA in cerebellum; (3) learning of the Ymaze task; (4) the glutamate-NO-cGMP pathway in cerebellum.

Chronic intracerebral administration of PregS normalizes motor coordination likely due to extracellular GABA reduction. PregS restores learning ability by restoring the glutamate-NO-cGMP pathway, likely due to both enhanced NMDA receptor activation and reduced GABAA receptor activation. We have identified an agent, PregS, which is able to restore motor coordination and learning ability in hyperammonemic rats and likely in patients with MHE.
This has been published in *Gonzalez-Usano, A, et al. ACS Chem Neurosci.(in press*)

Serum nitrotyrosine and psychometric tests as indicators of impaired fitness to drive in cirrhotic patients with minimal hepatic encephalopathy
Cirrhotic patients with minimal hepatic encephalopathy (MHE) show impaired driving ability and increased vehicle accidents. The neurological deficits contributing to impair driving and the underlying mechanisms are poorly understood. Early detection of driving impairment would help to reduce traffic accidents in MHE patients. It would be useful to have psychometric or biochemical parameters reflecting driving impairment. We assessed in 22 controls, 36 cirrhotic patients without and 15 with MHE, driving performance using a driving simulator (SIMUVEG) and Driver Test. Psychometric tests assessing different neurological functions were performed.

Patients with MHE showed impaired driving ability cor-



Felipo, V. (2013) 14(12):851-858

Targets and agents that could improve cognitive function in patients with MHE. Compounds that restore the function of the glutamate (Glu)–nitric oxide (NO)–cyclic GMP pathway and cGMP levels restore learning ability in rats. This may be achieved with phosphodiesterase 5 (PDE5) inhibitors, anti-inflammatory drugs, p38 inhibitors or modulators of type A GABA receptors (GABAARs).

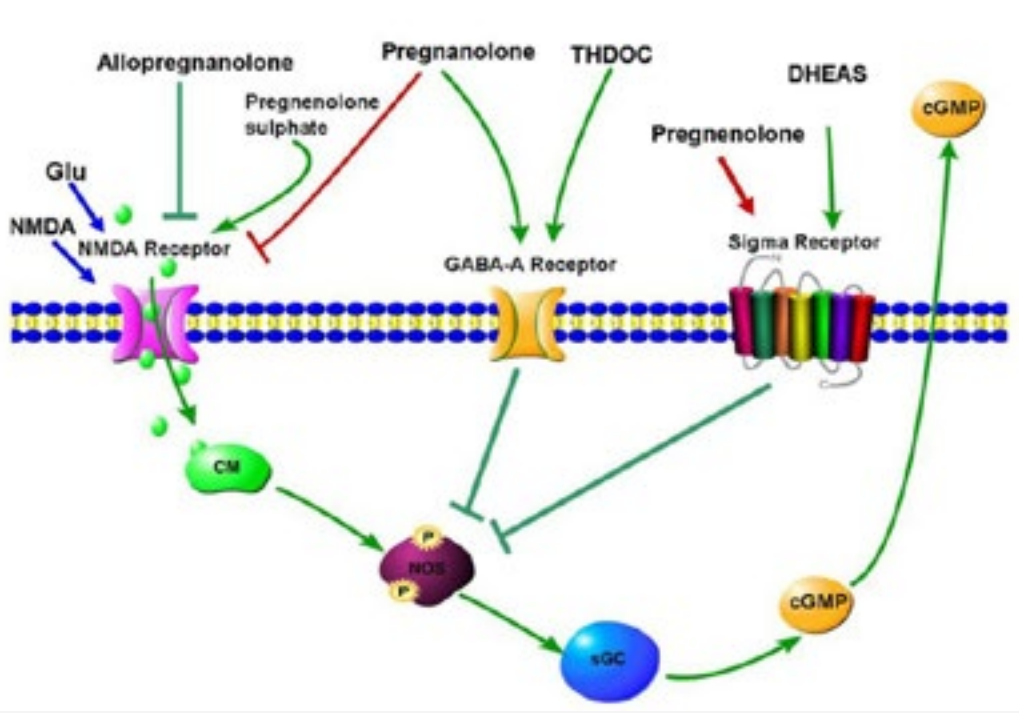
relating with MHE grade, with impaired vehicle lateral control in spite of reduced driving speed. Patients with MHE show psychomotor slowing, longer reaction times, impaired bimanual and visuo-spatial coordination and concentrated attention and slowed speed of anticipation and increased blood ammonia, cGMP, IL-6, IL-18 and 3-nitrotyrosine.
Conclusions: Impaired mental processing speed, attention and alterations in visuo-spatial and motor coordination seem main contributors to impaired driving ability in patients with MHE. Increased serum 3-nitrotyrosine is associated with impaired driving ability.
In collaboration with INCLIVA, INTRAS and Hospital Clínico Valencia
This has been published in *Felipo V, et al 2013. Liver International 33(10):1478-89*

How liver failure affects brain function.

Liver failure affects brain function, leading to neurological and psychiatric alterations; such alterations are referred to as hepatic encephalopathy (HE). Early diagnosis of minimal HE reveals an unexpectedly high incidence of mild cognitive impairment and psychomotor slowing in patients with liver cirrhosis — conditions that have serious health, social and economic consequences.

The mechanisms responsible for the neurological alterations in HE are beginning to emerge. New therapeutic strategies acting on specific targets in the brain (specifically, phosphodiesterase 5, type A GABA receptors, cyclooxygenase and mitogen-activated protein kinase p38) have been shown to restore cognitive and motor function in animal models of chronic HE, and NMDA receptor antagonists have been shown to increase survival in acute liver failure.

We have reviewed the latest studies aimed at understanding how liver failure affects brain function and potential ways to ameliorate these effects
in *Felipo, v, (2013) Nature Reviews Neuroscience. 14(12):851-858*.



Modulation of the glutamate-NO-cGMP pathway by different neurosteroids through NMDA, GABAA and sigma receptors in cerebellum of control and hyperammonemic rats. Green lines show the effects of each neurosteroid in cerebellum in vivo in control rats. Red lines show the effects that occur only in hyperammonemic rats but not in control rats.

González-Usano, A., et al (2013) J Neurochem125(1):133-43

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The Journal of biological Chemistry,9675-85. 2013

→ Cauli O, Llansola M, Agustí A, Rodrigo R, Hernández-Rabaza V, Rodrigues TB, López-Larrubia P, Cerdán S, Felipo V.
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Serum nitrotyrosine and psychometric tests as indicators of impaired fitness to drive in cirrhotic patients with minimal hepatic encephalopathy.
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Presence of diadenosine polyphosphates in microdialysis samples from rat cerebellum in vivo: effect of mild hyperammonemia on their receptors.
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Chronic hyperammonemia, glutamatergic neurotransmission and neurological alterations.
Metabolic brain disease,151-4. 2013

→ Montoliu C, Urios A, Forn C, García-Panach J, Avila C, Gimenez-Garzó C, Wassel A, Serra MA, Giner-Durán R, Gonzalez O, Aliaga R, Belloch V, Felipo V.
Reduced white matter microstrutural integrity correlates with cognitive deficits in minimal hepatic encephalopathy.
Gut,2013.

Conferences and meetings

→ Oral Communication, "Urinary NGAL outperforms KIM-1 and L-FABP for the diagnosis of AKI in patients with acute coronary syndrome or heart failure undergoing cardiac surgery or coronary angiography".ISN World Congress of Nephrology 2013,Hong-Kong,China.

→ Oral Communication,"Pacientes con encefalopatía hepática mínima presentan daño cortical focal, en paralelo con el deterioro cognitivo". XXXVIII Congreso de la Asociación Española de Estudio del Hígado (AEEH),Madrid,Spain.

→ Oral Communication,"Los pacientes con encefalopatía hepática mínima tienen alterado el mismatch negativity y esta alteración predice la disminución de la capacidad de atención".XXXVIII Congreso de la Asociación Española de Estudio del Hígado (AEEH),Madrid,Spain.

→ Keynote Speaker,"Jornada sobre Enfermedades Raras: El ciclo de la urea y sus patologías". Fundación Valenciana de Estudios Avanzados,Valencia,Spain

→ Steering committe, International Conference on Recent Advances inNeurorehabilitation,Valencia,Spain.

→ Keynote Speaker,International Symposium on “Brain ammonia homeostasis in health and diseases”,Copenague,Denmark.

Member of the Editorial Board of

→ Journal of Alzheimer´s Disease Hindawi publisging coorporation

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→ Open Gastroenterology Journal Bentham Science open

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→ Frontiers in Neuroenergetics Forntiers media

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→ Action Group A3 on "Prevention and early diagnosis of frailty and functional decline, both physical and cognitive, in older people" European Innovation Partnership on Active and Healthy Ageing, European Comission

→ Global Translatioanl Medicine Consortium (GTMC), European Society for Translational Medicine.

Rare and Genetic Disease Programme

Programme Coordinator: Palau, Francesc

Developmental Biology and Neuromuscular Disorders Models, lead by Galindo,Máximo Ibo

Genetics and Genomic of Neuromuscular Diseases, lead by Espinós, Carmen

Genetics and Molecular Medicine, lead by Palau, Francesc

Genetics And Physiopathology of Brain And Mental Disorders, lead by Hoenicka, Janet.

Intracellular Protein Degradation and Rare Diseases, lead by Knecht, Erwin.

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Developmental Biology and Neuromuscular Disease Models

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

Overview

Our group has two main lines of research in the fields of **developmental biology and pathophysiology of peripheral neuropathies.** Our main research model is the fruit fly Drosophila melanogaster.

In developmental biology, we are interested in the interplay of cell signaling and tissue polarity. The polarization of the epithelia in the appendages can influence the direction of signaling of the Notch pathway, and also the cross regulation of the Notch and EGFR pathways.

In disease models, our main line of research is the pathophysiology of the forms of Charcot-Marie-Tooth neuropathy caused by mutations in the GDAP1 gene. More generally, we are interested in the relationship of mitochondrial dysfunction and neurodegeneration, and we are also using our expertise in Drosophila genetics to model other inherited neuropathies and, in general, rare and genetic diseases that can be modeled in the fly.

Research results

EGFR-Ras signaling is down-regulated in planar cell polarity mutants.

The Drosophila leg joints are intercalated through activation of Notch signaling, activated by the Ser ligand. Notch activation is restricted towards cells distal to the expression of Ser by the planar cell polarity Fz/Stbm pathway. In Fz/Stbm pathway mutants Notch is activated in both directions, distal and proximal, and there are ectopic joints (Figure 1). Notch is also restricted by the EGFR/Ras pathway, so in EGFR/Ras mutants there is a similar phenotype. Using reporters for EGFR activation we have observed that in Fz/Stbm mutants EGFR is downregulated, which means that EGFR is downstream of Fz/Stbm. Our current hypothesis is that there is negative cross-regulation of EGFR/Ras and Notch, and Fz/Stbm tilts the balance towards Notch in distal cells and towards EGFR in proximal cells. Through genetic analysis we have discarded a role in this process for the Notch regulator Fringe and for the Dve transcription factor.

Alteration of Drosophila GDAP1 levels causes neural and muscular degeneration, and mitochondrial dysmorphology.

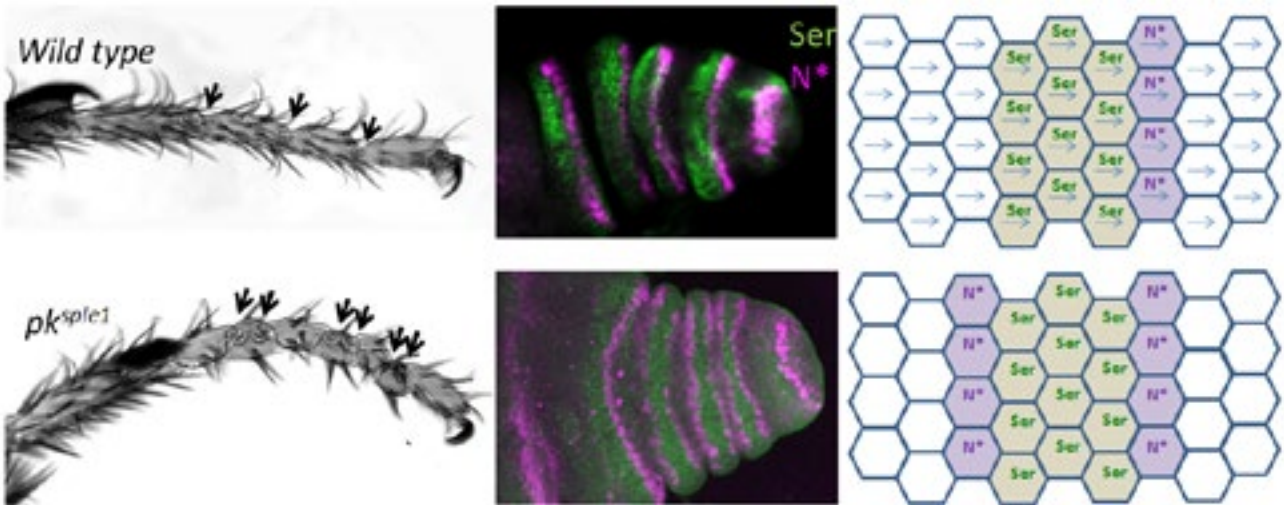

Figure 1. In a wild type leg (top) there are joints between the segments (arrows), which originate from the cells in which Ser activates Notch. This activation is restricted to those cells distal to Ser. En planar polarity mutants, Notch is activate on both sides and the number of joints is duplicated

We have used tissue-specific drivers to express wild type GDAP1 or an RNAi construct against the gene in order to increase and reduce the gene function, respectively. Using a retina-specific driver we have found out that both overexpression and silencing of GDAP1 induce neuronal death. The optic synapses at the lamina, the first optic neuropile, have aberrant morphologies, pre-synaptic elements are reduced, and there are preliminary signs of inadequate synaptic communication.

We used a second driver to direct expression in the muscle. In this tissue we have also observed that overexpression and silencing of CG4623 induce degeneration of the muscle fibers, and mitochondrial defects which are consistent with the CG4623 function: mitochondrial fragmentation upon overexpression and fusion upon interference (Figure 2).

Both in retina and muscle, the defects caused by RNAi can be rescued by co-expression of human GDAP1. Retinal and muscle degeneration are age-dependent, being much more accused in 35-day old flies than in young ones, so they represent a genuine degeneration rather than a developmental defect. We are also conducting physiological studies on the affected tissues to define the hallmarks of the cellular degeneration. Our preliminary results indicate a possible increase of oxidative stress.

Cell biology of Phf5A, a putative splicing factor.
Phf5A is a small protein of 100 aminoacids, which is extraordinarily conserved. Between humans and insects there is 95% aminoacid sequence identity. Phf5A seems to be involved in regulated splicing of transcripts, and has been identified as a possible target in glioblastoma and endometrial cancer.
We have generated a GFP-tagged Phf5A. Expression in cell culture shows strong nuclear and weaker cytoplasmic localization. We also obtained transgenic flies with an inducible RNAi construct. Restricted anatomical expression of the RNAi against the gene in the wing produces abnormal vein patterning: the relative positions of the veins are altered, which points towards an effect on cell signaling. Cells expressing the RNAi do not show increased levels of apoptosis, but there are fewer mitoses than in controls.

Phylogeny of Smyd genes.

This project is a collaboration with groups Genetics and genomic of neuromuscular diseases and
Genetics and molecular medicine, of the RareGen program. The Smyd4 gene was identified in a genetic screen of an inherited neuropathy. While humans have a single Smyd4 gene, flies have four copies. In order to identify which one would be adequate to carry out studies in the fly, we have performed a phylogenetic study of Smyd genes, which are divided into five families. This study has led us to identify the most likely Smyd4 ortholog in the fly, and to reconstruct the evolutionary history of the Smyd genes.

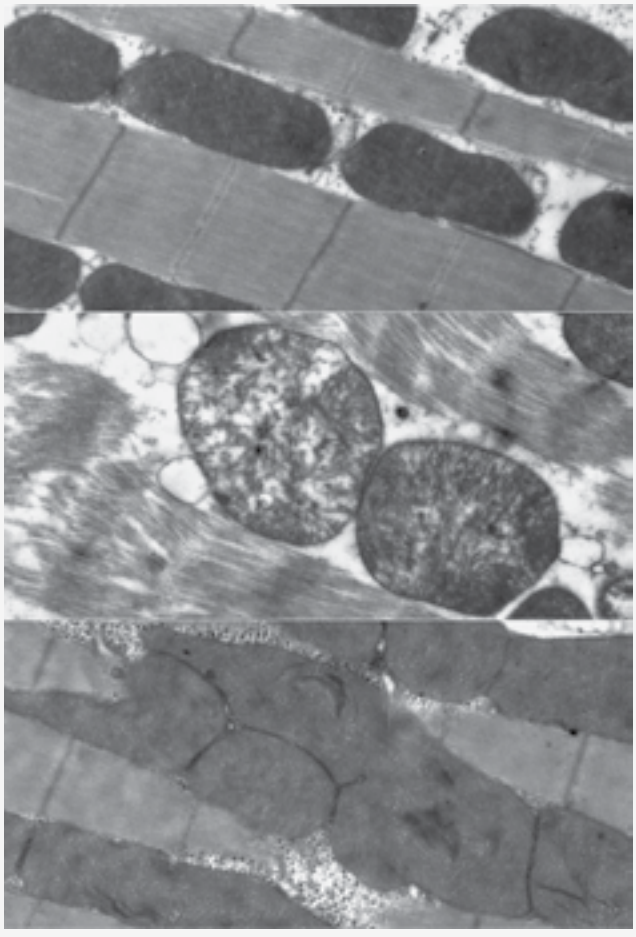

Figure 2. Mitochondrial morphology in wild type muscle fibers (top), GDAP1 over-expression (middle) and GDAP1 knock-down (bottom).

Conferences and meetings

- Keynote Speaker,A Drosophila model to study the Charcot-Marie-Tooth neuropathy caused by mutations in GDAP1. 23rd European Drosophila Research Conference. Institut de Recerca Biomédica, Institut de Biomedicina, Universitat de Barcelona e Institut de Biología Molecular de Barcelona (CSIC) ,Barcelona,Spain.
- Poster,Directional Notch signaling in the leg joints. 23rd European Drosophila Research Conference. Institut de Recerca Biomédica, Institut de Biomedicina, Universitat de Barcelona e Institut de Biología Molecular de Barcelona (CSIC),Barcelona,Spain.
- Keynote Speaker,Identification and characterization of the candidate gene responsible for a new clinical form of hereditary recurrent neuropathy. Fifth European and North American Charcot-Marie-Tooth Consortium Meeting. VIB - Department of Molecular Genetics University of Antwerp,Amberes,Belgium.
- Keynote Speaker, Generation and analysis of Drosophila models to study mitochondrial dynamics in CMT and other peripheral neuropathies. Fifth European and North American Charcot-Marie-Tooth Consortium Meeting. VIB - Department of Molecular Genetics University of Antwerp,Amberes,Belgium.
- Keynote Speaker, Caracterización del gen implicado en una nueva forma clínica de neuropatía hereditaria recurrente. XXVII Congreso Nacional de la Asociación Española de Genética Humana. Hospital La Paz y Hospital Ramón y Cajal,Madrid,Spain.

Genetics and Genomics of Neuromuscular Disorders

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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 diseases of the peripheral nervous system and in the investigation of these new genes and mutations in order to elucidate the mechanisms of disease. By genomic mapping and exome sequencing we determine the genetic causes that underlie new forms of neuromuscular diseases.

The discovery of these new genes and new mutations is the trigger that leads us to initiate several strategies to establish the relationship of this novel gene and neuropathy, and how the novel mutation causes disease. Our research is clearly translational and we are interested in developing new tools for genetic diagnosis.

Finally, besides of disease-causing mutation, 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 forms.

Research results

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 diseases of the peripheral nervous system and in the investigation of these new genes and mutations in order to elucidate the mechanisms of disease. By genomic mapping and exome sequencing we work in the identification of new mutations and new genes related to this group of neuropathies. We have recently reported the systematic analysis of known genes implicated in patients affected by Charcot-Marie-Tooth (CMT) disease of a clinical series supervised in Hospital U. i P. La Fe (Sivera et al Neurology 81: 1617-25, 2013). Related to this research, the private genetics of a relevant group of Gypsy patients with CMT have been reported in Sevilla et al Clin Genet 83: 565-70, 2013.

Within this research line and with an evident application in genetic testing, we have developed a diagnosis tool that consists of a panel of 58 genes/mutations involved in CMT and hereditary motor neuropathies (HMNs). We have also created a data base of CMT mutations in Spanish population (http://www.treat-cmt.es/db). 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.

The characterization of new genes and new mutations is the trigger that leads us to initiate studies of cellular biology, biochemical assays, and others in order to investigate the mechanisms of disease. That is, we are interested in determining how the novel mutation causes pathology and how a gene is implicated in a neuropathy. The in-depth investigation of the achieved findings in genetic analyses has led us to: (i) Investigation of the signaling pathways and interactors of SH3TC2, a protein that is part of multiprotein complexes associated with the transduction of the signal from axon to Schawnn cell, to better understand the regulator role of SH3TC2 in the development of peripheral nerves (Gouttenoire, Lupo et al Glia 61: 1041-51, 2013); (ii) Identification of a novel locus involved in a form of hereditary recurrent neuropathy; (iii) Identification of a novel mutation in the EGR2 gene

in a family with axonal CMT; (iv) Characterization of two new genes not previously related to neuropathies. We work on the identification of these genes using several approaches (genetic analyses, biochemical assays, and cellular studies) and with Drosophila melanogaster as model organism, in collaboration with Dr. I. Galindo.

Besides of the disease-causing mutation, other genetic factors contribute to the phenotype and modify it. We have recruited a clinical series with more than 30 families whose patients carry mutations in the GDAP1 gene and we have investigated candidate genetic factors that could modify the phenotype and could clarify the clinical inter and intrafamilial variability observed. We work on two loci: (i) Locus GDAP1 on chromosome 8, which comprises a gene involved in calcium metabolism as GDAP1 does. This is why that this gene is a good positional and functional candidate modifier. In collaboration with Dr. F. Palau we have observed that the sobre-expression of this candidate genes is able to restore the levels of calcium in the same way than the sobre-expression of GDAP1 in GDAp1-depleted cells by interference RNA; (ii) Locus on chromosome 10 identified by homozygosity mapping, which revealed common homozygous regions in patients with causative mutations in the GDAP1 gene.

Finally, we collaborate with research teams in the investigation of the molecular bases implicated in hereditary rare diseases. We collaborate closely with the group led by Dr. Rafael Artuch (Hospital de Sant Joan de Déu, Barcelona) and during 2013, we have published the genetics underlined in patients with 5'-oxoprolinase deficiency (*Calpena et al. JIMD Rep 7: 123-8, 2013*) and hyperlysinemia (*Tondo, Calpena et al. Mol Genet Metab 110: 231-6, 2013*). In addition, we have collaborated in the analysis of genes involved in the synthesis of coenzyme Q10 in patients with this deficiency (*Buján et al. J Inherit Metab Dis 37: 53-62, 2013*).

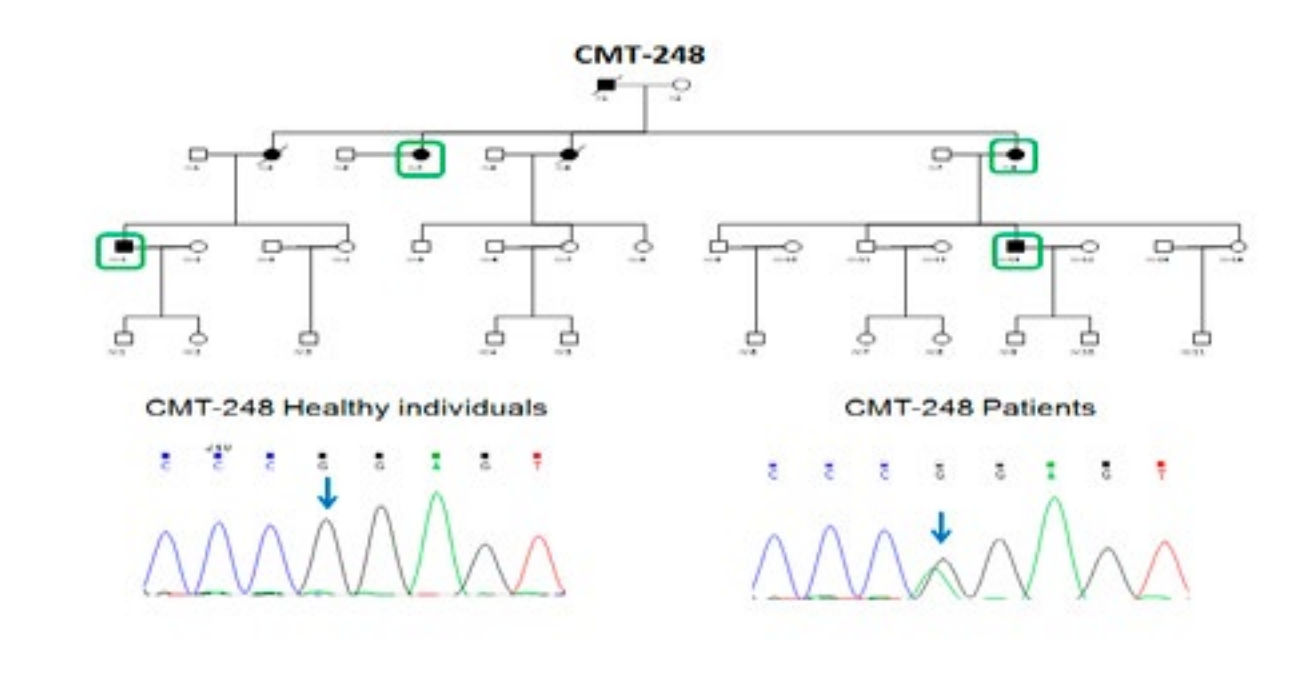

Figure 1. Pedigree of family CMT-248 and electrophoregrams that confirm the identified change by exome sequencing. Exome of patients marked in the figure was sequenced and revealed a novel nucleotide variant, c.1226G⇢A (p.R409Q), in the EGR2 gene. The mentioned change was confirmed by Sanger sequencing and analized in the remaining members of this family which allowed us to establish that disease cosegregates fully with the identified mutation: healthy individuals are homozygous for a guanine in position c.1226, whereas affected subjects are heterozygous for the change c.1226G⇢A.

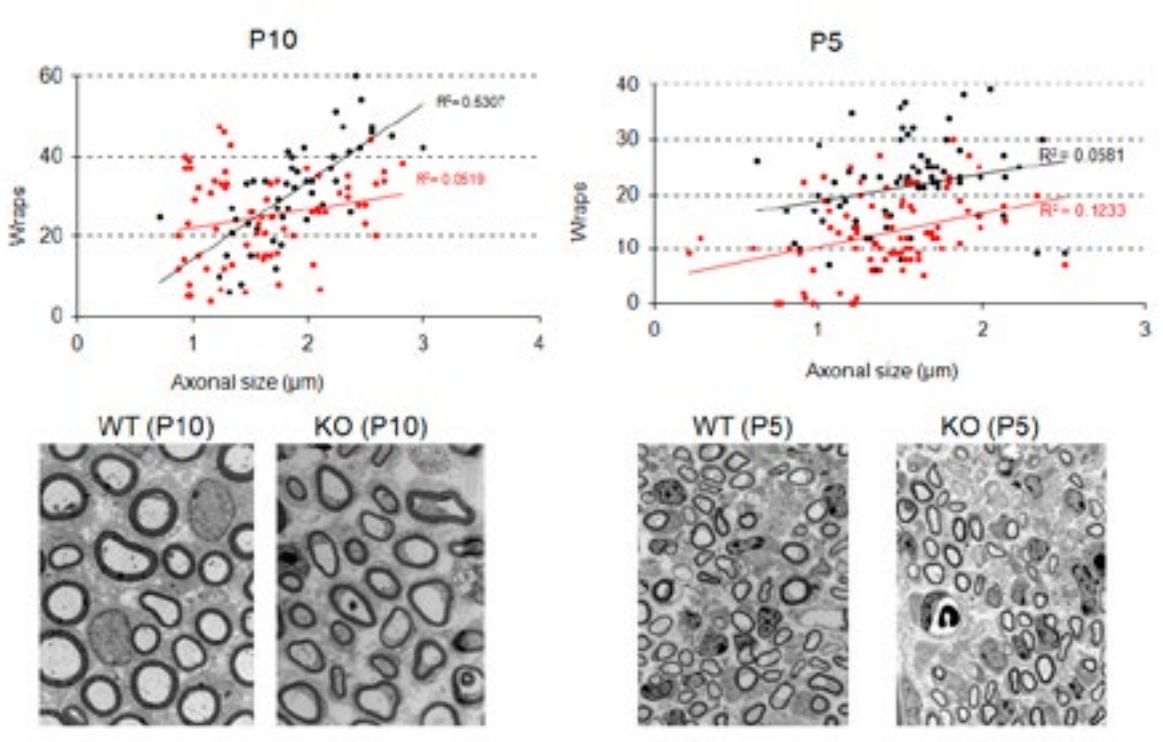

Figure 2. The onset of hypomyelinating phenotype in Sh3tc2-/- mice. Electron microscopy images of ultrathin cross-section of sciatic nerve from control (Sh3tc2+/+; WT, wild-type) and Sh3tc2-/- (KO, knock-out) mice at P10 and P5. The number of myelin wraps is represented as a function of the diameter of the correspondingf group of axons. The hypomyelination in Sh3tc2-/- nerves, as detected by overall reduction in number of myelin wraps around axons of all sizes, was already detectable at P5. This finding suggests a hypomylinating phenotype in an early stage of the development of myelin.

Publications

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- Sevilla T, Martínez-Rubio D, Márquez C, Paradas C, Colomer J, Jaijo T, Millán JM, Palau F, Espinós C. Genetics of the Charcot-Marie-Tooth disease in the Spanish Gypsy population: the hereditary motor and sensory neuropathy-Russe in depth. Clinical genetics, 83: 565-570.2013

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- Gouttenoire EA, Lupo V, Calpena E, Bartesaghi L, Schüpfer F, Médard JJ, Maurer F, Beckmann JS, Senderek J, Palau F, Espinós C, Chrast R. Sh3tc2 deficiency affects neuregulin-1/ErbB signaling. Glia 61: 1041-51.2013

- Calpena E, Casado M, Martínez-Rubio D, Nascimento A, Colomer J, Gargallo E, García-Cazorla A, Palau F, Artuch R, Espinós C. 5-Oxoprolinuria in Heterozygous Patients for 5-Oxoprolinase (OPLAH) Missense Changes. JIMD reports 7:123-8.2013

Conferences and Meetings

- Oral Communication, "Identification and characterization of the candidate gene responsible for a new clinical form of hereditary recurrent neuropathy". The 5th International CMT Consortium. Amberes,Belgium.

- Oral Communication, "Sh3tc2 deficiency affects neuregulin-1/ErbB signalling". ,The 5th International CMT Consortium. Amberes,Belgium.

- Oral Communication, "EGR2 is involved in axonal Charcot-Marie-Tooth disease". The 5th International CMT Consortium. Amberes,Bélgica.

- Oral Communication,"Charcot-Marie-Tooth disease: Genetic and clinical spectrum in a Spanish clinical series". The 5th International CMT Consortium. Amberes,Belgium

- Oral Communication, "TREAT-CMT, the Spanish Consortium on Charcot-Marie-Tooth disease". The 5th International CMT Consortium. Amberes,Belgium

- Oral Communication, "Vestibular and auditory function in patients with Charcot-Marie-Tooth disease due to mutations in the SH3TC2 gene". The 5th International CMT Consortium. Amberes,Belgium.

- Oral Communication,"Candidate gene responsible for a new clinical form of hereditary recurrent neuropathy". VI Reunión Científica Anual CIBERER. Madrid,Spain.

- Oral Communication, "Caracterización del gen implicado en una nueva forma clínica de neuropatía hereditaria recurrente". XXVII Congreso Nacional de la Asociación Española de Genética Humana. Madrid,Spain.

Genetics and Molecular Medicine

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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).

Specifically, we investigated (i) the role of mitochondrial dynamics and calcium homeostasis in the CMT neuropathy associated with mutations in the GDAP1 gene using cell models by RNA interference in SH-SY5Y cells and the Gdap1-/- knockout mouse developed in the laboratory, (ii) the metabolic and pathophysiological consequences of frataxin deficiency in SH- SY5Y cells and in the humanized mouse YG8R as cellular or animal models of FRDA, respectively , and (iii) analysis of the biology of ANKK1 in the myogenesis and satellite cells, in collaboration with Dr. J. HoenickaAlso within the RareGene Program, we have collaborated with Dr. C. Espinós in the search for new genes associated with peripheral neuropathies.

Research results

We are highlighting some results related to our research on the mechanisms of genetic disease in peripheral neuropathies and Friedreich's ataxia, and translational research in the field of genetic diagnosis and molecular epidemiology of Charcot-Marie-Tooth disease. Below we are summarizing several reports published in 2013.

Silencing of the Charcot-Marie-Tooth disease-associated gene GDAP1 induces abnormal mitochondrial distribution and affects Ca2+ homeostasis by reducing store-operated Ca2+ entry.
GDAP1 is an outer mitochondrial membrane protein that acts as a regulator of mitochondrial dynamics. Mutations of the GDAP1 gene cause Charcot-Marie-Tooth (CMT) neuropathy. We show that GDAP1 interacts with the vesicle-organelle trafficking proteins RAB6B and caytaxin, which suggests that GDAP1 may participate in the mitochondrial movement within the cell. GDAP1 silencing in the SH-SY5Y cell line induces abnormal distribution of the mitochondrial network, reduces the contact between mitochondria and endoplasmic reticulum (ER) and alters the mobilization of mitochondria towards plasma membrane upon depletion of ER-Ca(2+) stores. GDAP1 silencing does not affect mitochondrial Ca(2+) uptake, ER-Ca(2+), or Ca(2+) flow from ER to mitochondria, but reduces Ca(2+) inflow through store-operated Ca(2+) entry (SOCE) following mobilization of ER-Ca(2+) and SOCE-driven Ca(2+) entry in mitochondria. Our studies suggest that the pathophysiology of GDAP1-related CMT neuropathies may be associated with abnormal distribution and movement of mitochondria throughout cytoskeleton towards the ER and subplasmalemmal microdomains, resulting in a decrease in SOCE activity and impaired SOCE-driven Ca(2+) uptake in mitochondria.

Mitochondrial pathophysiology in Friedreich's ataxia
Neurological examination indicates that Friedreich's ataxia corresponds to a mixed sensory and cerebellar ataxia, which affects the proprioceptive pathways. Neuropathology and pathophysiology of Friedreich's ataxia involves the peripheral sensory nerves, dorsal root ganglia, posterior columns, the spinocerebellar, and corticospinal tracts of the spinal cord, gracile and cuneate nuclei, dorsal nuclei of Clarke, and the dentate

nucleus. Involvement of the myocardium and pancreatic islets of Langerhans indicates that it is also a systemic disease. The pathophysiology of the disease is the consequence of frataxin deficiency in the mitochondria and cells. Some of the biological consequences are currently recognized such as the effects on iron-sulfur cluster biogenesis or the oxidative status, but others deserve to be studied in depth. Among physiological aspects of mitochondria that have been associated with neurodegeneration and may be interesting to investigate in Friedreich's ataxia we can include mitochondrial dynamics and movement, communication with other organelles especially the endoplasmic reticulum, calcium homeostasis, apoptosis, and mitochondrial biogenesis and quality control. Changes in the mitochondrial physiology and transport in peripheral and central axons and mitochondrial metabolic functions such as bioenergetics and energy delivery in the synapses are also relevant functions to be considered. Thus, to understand the general pathophysiology of the disease and fundamental pathogenic mechanisms such as dying-back axonopathy, and determine molecular, cellular and tissue therapeutic targets, we need to discover the effect of frataxin depletion on mitochondrial properties and on specific cell susceptibility in the nervous system and other affected organs.

Charcot-Marie-Tooth disease: genetic and clinical spectrum in a Spanish clinical series
The objective is to determine the genetic distribution and the phenotypic correlation of an extensive series of patients with Charcot-Marie-Tooth disease in a geographically well-defined Mediterranean area. A thorough genetic screening, including most of the known genes involved in this disease, was performed and analyzed in this longitudinal descriptive study. Clinical data were analyzed and compared among the genetic subgroups. Molecular diagnosis was accomplished in 365 of 438 patients (83.3%), with a higher success rate in demyelinating forms of the disease. The CMT1A duplication (PMP22 gene) was the most frequent genetic diagnosis (50.4%), followed by mutations in the GJB1 gene (15.3%), and in the GDAP1 gene (11.5%). Mutations in 13 other genes were identified, but were much less frequent. Sixteen novel mutations were detected and characterized phenotypically. In conclusion,
the relatively high frequency of GDAP1 mutations, coupled with the scarceness of MFN2 mutations (1.1%) and the high proportion of recessive inheritance (11.6%) in this series exemplify the particularity of the genetic distribution of Charcot-Marie-Tooth disease in the Region of Valencia.

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. Especial mention deserves the long time collaboration with the Department of Neurology of the La Fe University Hospital in Valencia.

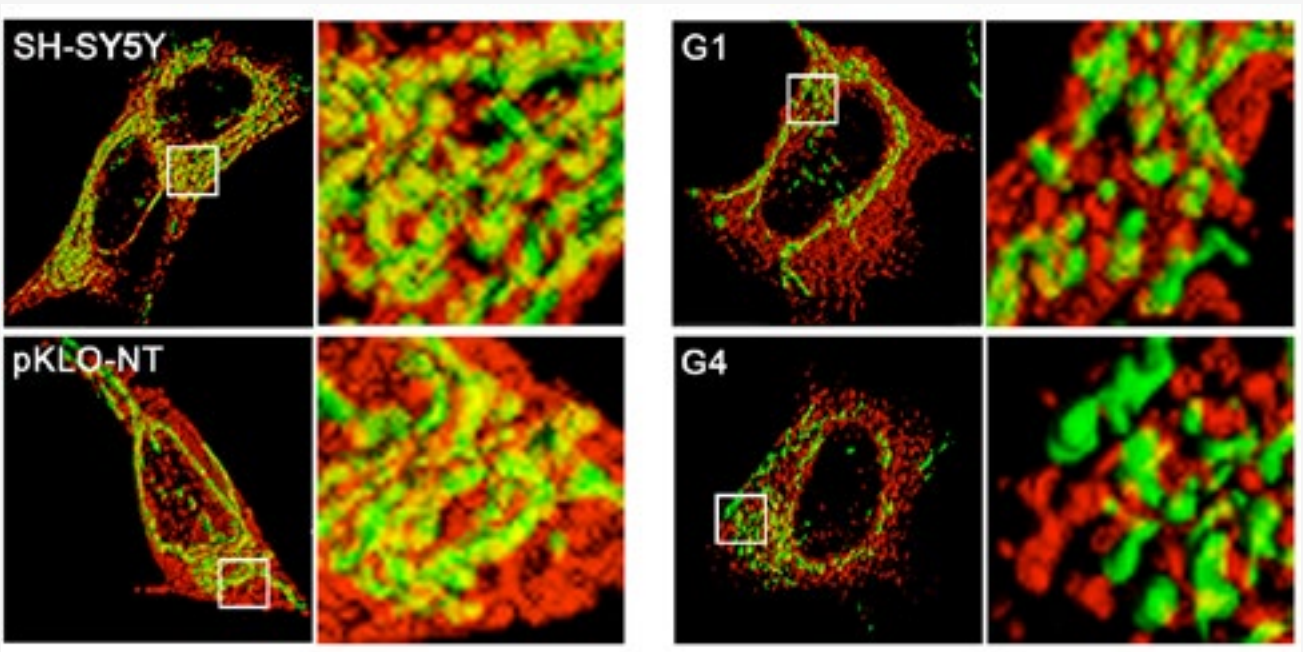

Figure A

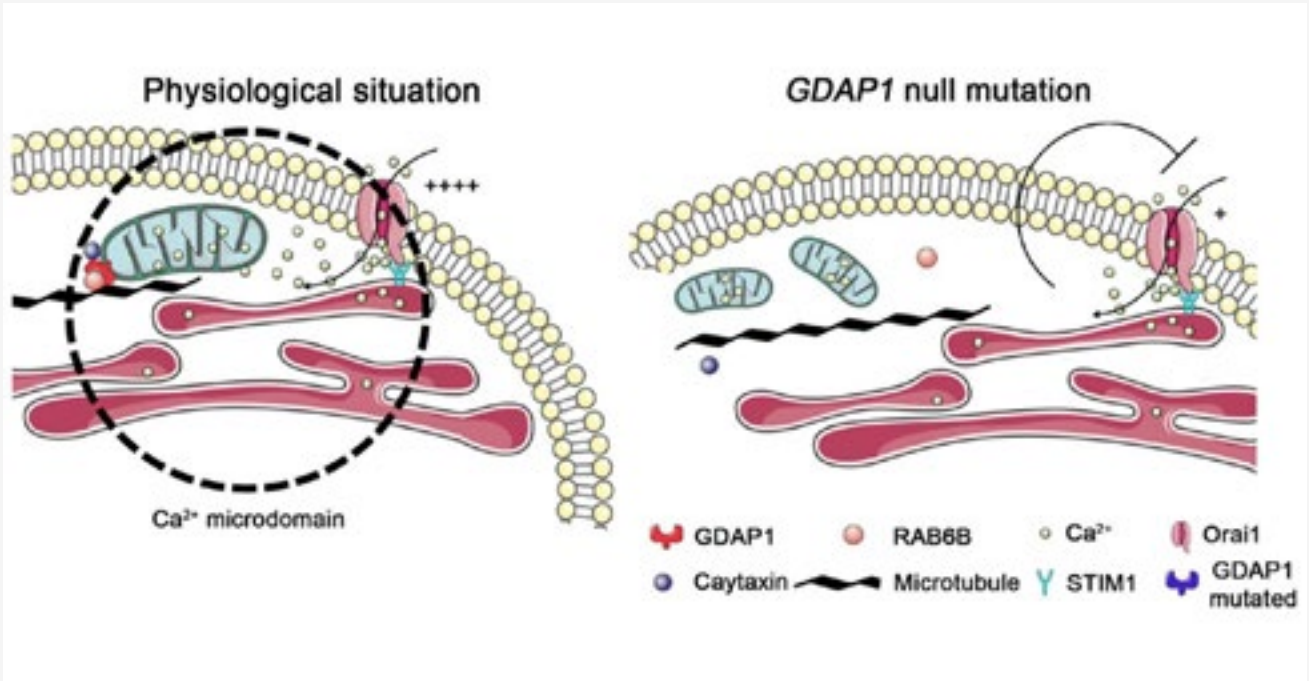

Figure B

Publications

- Calpena E, Casado M, Martínez-Rubio D, Nascimento A, Colomer J, Gargallo E, García-Cazorla A, Palau F, Artuch R, Espinós C.
  5-Oxoprolinuria in Heterozygous Patients for 5-Oxoprolinase (OPLAH) Missense Changes .
  JIMD reports,2013

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  Charcot-Marie-Tooth disease: Genetic and clinical spectrum in a Spanish clinical series.
  Neurology,2013 Sep 27

- Sevilla T, Martínez-Rubio D, Márquez C, Paradas C, Colomer J, Jaijo T, Millán JM, Palau F, Espinós C.
  Genetics of the Charcot-Marie-Tooth disease in the Spanish Gypsy population: the hereditary motor and sensory neuropathy-Russe in depth.
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- Pozo-Rubio T, Capilla A, Mujico JR, de Palma G, Marcos A, Sanz Y, Polanco I, García-Novo MD, Castillejo G, Ribes-Koninckx C, Varea V, Palau F, Ortigosa L, Peña-Quintana L, Nova E.
  Influence of breastfeeding versus formula feeding on lymphocyte subsets in infants at risk of coeliac disease: the PROFICEL study.
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  The TREAT-NMD Duchenne Muscular Dystrophy Registries: Conception, Design, and Utilization by Industry and Academia.
  Human mutation,2013 Nov

Conferences and meetings

- VI Reunión anual del CIBER de Enfermedades Raras. Escorial
  Fifth European and North American Charcot-Marie-Tooth Consortium Meeting
  EMBO Meeting 2013, Septiembre 21 a 22, Amsterdam, Holland.

- XXVII Congreso Nacional de Genética Humana. Madrid, Spain.

- VI Reunión Anual CIBERER. Madrid. 2013

- EMBO Meeting 2013, Amsterdam, Holland.

- Gordon Research Conference & Seminar, Calcium Signalling, Luca, Italy.

- VI Reunión Anual CIBER de Enfermedades Raras, Febrero 2013. Madrid, Spain

Genetics and Molecular Physiopathology of Mental and Brain Disorders

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Estela Pérez Santamarina

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Irene Pla Navarro
Magdalena Martínez

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Overview

Our group researches the **genetics and physiopathology** underlying neuropsychiatric disorders, focusing on the study of ANKK1 gene biology, and its role in human diseases.

Although the ANKK1 gene has been traditionally highly associated to addictions and dopaminergic traits; our group aims to expand the biological spectrum of ANKK1 (gene and protein) into other pathologies related to the striatum dysfunction such as Parkinson's disease (PD), and neuromuscular disorders. In order to do this, ANKK1's relation with the dopaminergic system has been studied, as well as its expression pattern and function in different tissues during development and adulthood. In particular, we have studied: (i) ANKK1's biology and pathophysiology in the context of addictive behaviours, assessing ANKK1's expression in the neurogenic niches of the adult brain and in neuronal precursors using the murine model; (ii) ANKK1's association with the dopaminergic system and biomarkers for PD; (iii) genomic analysis in schizophrenic individuals; and (iv) the biological role of ANKK1 during myogenesis and its relationship with Duchenne muscular dystrophy (DMD).

Research results

i) ANKK1's biology and physiopathology of addictive behaviours

Although TaqIA ANKK1 polymorphism is the most studied genetic variant in psychiatry, the role of this gene remains unknown. Our group has characterized ANKK1's expression pattern in humans and rodents, demonstrating that ANKK1 is expressed during neurodevelopment and the adulthood in astroglial cells (Fig.1). Moreover, unpublished data from our team have shown that ANKK1 is also expressed in the adult neurogenic niches and by neuronal stem cells in the murine model (manuscript in preparation; España-Serrano, et al., ANKK1 gene cellular expression relates addictions and neural stem cells), supporting the importance of ANKK1, and its polymorphic variants, in the structure and plasticity of the brain. These findings are consistent with novel paradigms in psychiatry according to which differences in the structure of the brain could be the basis of distinct susceptibilities to psychiatric disorders.
In collaboration with Prof. Harker Rhodes (Geisel School of Medicine and Dartmouth-Hitchcock Medical Centre, New Hampshire, USA), a conditional knock-out mouse model for ANKK1 is being developed. DNA constructs have been sent to Prof. Rhodes, who obtained ES cells effectively transformed and are currently working on the mating between chimeric males and C57BL/6J females in order to obtain the F1.

ii) ANKK1's dopaminergic association and biomarkers for Parkinson's Disease

Using the murine model, we assessed how the levels of ANKK1's expression in the brain can be modulated by different treatments with dopamine antagonists. Despite the fact that the stimulation of the dopaminergic receptor D2 (D2) could not alter ANKK1's expression in a direct manner, an intimate association between this gene's expression and the activation of the dopaminergic system, via the dopaminergic receptors D1-like and D3, was established (submitted for publication). These observations suggested an association between ANKK1 and the dopaminergic system that was further analyzed in the context of two human pathologies characterized by striatum deficiencies: alcoholism and Parkinson's disease.

In collaboration with Dr. Guillermo Ponce (Instituto de Investigación Sanitaria Hospital 12 de Octubre (IISH12O), Madrid, Spain), the relationship between the TaqIA ANKK1 polymorphism and the C957T DRD2 polymorphism (nearby the ANKK1 gene) is being assessed in peripheral blood lymphocytes from healthy controls and alcoholic patients. Preliminary results have shown basal differences in ANKK1's expression in the control group, which may be related to the genotype and the differential response to apomorphine (dopamine antagonist).

Regarding the study of ANKK1 gene in the context of PD we have found, in collaboration with Dr. Pedro García Ruiz (Neurology Service of the Fundación Jiménez Díaz, Madrid, Spain) polymorphic variants in the upstream sequence of regulatory regions of ANKK1 associated with this disease. These variations have been previously described in the ENCODE project. Since these changes have been associated with an increased susceptibility to PD, functional in vitro studies with dopaminergic treatments are been conducted in order to validate them. This study received the award for Genetic Innovation (Sistemas Genómicos/ Universidad de Valencia, 2013) granted to the Msc project "Study of the ANKK1 gene in Spanish patients with Parkinson's Disease" (Estela Pérez Santamarina).

iii)Genomic analysis in schizophrenic individuals
Our team has also participated in a Genome Wide Association Study (GWAS) in collaboration with the "Grupo de Estudio de Psicosis del CIBERSAM" (*manuscript under review; Ivorra et al., Replication of previous genome-wide association studies of psychiatric diseases in a large schizophrenia case-control sample from Spain*). In addition, we are also collaborating with the IISH12O and the Hospital de la Ribera Psychiatry Service in the genetic characterization of schizophrenic families by exome analysis and Comparative Genome Hybridization (CGH).

iv)The biological role of ANKK1 in the satellite cell and in the Duchenne muscular Dystrophy
Our team has also researched into satellite cell's specific markers in murine muscle, in collaboration with Dr. Francesc Palau and Dr. Juan Vílchez (Programa de Enfermedades Raras y Genéticas). Since ANKK1 is expressed in muscular progenitor cells during embryogenesis, we have chosen to study this protein in order to clarify the molecular mechanisms underlying the decreased cellular regeneration described in patients. Double labelling experiences with α-ANKK1 and embryonic α-myosin have revealed co-localization of these two proteins, as well as ANKK1's cellular pattern in murine myoblasts during development (Fig.2). In addition, our preliminary results show ANKK1's implication in muscular differentiation.
Both, experimental biology and translational research, have been funded by the Instituto de Salud Carlos III (Fondo de Investigaciones Sanitarias; Acción Estrategia en Salud [AES]) and the Fundación privada Isabel Gemio.

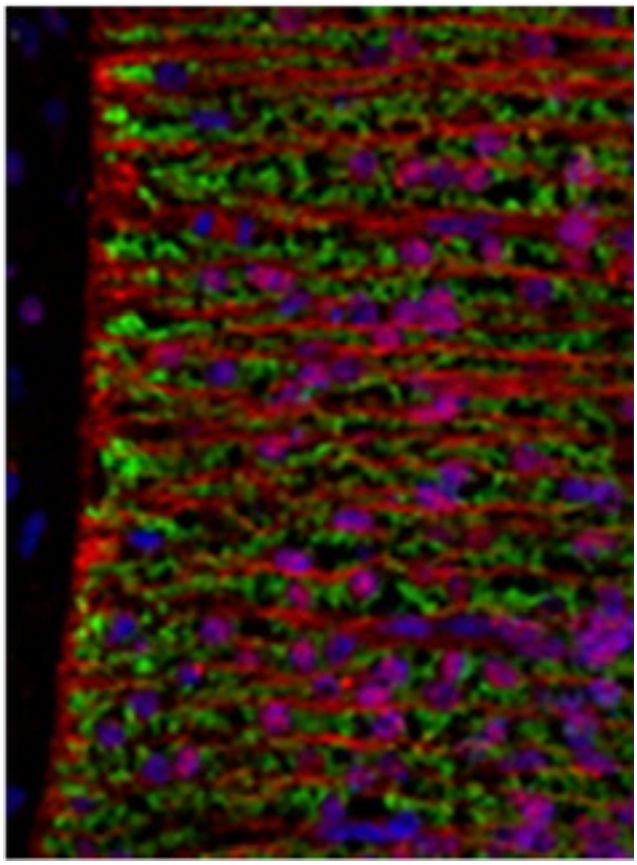


Figure 1: Ankk1 expression pattern in mice embryo radial glial (red).

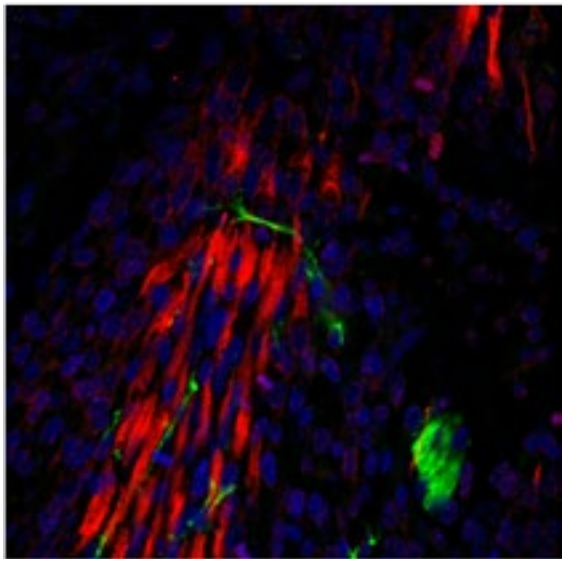


Figure 2: Ankk1 cellular expression pattern in mice embryo mioblasts (red)

Conferences and meetings

- Oral Communication,"De la molécula del ADN a la individualidad genética en la medicina". VII Congreso Nacional de los Servicios de Prevención de Riesgos Laborales en el Ámbito Sanitario, Madrid,Spain.
- Oral Communication,"Genetic Vulnerability Of The Pathological Gambling And Dual Pathology". III Congreso Internacional de Patología Dual, Madrid,Spain.
- Oral Communication,"Investigando el Genoma en Pacientes con Esquizofrenia, Jornada de Investigación". Hospital Universitario de la Ribera,Valencia,Spain.
- Oral Communication "Genética Básica: Todo lo que debe saber un psiquiatra sobre Genética". IX Foro Venezolano de Psiquiatría , Margarita,Venezuela.
- Oral Communication, "Genética de los trastornos psiquiátricos:¿Qué se hereda?". IX Foro Venezolano de Psiquiatría, Margarita,Venezuela.

INDEX

Intracellular Protein Degradation & Rare Diseases

Group Leader

Erwin Knecht Roberto

→ Researchers
- Carmen Aguado Muñoz (CIBERER)
- Eva Pérez-Jiménez

→ Graduate students
- Ghita Ghislat
- José Félix Moruno Manchón
- Alihamze Fathinajafabadi
- Marcos Lahuerta Ferreres

→ Technicians
- Asunción Montaner Fayos
- Mari Paz Rubio Rodríguez

→ Collaborators
- José Manuel Vidal Donet
- María Ciria Calduch
- Cristian García Ruiz

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

Overview

Our laboratory investigates how the main mechanisms of intracellular protein degradation (more specifically autophagy and the ubiquitin-proteasome system) are regulated. In addition, we also investigate the relevance for the pathogenesis of various rare diseases of alterations in these mechanisms and their regulation. Although rare diseases have a low prevalence, their study can allow the understanding of the pathogenesis of more frequent pathologies.

Intracellular proteolytic mechanisms, especially autophagy, can be induced or inhibited by a variety of molecules, such as growth factors, nutrients and hormones, and this occurs through a complex and still poorly known signaling network. Since both autophagy and the ubiquitin-proteasome system play essential roles in cellular homeostasis, alterations in their regulation and normal functions have been found to be important in several human pathologies, including cancer and neurodegenerative, muscle and cardiovascular diseases.

Accordingly, in addition to investigate and to increase our knowledge on the regulation and main mechanisms of intracellular protein degradation, we collaborate with other groups of a Spanish public consortium (Centre for Biomedical Network Research on Rare Diseases, CIBER-ER) in elucidating the molecular bases of those rare diseases in which undegraded proteins, polysaccharides or lipids accumulate within cells, as well as in developing new diagnostic methods and therapeutical treatments.

Research results

During this year we have focused our research in two main areas:
1. The regulation of the mechanisms of intracellular protein degradation (in particular autophagy and the ubiquitin-proteasome system). More specifically:
- We have studied the regulation of macroautophagy by glucose and we have shown that, contrary to what we expected based on the generally accepted idea that nutrient withdrawal induces autophagy, glucose clearly increases the autophagic flux, from autophagosomal formation to degradation of the lysosomal content. Although this increase is in part mediated by ATP production from glucose, this is not the only cause, as we have identified a signaling pathway that depends on p38 MAPK, and not on AMPK or mTORC1, which are the main regulatory kinases of autophagy.
- We have found that three calcium-dependent phospholipid binding proteins, i.e. annexin A1, annexin A5 and probably copine 1, are implicated in autophagosome maturation and, therefore, ease autophagy. In addition, we have shown that Ca^{2+} acts as a second messenger in autophagy induction due to withdrawal of essential amino acids. These results allow us to propose a new signaling pathway that is induced in the absence of amino acids by an increase in the intracellular calcium concentration and that involves the kinases CaMKK-ß, AMPK, ULK-1 and $mTORC_1$.
- We have established that, in human glioblastoma cells, the tumor suppressor PTEN, which is known to negatively regulate the PI3K class I-AKT-mTOR signaling pathway through its activity of lipid kinase, induces autophagy and inhibits the ubiquitin-proteasome system independently of this activity. Hence, we have uncovered a new signaling pathway that is PTEN-dependent and mTORC1-independent and that probably relies on the protein kinase activity of PTEN. This new signaling pathway can regulate, in opposing directions, the two main intracellular protein degradation mechanisms, i.e. autophagy and the ubiquitin-proteasome system.

2. The analysis of the relevance of alterations in these degradation mechanisms and their regulation in several rare diseases. In particular we have studied:
- Neuronal ceroid lipofuscinosis (NCL), which refers to a group of fatal neurodegenerative inherited disorders characterized by the accumulation of an autoflu-

orescent material inside the lysosomes. During this year we have focused our interest on two of these disorders: LINCL (CLN2, late infantile) and JNCL (CLN3, juvenile NCL), that are due to mutations in CLN2 and CLN3 genes, respectively, and that represent 90% of all cases of NCLs diagnosed worldwide.

Apart of similarities, we have found several differences in the phenotypes of LINCL and JNCL patient-derived fibroblasts, which could explain the earlier appearance of the symptoms in the former of the two NCL forms: i) an inhibition in autophagosome formation in CLN2 compared to a simple alteration in autophagosome-endosome/lysosome fusion in CLN3, which is due to an increase in lysosomal pH , and ii) an increased accumulation of reactive oxygen species in CLN2 fibroblasts due to a direct alteration in TPP1 activity, which would be only partial in CLN3 and again due to the increased lysosomal pH.

- Lafora disease (LD), which is an especially severe and relatively common kind of progressive myoclonus epilepsy.

The prevalence of the disease is variable and cases are distributed worldwide, although they are more commonly found in particular geographic areas, including Spain. Ninety-five percent of cases are caused by mutations in either EPM2A (that codifies the dual phosphatase laforin) or EPM2B (that codifies the ubiquitin ligase malin) genes.

Our group, in collaboration with six other laboratories belonging to the CIBER-ER (Drs. P. Bovolenta, F. Pallardó, V. Rubio, S. Rodríguez de Córdoba, P. Sanz and J. Serratosa), is part of the Lafora Consortium, which studies in a coordinated manner and from different perspectives this condition.

During this year, and in all of the experimental models of the disease available to us we have found: i) a defect in autophagosome formation and, therefore, a reduced autophagy, ii) an increase in oxidative stress probably caused by defects in autophagy and in the antioxidant defence system, mainly the mitochondrial isoform of superoxide dismutase (MnSOD) and the catalase enzymes. Therefore, both a defect in autophagy and an increase in the oxidative stress seem to play an important role in the pathogenicity of this disease.

Last but no least, we collaborate with other groups that belong to the CIBER-ER in the study of other rare diseases, such as X-linked adrenoleukodystrophy (Dr. A. Pujol's group), retinitis pigmentosa (Dr. R. González-Duarte's group) and the mitochondrial disease MELAS (Dr. E. Armengod's group). These collaborations have lead to conclusions that will be mentioned in the next report, given that the results have been or will be published in 2014.

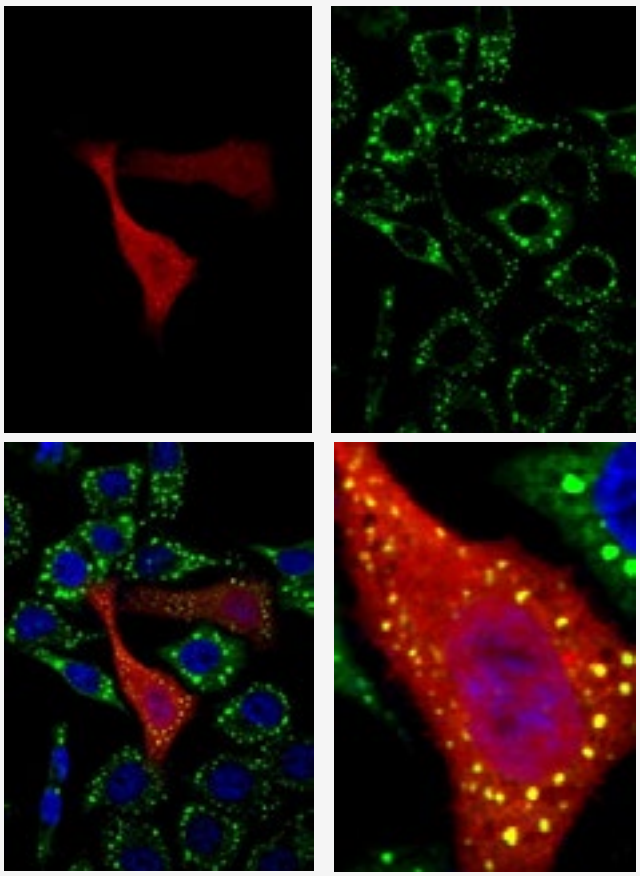


Figure A COS-7 cells were transfected with CERKL-HA and its colocalization was analyzed by immunofluorescence with the marker of stress granules PABP. Image shows colocalization of both proteins in the cytoplasm.

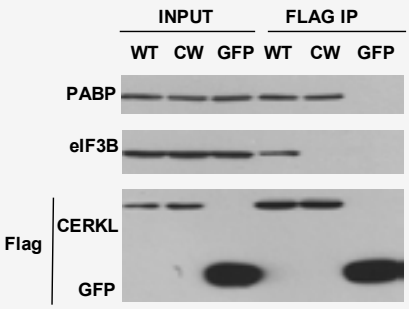


Figure B - Flag-tagged GFP, CERKL WT (WT) or the mutant CERKL-C125W (CW) were immunoprecipitated and interaction with PABP or eIF3b was analyzed. Both the wild type and the mutant protein interact with PABP, but not with eIF3b.

Publications

→ Errafiy R, Aguado C, Ghislat G, Esteve JM, Gil A, Loutfi M, Knecht E.
PTEN increases autophagy and inhibits the ubiquitin-proteasome pathway in glioma cells independently of its lipid phosphatase activity.
PLoS One. 2013 Dec13; 8(12):e83318.

→ García-Giménez JL, Seco-Cervera M, Aguado C, Romá-Mateo C, Dasí F, Priego S, Markovic J, Knecht E, Sanz P, Pallardó FV.
Lafora disease fibroblasts exemplify the molecular interdependence between thioredoxin 1 and the proteasome in mammalian cells.
Free Radic Biol Med. 2013 Dec; 65:347-59.

→ Launay N, Ruiz M, Fourcade S, Schlüter A, Guilera C, Ferrer I, Knecht E, Pujol A.
Oxidative stress regulates the ubiquitin-proteasome system and immunoproteasome functioning in a mouse model of X-adrenoleukodystrophy.
Brain. 2013 Mar; 136(3):891-904.

→ Ghislat G, Knecht E.
Ca^{2+}-sensor proteins in the autophagic and endocytic traffic.
Curr Protein Pept Sci. 2013 Mar; 14(2):97-110.

→ Moruno-Manchón JF, Pérez-Jiménez E, Knecht E.
Glucose induces autophagy under starvation conditions by a p38 MAPK-dependent pathway.
Biochem J. 2013 Jan 15; 449(2):497-506.

→ Vidal-Donet JM, Cárcel-Trullols J, Casanova B, Aguado C, Knecht E.
Alterations in ROS activity and lysosomal pH account for distinct patterns of macroautophagy in LINCL and JNCL fibroblasts.
PLoS One. 2013; 8(2):e55526.

Conferences and Meetings

→ Co-organizer of the International Workshop "Intracellular protein degradation in neurodegenerative diseases" (VLC/ CAMPUS), November 2013, Valencia, Spain.

CIPF facilities

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

Coordinated management and collaborative nature of technical specialists the CIPF services are designed to promote networking and to enhance the competitiveness of its users, whether research or industrial projects, due to the interdisciplinarity.
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 projects of large companies, absorbing the know-how to incorporate it into their background.
Resulting from this the 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.

Animal Facility

Animal Facility works for the care and maintenance of the laboratory animals. Quality in research and scientific advances require an ethic use of laboratory animals 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 in scientific purposes are required.
Our Animal Facility is registered as **Breeding, User and Supplier Center for Experimentation with Animals** with **no. ES 46 250 0001 002** and it is made up of qualified and accredited personnel in Laboratory Animal Science.

Confocal Microscopy

The Optical and Confocal Microscopy Service (OCMS) provides a central facility at CIPF for microscopy imaging and analysis with dedicated support from an experienced microscopy professionals. The confocal microscopy is a standard and valuable tool in life science as well as material science, for this reason, the OCMS has contributed to the research projects of several groups and it has collaborated with other important 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 Facility 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 antigensm, analysis of the cell cycle, cytotoxicity assays for assessing cell death and investigate specific apoptosis pathways, functional tests applied to established cell lines and primary cultures and ex vivo samples, cell analysis of microorganisms for clinical applications in Biotechnology and Environmental Sciences, functional characterization and immunophenotype of stem cells, analysis of real time kinetic 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. Detection and quantification of different cell parameters. Trials of special relevance in the fields of toxicology and drug discovery.

Genomics

The Genomics Service 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

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:
Images of neural stem cells marked with GFP and injected/transplanted into the previously injured spinal cord of mice were taken with the TEM, in order to monitor their development/evolution in the target tissue. It was observed that transplanted neural stem cells remained undifferentiated in the lesioned tissue and established contacts with endogenous macrophages. These studies are part of a research project of the University of Cambridge and were published on the *Journal Brain*.
In order to study how the final number of interneurons in the brain is determined from birth to adult age, cultivated GFP-marked interneurons were transplanted into the cerebral cortex of a mouse. These cells were thereafter studied under the TEM, revealing that GFP-positive interneurons established synaptic contacts with endogenous neurons, which has helped to better understand the underlying mechanism of the regulation of the neuronal population. These studies are part of a research project of the University of San Francisco and were published on the *Journal Nature*.

NMR

The NMR facility 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.

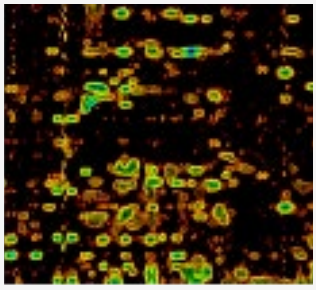
Proteomics

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.

Traslational Genetics Service

The Translational Genetics Service 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 Centre (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.

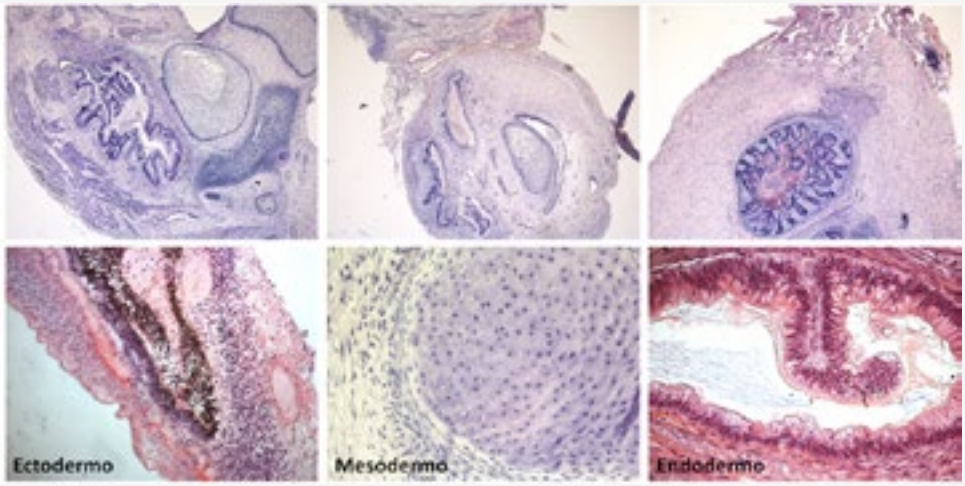


PICTURE 10 - Three-dimensional protein structure obtained by Nuclear Magnetic Resonance.

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

Stem Cell Bank

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 conditionsI.

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, Parkinson, macular degeneration, Alzheimer, autism, severe anemia, muscular atrophy, spinal cord damage and cardiac diseases.

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 miotó-nica 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

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.

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 an 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.

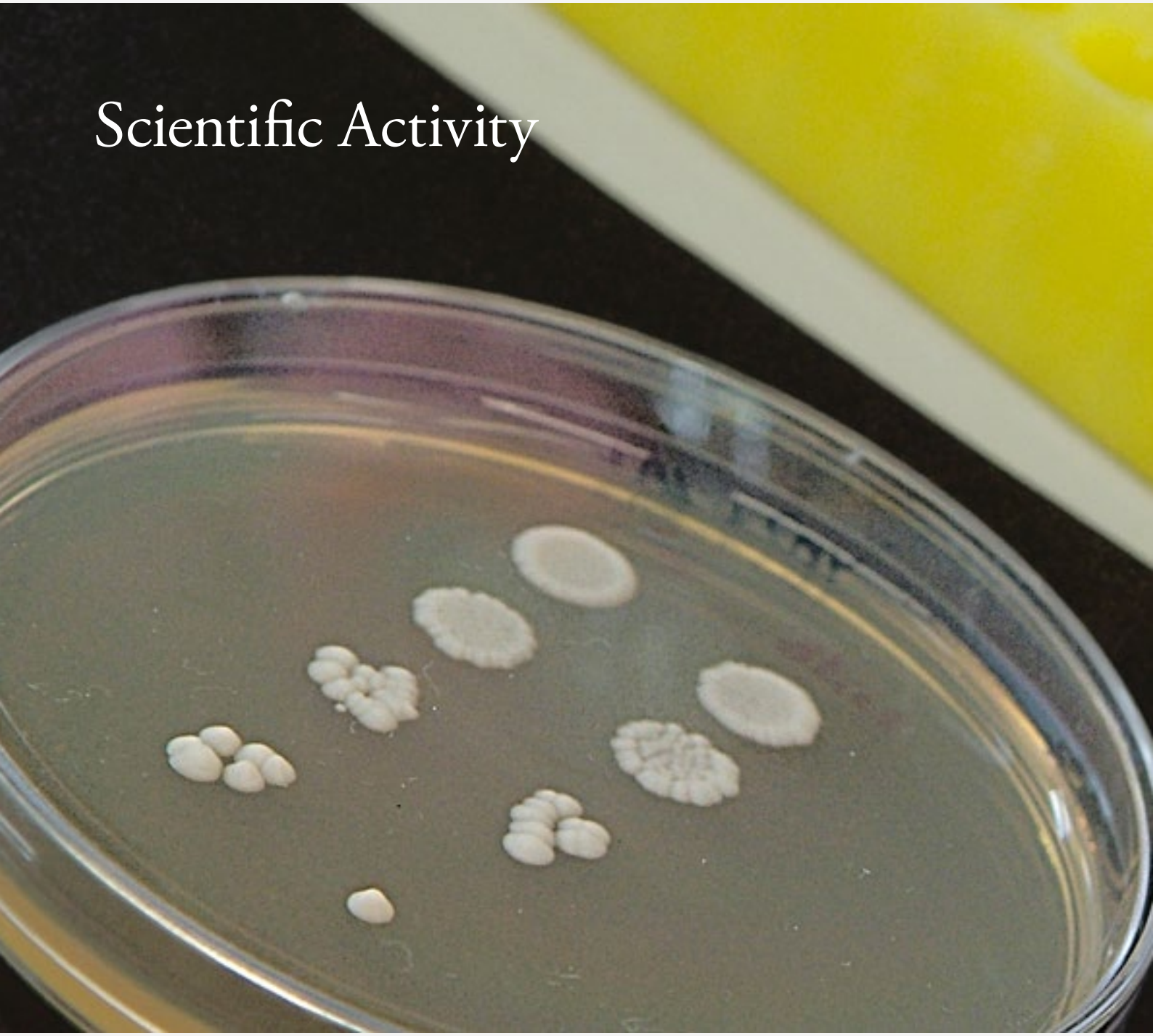
Monopolar and bipolar electrosurgical generator (Valleylab / Storz).

Two surgical tables with temperature control system and integrated mobility.

Computational Genetics

Our broad goal is developing and applying 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	Investigador Principal proyecto
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
Alba González Usano	Formación personal investigador	Felipo, Vicente
Alihamze Fathinajafabadi	Formación personal investigador	Knecht Roberto, Erwin
Patricia Sebastian Leon	Formación personal investigador	Dopazo Blazquez, Joaquín
Micaela Molina Navarro	Contrato Juan de la Cierva	Rodríguez Navarro, Susana
Antonio Diez Juan	Contrato Ramón y Cajal	Diez Juan, Antonio
Rodrigo Carbajo	Programa de incentivación de la incorporación e intensificación de la actividad investigadora	Carbajo Martínez, Rodrigo José
Susana Rodriguez Navarro	Programa de incentivación de la incorporación e intensificación de la actividad investigadora	Rodríguez Navarro, Susana
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

Carlos III Institute of Health

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

Education Cancillery (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, Vicente
Jorge Montesinos	Ayudas para la contratación de personal investigador en formación en fase predoctoral	Guerri Sirera, Consuelo
Antonio Diez Juan	Programa de Estabilización de investigadores y de intensificación de la actividad investigadora en el Sistema Nacional de Salud	Diez Juan, Antonio
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
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
Navarro Rey, Carmen	Ayudas a la movilidad predoctoral para la realización de estancias breves	Armengod, Eugenia
Amaya Niño Pariente	Ayudas a la movilidad predoctoral para la realización de estancias breves	Vicent Docon, Mª Jesus

European Comission

Grantee	Type of grant	Principal Investigator
Ana Conesa	Cost Action	Conesa Cegarra, Ana

Research projects grants

7th Framework Programme

Type of grant	Title	Principal Investigator
Collaborative Project	Inanonak	Vicent Docon, Mª Jesus
Collaborative Project	Livimode	Vicent Docon, Mª Jesus
Collaborative Project	Eurocondor	Burks , Deborah Jane
Collaborative Project	Innovaliv	Burks , Deborah Jane
Collaborative Project	Denamic	Felipo Orts, Vicente
Collaborative Project	MLPM2012— Machine Learning for Personalized Medicine	Dopazo Blazquez, Joaquín
Collaborative Project	Stategra	Conesa Cegarra, Ana Victoria
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	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 de Investigación fundamental no orientada	Bases Moleculares de las alteraciones neurológicas en hiperamonemia y encefalopatía hepática. Implicaciones terapéuticas	Felipo Orts, Vicente
Proyectos de Investigación fundamental no orientada	Caracterización de la variabilidad genética, genómica y metabólica de los cítricos y su aplicación en la obtención de nuevas variedades	Dopazo Blazquez, Joaquín
Programa Consolider	Consolider SICI	Felipo, Vicente
Programa Consolider	Consolider SICI	Moreno Manzano, Mª Victoria
Programa Consolider	Consolider SICI	Orzaez Calatayud, Mª Del Mar
Proyectos de Investigación fundamental no orientada	Degradación Intracelular De Proteínas: Regulación y alteraciones en Enfermedades Raras	Knecht Roberto, Erwin
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	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	Estudio de nuevas relaciones genoma-transcriptoma mediante técnicas de ultrasecuenciación	Conesa Cegarra, Ana Victoria
Acciones Bilaterales	Genómica y transcriptómica de las rutas de detoxificación en drosophila	Conesa Cegarra, Ana Victoria
Proyectos de Investigación fundamental no orientada	La Regulación de Células Progenitores por las Señales de insulina/IRS2: Implicaciones en enfermedades metabólicas	Burks , Deborah Jane
Proyectos de Investigación fundamental no orientada	Mecanismos moleculares de moduladores de apoptosis	Orzaez Calatayud, Mª Del Mar
Proyectos de Investigación fundamental no orientada	Nanofármacos poliméricos utilizados como agentes simples y en terapía de combinación. Plataforma tecnológica versátil para la regeneración tisular y tratamientos anticancerígenos	Vicent Docon, Mª Jesus
Proyectos de Investigación fundamental no orientada	Nuevas tecnologias para toxicologia in Vitro: Diseño y validación de una plataforma integrada Admetox de ensayos celulares para predicción de riesgo químico en humanos	Oconnor Blasco, Jose Enrique
Proyectos de Investigación fundamental no orientada	Papel de las anafilatoxinas del complemento en la recuperación del daño cardiaco	Diez Juan, Antonio
Proyectos de Investigación fundamental no orientada	Papel de los receptores TLRs y el Inflamasoma en el daño que induce el etanol en el cerebro	Guerri Sirera, Consuelo
Acciones complementarias	Pathogenomics - Metabolomica e Interactomica de la relación húesped-patógeno	Conesa Cegarra, Ana Victoria
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	Importancia de los mecanismos de degradación de proteinas en la neurodegeneración causada por etanol	Guerri Sirera, Consuelo
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 multilaterales	Una aproximacion omica al diagnóstico de la Tuberculosis	Pineda Lucena, Antonio
Proyectos de Investigación fundamental no orientada	Understanding the mechanisms of the disease and prioritizing candidate genes under a sys-tems perspective	Dopazo Blazquez, Joaquín
Proyectos de Investigación fundamental no orientada	Disección de la fisiopatología de la enfermedad de Charcot-Marie-Tooth	Palau, Francesc

Carlos III Institute of Health

Type of grant	Title	Principal Investigator
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	Development Of Tools Of New Generation For Gene Ex	Dopazo Blazquez, Joaquín
Redes de Investigación	Red De Trastornos Adictivos	Guerri Sirera, Consuelo
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
Proyectos de Investigación en salud	Biología celular y función de ANKK1 en el cerebro: relación con el sistema depaminérgico y la neurogénesis	Hoenicka, Janet
Redes de Investigación	Red De Cáncer	Dopazo Blazquez, Joaquín
Redes de Investigación	Red De Transtornos Adictivos	Guerri Sirera, Consuelo
Proyectos de Investigación en salud	Regeneración de la función motora tras lesión medular traumática: activación del potencial regenerador endógeno	Moreno Manzano, Mª Victoria
Centro de Investigación Biomédica en Red – CIBER'	CIBERER	Dopazo Blazquez, Joaquín; Knecht, Erwin; Palau, Francesc
Centro de Investigación Biomédica en Red – CIBER'	CIBERDEM	Burks , Deborah Jane
Proyectos de Investigación en salud	Translational Research, Experimental Medicine and Therapeutics on Charcot-Marie-Tooth	Galindo, Máximo Ibo

Spanish Agency for International Cooperation

Type of grant	Title	Principal Investigator
Acciones integradas	Acción para el fortalecimiento científico tecnológico en áreas relacionadas con la genómica y bioinformática aplicadas	Dopazo Blazquez, Joaquín
Proyecto de Investigación	Papel de la activacion del Sistema Innato Inmunitario y de la glia en el daño cerebral inducido por el consumo de alcohol	Guerri Sirera, Consuelo

Education Cancillery (GVA)

Type of grant	Title	Principal Investigator
Acciones complementarias	Aplicaciones de la RMN y de la Metabolomica al desarrollo de nuevos agentes antineoplásicos dirigidos molecularmente	Pineda Lucena, Antonio
Ayudas a congresos	Catedra Grisolia	Felipo Orts, Vicente
Prometeo	Bases Moleculares de las Alteraciones Neurologicas en Hiperamonemia y Encefalopatia Hepatica. Implicaciones terapeuticas	Felipo Orts, Vicente
Prometeo	Estructura y función de reguladores de expresión génica	Armengod González, Mª Eugenia
Prometeo	Estructura y función de reguladores de expresión génica	Rodríguez Navarro, Susana
Prometeo	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
Prometeo	Identificacion de nuevas dianas Terapéuticas en angiogenesis y apoptosis basadas en interacciones proteína-proteína	Orzaez Calatayud, Mª Del Mar
Acciones complementarias	Nanofármacos poliméricos utilizados como agentes simples y en terapía de combinación. Plataforma tecnológica versátil para la regeneración tisular y tratamientos anticancerígenos	Vicent Docon, Mª Jesus
Acciones complementarias	Nuevas tecnologias para toxicologia in Vitro: Diseño y validación de una plataforma integrada Admetox de ensayos celulares para predicción de riesgo químico en humanos	Oconnor Blasco, Jose Enrique
Acciones complementarias	The Spanish Ion Channel	Felipo Orts, Vicente

Foundations and other Private Entities

Type of grant	Title	Principal Investigator
Fundacion Gent per gent	Analisis de gemelos discordantes para investigar la correlación entre alteraciones en expresión y metilación de DNA en Lupus eritematoso sistémico	Conesa Cegarra, Ana Victoria
Fundación La Marato TV3	Epilepsia progresiva mioclónica de Lafora: Bases fisiopatológicas de la enfermedad y aproximaciones terapéuticas	Knecht Roberto, Erwin
Fundación La Marato TV3	Study of cohesin functions in Cornelia de Lange Syndrome	Rodríguez Navarro, Susana
Donations	Proyecto Paula	Burks , Deborah Jane

Contracts Research

Entity	Title	Principal Investigator
Umecrine Cogninition AB	Research contract	Felipo Orts, Vicente
Asociación de Investigación de la Industria textil	Research contract	Felipo Orts, Vicente
BULL	Cátedra Genómica Computacional	Dopazo Blazquez, Joaquín
Salvat	Research contract	Vicent Docon, Mª Jesus
Salvat	Research contract	Vicent Docon, Mª Jesus
Jannsen Cilag S.A.	Research contract	Fustero Lardíes, Santos
Jannsen Serine	Research contract	Pineda Lucena, Antonio
Jannsen Cilag S.A.	Research contract	Pineda Lucena, Antonio
Jannsen Cilag S.A.	Research contract	Pineda Lucena, Antonio
Jannsen Cilag S.A.	Research contract	Pineda Lucena, Antonio
Fundación Cugat	Research contract	Moreno Manzano, Mª Victoria
Europath Bioscience	Research contract	Dopazo Blazquez, Joaquín
BCN Time4Research	Research contract	Vicent Docon, Mª Jesus
BCN Time4Research	Research contract	Moreno Manzano, Mª Victoria
Fundación Pública Andaluza Progreso y Salud	Research contract	Moreno Manzano, Mª Victoria

CIBERER Competitive funding

Human resources grants

Education Cancilleri (GVA)

Grantee	Type of Grant	Investigador Principal proyecto
Carmén Espinós	Programa de Estabilización de investigadores y de intensificación de la actividad investigadora en el Sistema Nacional de Salud	Espinós, Carmén

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
Collaborative Project	European Friedreich's Ataxia Consortium for Translational Studies (EFACTS)	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

Education Cancilleri (GVA)

Grantee	Type of Grant	Investigador Principal proyecto
Prometeo	Genes, proteínas y rutas de señalización en enfermedades raras (BioMeder)	Palau, Francesc

Foundations and other Private Entities

Type of grant	Title	Principal Investigator
Fundacion Alicia Koplowtiz	Identificación de biomarcadores asociados con la Ataxia de Friedreich	González Cabo, Pilar
Fundación La Marato 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
Difluorobenzyl ethanolamine derivatives with antimicrobial activity	P201000997	Spanish patent
Triazine derivatives and their uses as TRPV1 inhibitors	WO/2012/136873	PCT application
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

Spin-offs

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.

During 2013, the 3 new spin-off companies created in 2012 had started to grow in their activities, and , as a member of the companies, the CIPF has attended to all the meetings related to their management.

The three 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

Scientific collaboration

During 2013, the CIPF has strengthened its cooperation with other national and international, both public and private companies, research centers and institutions:

Collaboration agreements or institutional framework:

- Collaboration Agreement CVBAN.
- Collaboration Agreement with Alumni.net.
- Collaboration Agreement with Bancaja Foundation to promote a research program.
- Collaboration Agreement with the Catholic University of Valencia.
- Collaboration Agreement with the Spanish Society of Laparoscopic Surgery.
- Collaboration Agreement with the Technological Institute of Informatics ITI (UPV).
- Collaboration Agreement with the C.A.C.,S.A.
- Collaboration Agreement with Ministry of Education for the establishment of the general conditions for a better grants management.
- Collaboration Agreement with University CEU- Cardenal Herrera (Valencia)
- Memorandum of understand between UPV, UVEG, IBV, ISS La Fe, CSIC, FISABIO, FIVI, INCLIVA, FIHGU, FIVO to regulate the VLC/ Campus innovation Platform

Agreements for the implementation of projects and specific actions

- Collaborationn Agreement with the University of Valencia to stablish a PhD in Neurosciences.
- Collaboration Agreement with Agencia Valenciana de Salud for the enforcement of the research activity in the National Health Service.
- Collaboration Agreement with Neuraltech Biopharma S.L.
- Collaboration Agreement with INCLIVA for the Neuroendocrinology Laboratory.
- Collaboration Agreement with INCLIVA for the Neurobiology Laboratory.
- Collaboration Agreement with the Catholic University of Valencia to created the Unit of Molecular Phatology and Tranlational research on oncology.
- Agreement with the Resarch Foundation of the Hospital General to carry out a training programme in surgery.
- Aggrement with Asociation for the research in tumors Uro-oncologics to carry out a specilized programme on surgery
- Agreement with CIBERER to carry out a research project on Charcot- Marie- Tooth dissease.
- Agreement with INCLIVA to carry out the estabilitation of researchers in the National Health Service
- Agreement with the Foundation for Research of the Hospital Universitario 12 de Octubre, "Bases moleculares de los trastornos psiquiátricos".
- Agreement with Universidad de Valencia to create the Nodo of Proteomics.
- Agreement with RESEARCH EXECTIVE AGENCY to carry out a research project.
- Agreement with the Catholic University of Valencia to carry out the research project " Escoliosis induction in animals by experimental surgery"
- Agreement with BCN TIME4RESEARCH to carry out the research Project "Treatment of chronic sci based on synergistic cell replacement and nanomedicine approaches" .
- Research contract with University Tubingen, Katholieke Universitit Leuven, CEGATHMBH, Eagle Genomics- Research netwok services LTD.
- Research contract with UMICRINE COGNOTION AB.
- Research contract with Jannsen Cilag.
- Research contract with the Catholic University of Valencia.
- Research contract with AITEX.

•Research contract with University of Valencia for a Scientific managamente in the project "Route development for the steros elective synthesis of beta-amino alcohols".
•Contract with EQUIPNET, INC.
•Contract with Provalentia Congresos S.L. for the Congress in Rare Diseasses cellebrated in April.
•Contract with the University of Valencia for the use of the Animal facility.

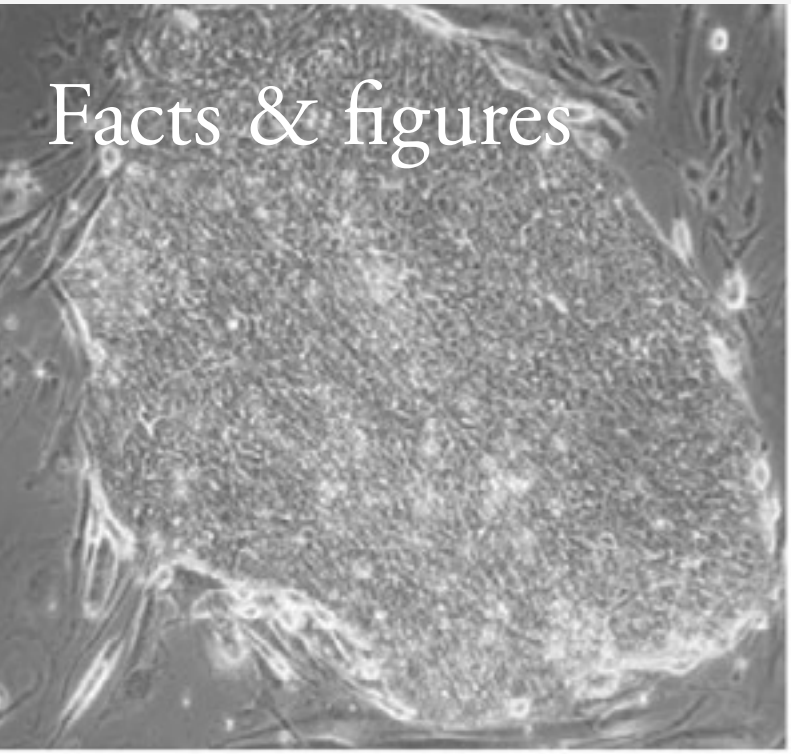
Patent and research results ownership

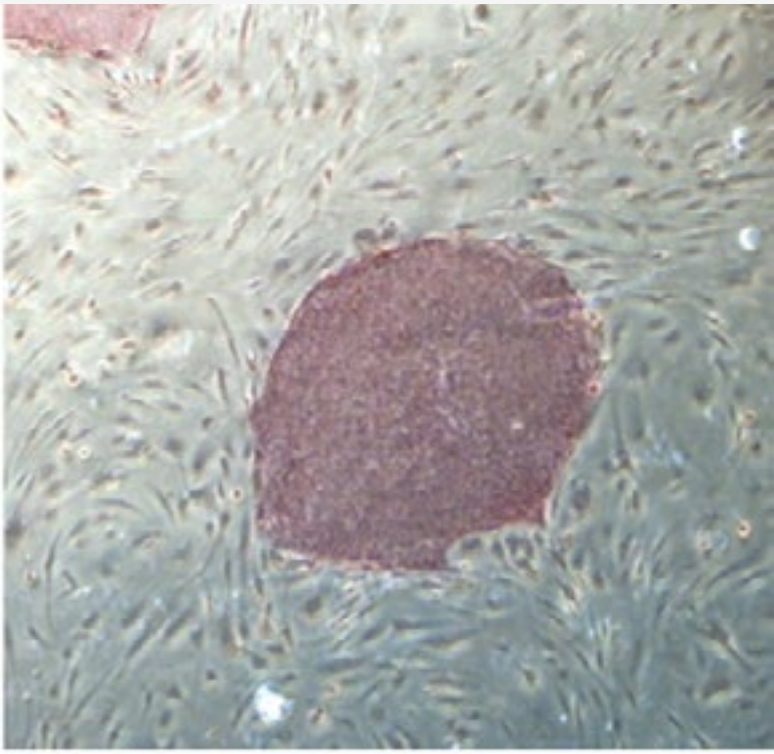
•Contract on joint Ownership with Genera Biotech S.L.
•Agreement on joint Ownership of the patent "New Hexakis ortho-substituted p-Terphenyls for the treatment of human immunodeficiency virus type-1 (HIV-1) infections and related disease" with UCV, UV and ISCIII.

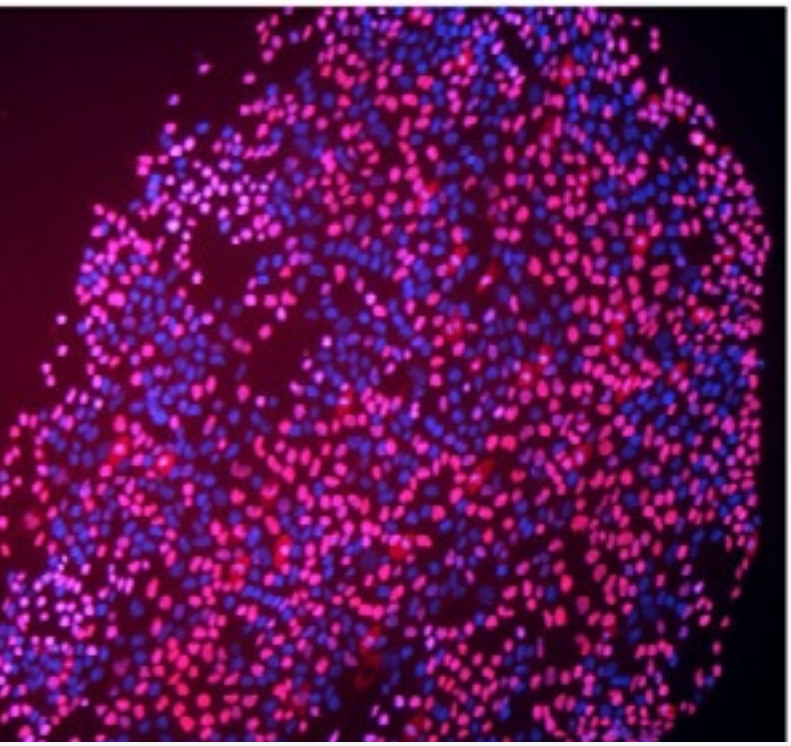
HR agreements

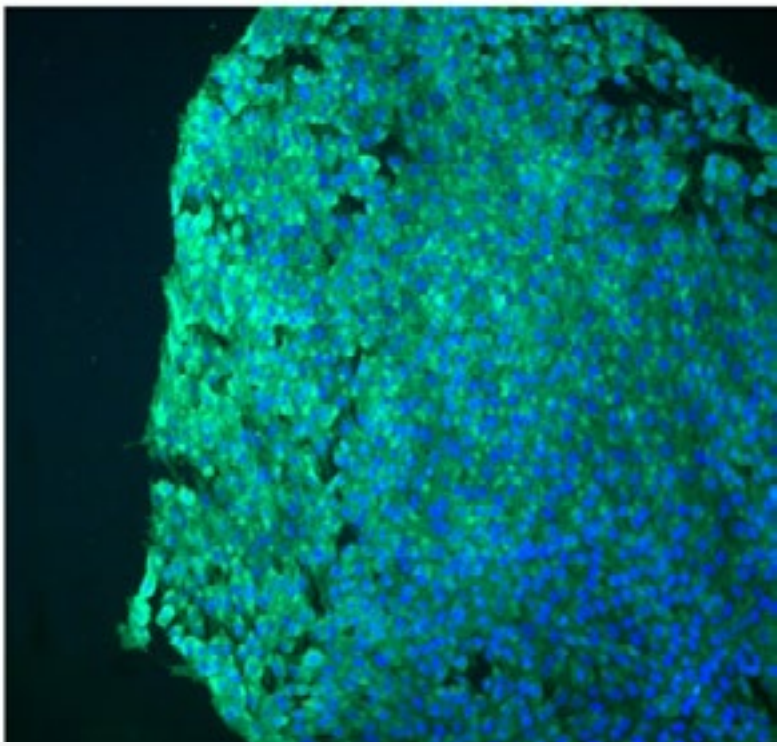
•Collaboration agreement with UNED Sevilla extra curricular activitues.
•Research contract with Jannsen Cilag adding two researchers to the project.
•Collaboration agreement with University CEU-San Pablo for hosting students.

Facts & figures









Personnel

Table 1 - Research & Support Personnel

Research Staff	**89**	25%
Support Staff	**77**	21%
Technical	57	16%
Staff Research Management	20	6%
Collaborators	**196**	54%
Researchers	99	27%
Students	97	27%
Total	**362**	**100%**

Charter 1 - Research and support staff .

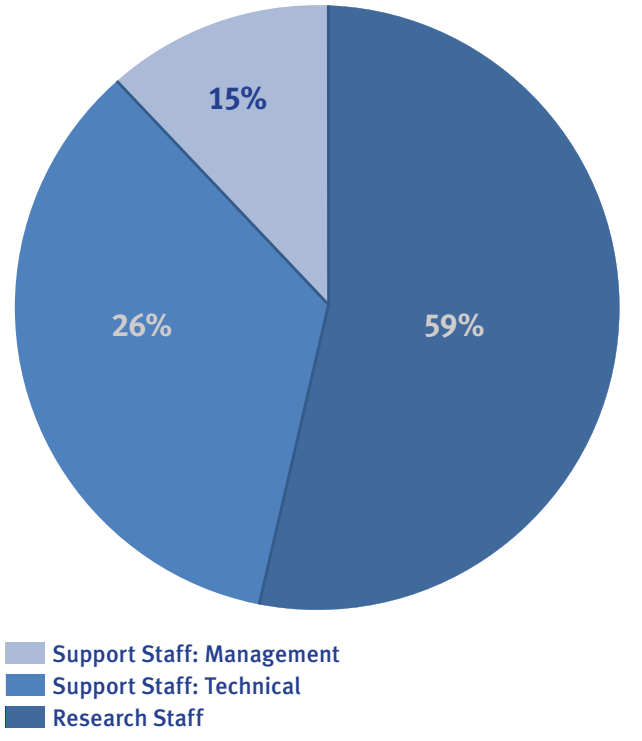


Table 2 - Research & Support Personnel by academic studies

	Nr. of persons
Research Staff	188
Genetics	1
Agronomist	1
Bioinformaticians	2
Biologists	78
Biotechnologists	18
Statisticians	3
Pharmacists	32
Physicists	1
Computer Science	5
Mathematicians	1
Medicine	3
Chemists	25
Veterinarians	3
Biochemists & Biomedicine	15
Support Staff	77
Technical Research	57
Technical Resources	11
Higher laboratory Technicians	37
Project manager	2
Pathology technical Specialist	5
Veterinary Technician Assistant	3
Ade/Economist/Finance	4
Lawer/Attorneys/Lab. Rel.	3
Computer Science	2
Medicine and Pharmacology	1
Telecomumnications	1
Others	8
Students	97
TOTAL	**362**

Table 3 - Research Personnel (staff and collaborator) with PhD

	Nr. of persons
PhD Research Personnel	91
Non PhD Research Personnel	97
TOTAL	**188**

Charter 2 - Research personnel with PhD

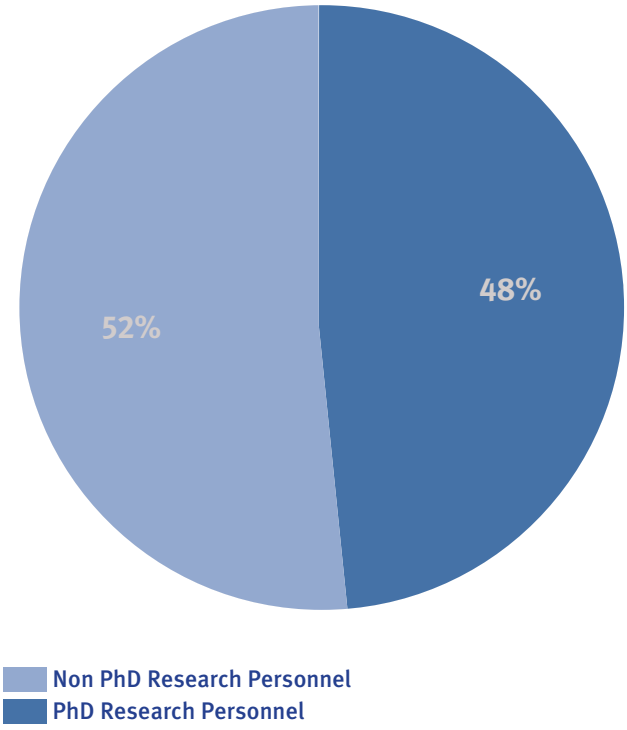
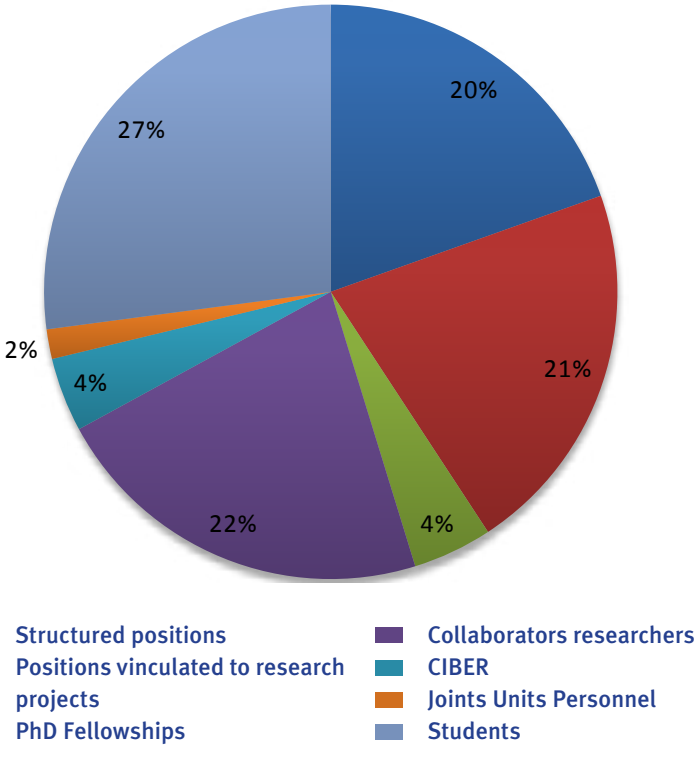


Table 4 - Clasification by labor relationship

	Nr. of persons
Staff	166
Structured positions	70
Positions vinculated to research projects	80
PhD Fellowships	16
FPI	7
FPU	3
Becas Pre del ISCIII	1
Val i+d C.Educación	3
GVA (Prometeo)	2
External Research Staff	196
Collaborators researchers	78
CIBER	15
Joints Units Personnel	6
Students	97

Charter 3 - Personnel by labor relationship



Funding

Table 1 - Income in 2013

Source	Amount (in K€)
European and International Funding	346
National competitive funding	1.220
Instituto de Salud Carlos III	1.043
Education Cancillery/ Regional competive Funding	295
Contract and Privately-funded research	506
Donations	12
Health Cancillery	4.400
TOTAL	7.822

Charter 1 - Income in 2013

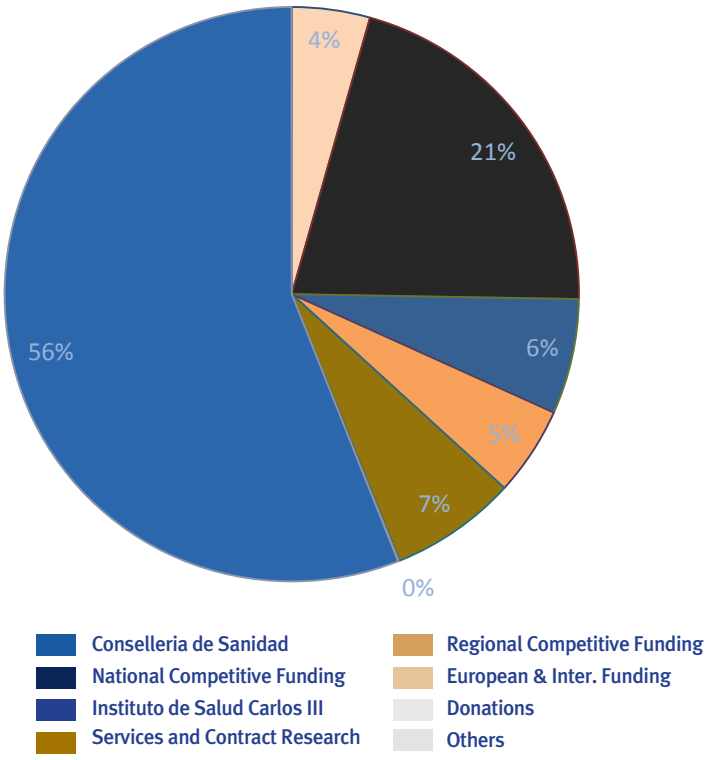


Table 2 - Funding Agencies in 2013: New grants and Contracts.

Fundig Entities	New grants awarded in 2013
International	
EC-Directorate General of research & Innovation	2
Privates	1
National	
Ministry of Economiy and Competitiveness	4
Carlos III institute of Health	3
Privates	6
Regional	
Generalitat Valenciana (GVA)	7

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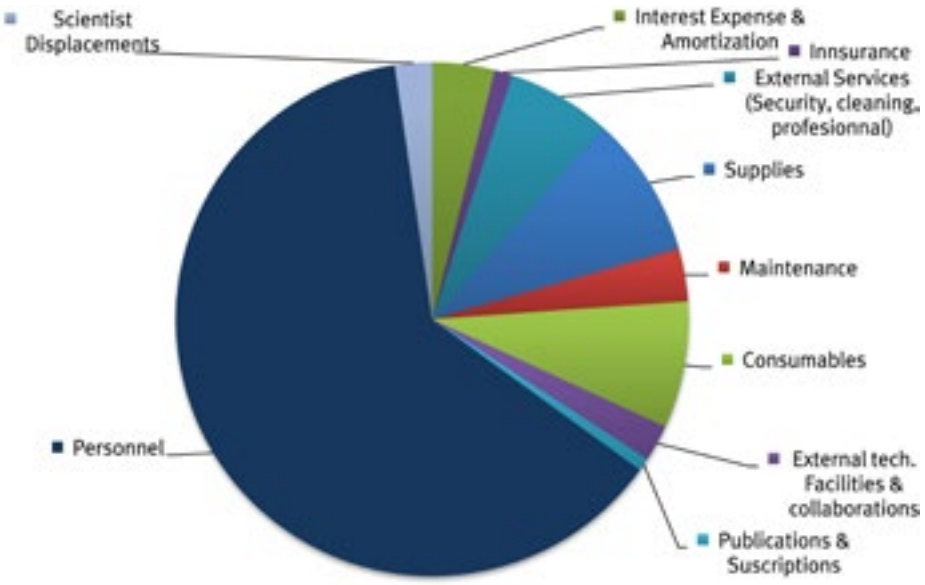
Table 3 - Active Grants and Contracts by Agency in 2013

Funding Entities	Grants in 2013
International	
EC-Directorate General of research & Innovation	8
Privates	1
National	
Ministry of Economiy and Competitiveness	36
Ministry of Education	5
Spanish Agency for Internatioanl Cooperation	2
Carlos III institute of Health	12
Privates	13
Fundacion La marato	2
Fundacion Gent per Gent	1
Contracts	10
Regional	
Generalitat Valenciana (GVA)	19
Privates	4
Total	**100**

Table 4 - Execution of Funds 2013

Expenses	%
Personnel	61,9%
Supplies	8,9%
Consumables	7,9%
External Services (Security, cleaning)	6,5%
Interest Expense	3,9%
Maintenance	3,3%
Others : External tech. facilities	2,3%
Scientist Displacements	2,3%
Amortization	1,3%
Innsurance	1,1%
Publications & Suscriptions	0,8%
Collaboration related with grants	0,4%
Profesionnal counseling Services	0,4%
Total	100%

Charter 2 - Execution of Funds

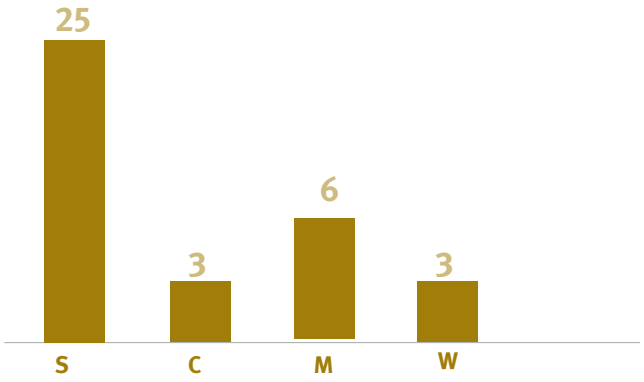


Events

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

	Nr.
Seminars	25
Congresses	3
Meetings	6
Workshops	3
TOTAL	**37**

Charter 1 - Events



S: Seminars
C: Congresses
M: Meetings
W: Workshops

Thesis

Title	PhD Student	Director	Institution
Polymer Conjugates for the Treatment of Neurodegenerative Disorders	Conejos Sánchez, Inmaculada	Dr. Maria Jesus Vicent Docon	Universidad de Valencia
Polymer-Based Combination Conjugates for the Treatment of hormone-dependent Breast Cancer	Deladriere, Coralie	Dr.Maria Jesus Vicent Docon, Dr. Ruth Lucas Dominguez	Universidad de Valencia
Las Células Troncales de pulpa dentaria como alternativa Terapeútica en eltratamiento del Infarto Agudo de Miocardio	Gandía Ventura, Carolina	Dr.Luke Noon	Universidad de Valencia
Papel del Sus 1 y Proteinas relacionadas en el proceso de Expresión Génica	Garcia Oliver, Encarnación	Dr.Susana Rodriguez Navarro	Universidad de Valencia
Regulation of lysosomal degradation by calcium and calcium-binding proteins	Ghita Ghislat	Dr.Erwin Knecht Roberto	Universidad Politecnica de Valencia
Ph-Responsive Polyacetals for the Treatment of Hormone-Dependent Cancer	Giménez Navarro, Vanesa	Dr. Maria Jesus Vicent Docon	Universidad de Valencia
Papel de los Neuroesteroides en las alteraciones en la Activación de los receptores NMDA, GABAA y Sigma y en las alteraciones cognitivas y motoras en Encefalopatía Hepática. Mecanismos Moleculares e implicaciones terapéuticas.	Gonzalez-Usano, Alba	Dr.Vicente Felipo Orts, Dr. Esteban Morcillo, Dr. Omar Cauli	Universidad de Valencia
Methodological Advances in the Functional Profling of Genomic Studies	Montaner Gonzalez, David	Dr.Juaquín Dopazo Blázquez	Universidad de Valencia
Role of IRS2 in Obesity and Adipogenesis	Moreno Viedma, Veronica	Dr.Deborah Burks	Universidad de Valencia
Regulación de la degradación intracelular de Proteínas por Glucosa.	Moruno Manchón, Jose Felix	Dr. Erwin Knecht Roberto, Dr. EvaPérez-Jiménez	Universidad Politecnica de Valencia
Dendritic Derivatives as building blocks for Biomedical Applications	Movellan, Julie	Dr. Maria Jesus Vicent Docon	Universidad de Zaragoza
Regulation des differences voies de la degradation intracellulaire des proteines produites par des variations dans le niveau d´expression du suppresseur tumoral, PTEN	Rajaa Errafyi	Dr. Erwin Knecht Roberto, Dr. Mohamed Loutfi	Universidad Hassan II-Casablanca

Thesis & Publications

Table 1 - Thesis

	Nr.
Thesis in progress	42
PhDs	12

Table 2 - Nr. of publications by type of journal

	Nr.
Journals	107
Books	2
Books chapters	1
TOTAL	**110**

Table 3 - Nr. of publications included in JCR

	Nr.
Included in JCR	104
Not included in JCR	3
TOTAL	**107**

Charter 1 - Publications included in JCR

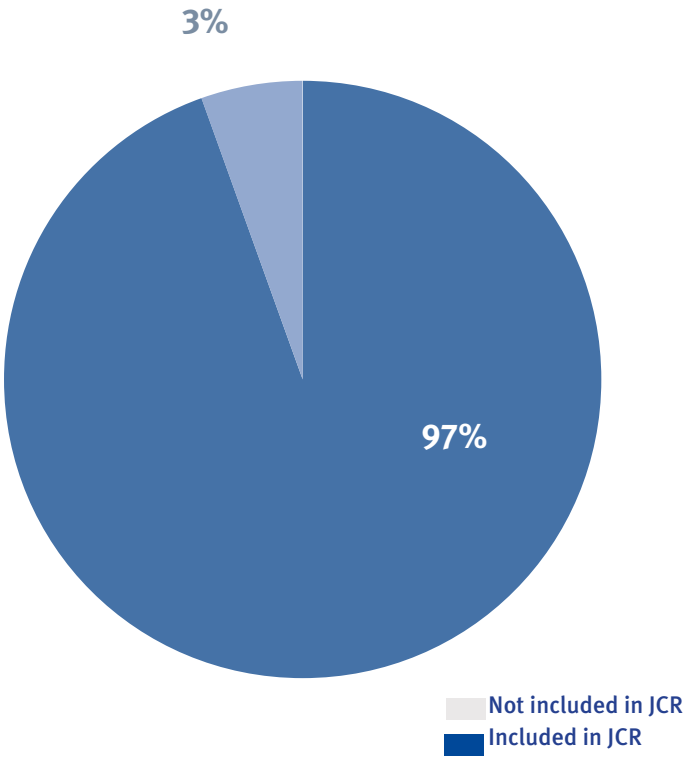


Table 4 - Publications by Laboratory

Laboratory	Nr.
Organic Molecules	15
Systems Biology	12
Neurobiology	9
Polymer Therapeutics	9
Molecular Endocrinology	9
Structural Biochemistry	8
Genetics and Molecular Medicine	8
Gene Expression Coupled to RNA Transport	6
Genetics and Genomic of Neuromuscular Diseases	6
Intracellular Protein Degradation & Rare Diseases	6
Cellular Pathology	5
Peptide & Protein Chemistry	5
Neuronal & Tissue Regeneration	4
Oncogenic Signalling	3
RNA Modification & Mitochondrial Diseases	2
Genomics of Gene Expression	2
Rho Signaling in Neuropathologies	1
Genetics and Physiopatoloy of Brain and Mental Disorders	0
Developmental Biology and Neuromuscular Diseases models	0

Charter 2 - Publications by Laboratory

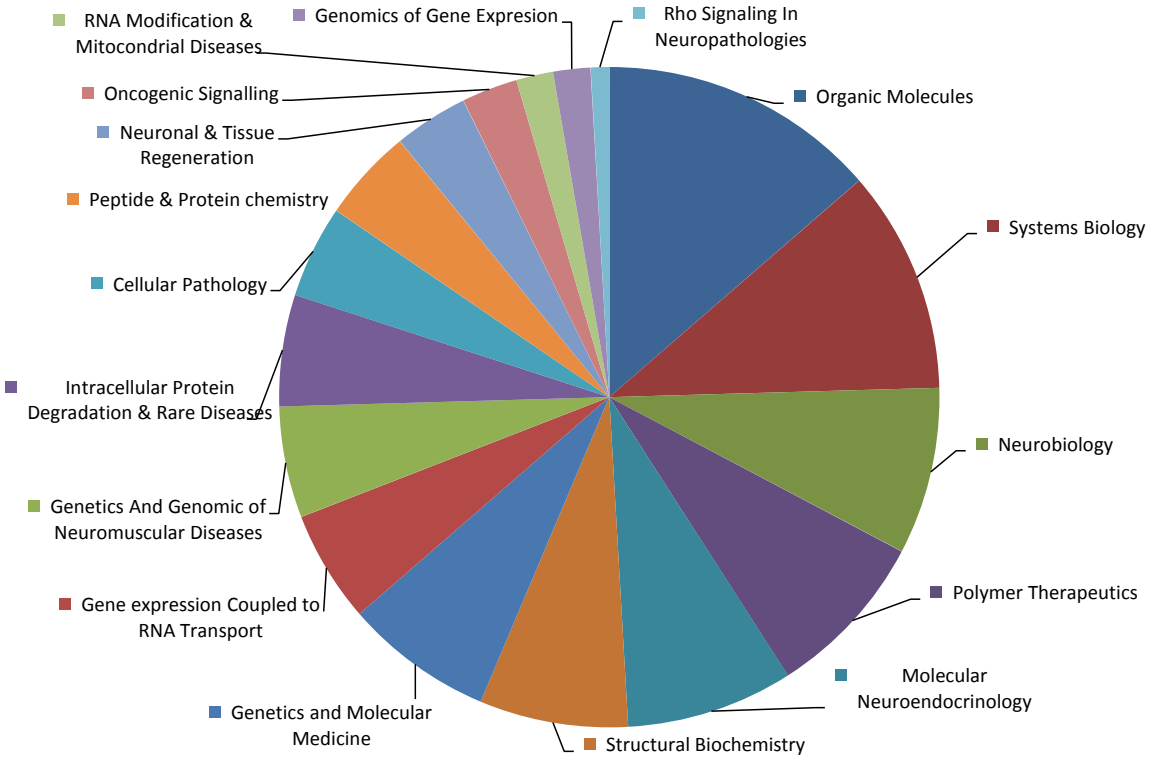


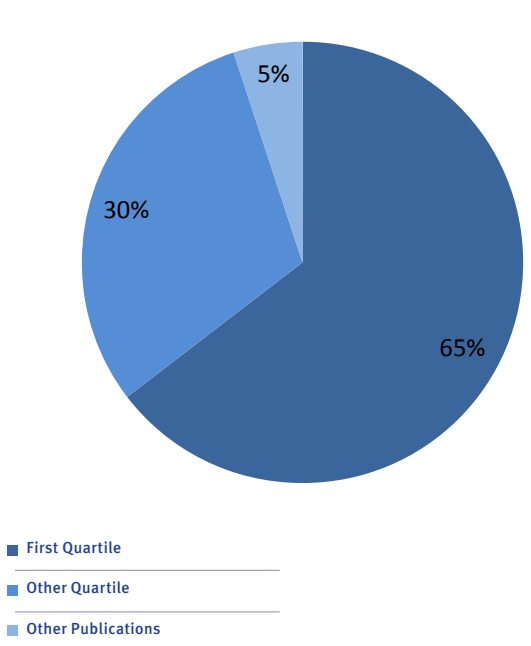
Table 5 - Publications by Quartile 2009-2013 *(Actual Researchers)*

	Nr.	%
First Quartile	370	65%
Other Quartile	174	30%
Other Publications	29	5%
Total	573	

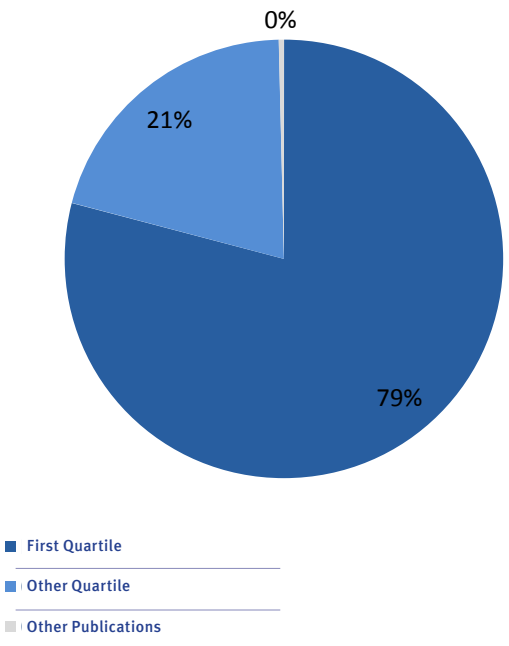
Table 6 - Cites by Quartil 2009-2013 *(Actual Researchers)*

	Cites	%
First Quartile	3.721	79%
Other Quartile	965	21%
Other Publications	18	0%
Total	4.704	

Charter 3 - Percentage of Publications by Quartile, `period 2009 - 2013



Charter 4 - Percentage of cites by Quartile , period 2009 - 2013



PRINCIPE FELIPE

CENTRO DE INVESTIGACION