During 2012, the CIPF has successfully overcome the organizational and financial challenges in order to keep scientific excellence while ensuring sustainability and optimization of resources. According to the CIPF Strategic Plan adopted in 2012, the research activity has been divided into 4 following strategic areas of work:

- **The Computational Medicine Programme**, led by Dr. Joaquín Dopazo, has conducted cutting-edge research in the field of integrative bioinformatics applied to translational and personalized medicine from different perspectives covering from genomics to medical imaging.

- **The Biomedicine Programme**, led by Dr. Vicente Felipo, has conducted basic and translational research on mechanisms, diagnosis and treatment of various human diseases.

- **The Biomedical Biotechnology Programme**, led by Dr. Antonio Pineda Lucena, contributed to biomedical research through knowledge of the molecular basis of diseases and the development of biochemical tools that enable the discovery of new diagnostic procedures, drug targets and principles active against them.

- **The Bioincubator**, led by Dr. Susana Rodríguez-Navarro, has promoted emerging groups and disciplines such as stem cell research applied to various pathologies, with capacity to add value and innovation.

In addition to scientific highlights, which are detailed in this report, CIPF has focused its efforts in internationalizing its research. In this regard, the Genomics of Gene Expression laboratory, headed by Dr. Ana Conesa, is coordinating the STATegra European project. STATegra aims to develop new statistical methods and tools for the integrative analysis of diverse omics data for a more efficient use of the genomics technologies, as well as to make them readily available to the research community through rapid and efficient implementation as user-friendly software packages.

Research on Regenerative Medicine has been funded by the Instituto de Salud Carlos III. During 2012 the CIPF has inaugurated its GMP facilities, which offer a high-end infrastructure for research and development in the field of Cell Therapy and Regenerative Medicine. The facilities consist of 4 classified manufacturing rooms, 1 cryogeny room, 1 labeling room, 1 sterility room and 1 quality control laboratory and 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, among others.

Translating research in clinical practice has been a priority for CIPF researchers that have collaborated with clinical partners in a significant number of projects. The CIPF has been involved in the FutureClinic project, funded by the Regional Ministry of Health, which aims to foster the adoption of Personalized Medicine based on Genomics. In order to support research in this field, the Chair on Computational Genomics has been created in collaboration with Bull, a world-class IT player.

Last but not least, the valorization of applied research results and scientific capabilities has been a top priority. During 2012, the CIPF has strengthened ties with the industry and involved both national and international companies in its research projects. In particular, collaboration and licensing agreements have allowed the creation of 3 spin-off companies -founded by researchers at the CIPF- that will exploit and commercialize research results, ranging from patents to applied software and know-how.
Organization
Organization chart

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SCIENTIFIC DIRECTOR
Vicente Felipo Orts (until March 2012)
Joaquín Dopazo Blázquez (since April 2012)

SCIENTIFIC DEPUTY DIRECTORS
Antonio Pineda Lucena
Susana Rodríguez-Navarro

COMPUTATIONAL MEDICINE PROGRAMME
Joaquín Dopazo Blázquez

BIOMEDICAL BIOTECHNOLOGY PROGRAMME
Antonio Pineda Lucena

BIOMEDICINE PROGRAMME
Vicente Felipo Orts

BIOINCUBATOR
Susana Rodríguez-Navarro

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Director de la Fundación Hospital Provincial de Castellón

PROF. JOSÉ VTE. CASTELL RIPOLL
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Vocal
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DR. JOSÉ MIR PALLARDO
Vocal
Jefe de la Unidad Hepática del Hospital La Fe

Dr. Francisco Murcia García
Vocal
Presidente de la Fundación Valenciana de Estudios Avanzados

DR. ANTONIO PELLICER MARTÍNEZ
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Director del Instituto Valenciano de Infertilidad

D. JUAN ANTONIO PÉREZ ESLAVA
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Gerente de la Fundación Bancaja

D. ÁNGEL VILLANUEVA PAREJA
Vocal
Vocal del Consejo de Administración de Bancaja
CIPF Research in 2012
Systems Biology

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Carlos Javier Santoyo López
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Martina Marbá Maya
Rubén Sánchez García

Collaborators
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David V. Conesa Guillem
Mª Amparo Ibáñez Comany
Flavia Martínez Carvalho
Overview

Medicine and Biology are increasingly becoming massive data sciences because of the application of the new omic technologies and the medical imaging. Innovative approaches in these areas must target the management and exploitation of genomic data from a Systems Biology perspective. Following this, the general objective we seek through the main lines of research is to relate variation at genomic level (punctual or structural variants, methylation changes, gene expression differences, etc.) to their effect at both cellular and phenotypic level trying to understand the underlying mechanisms that govern the network of molecular interactions in the cell.

Our Program carries our groundbreaking research by applying translational bioinformatics to personalized medicine integrating genomics and medical imaging. 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. 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. The program is structured in three laboratories, plus three core units:

**Computational Genomics Department**

<table>
<thead>
<tr>
<th>Genomics of Gene Expression Laboratory</th>
<th>Systems Genomics Laboratory</th>
<th>Bioimaging Laboratory</th>
</tr>
</thead>
<tbody>
<tr>
<td>Computational Biology Unit</td>
<td>IT Support Unit</td>
<td>Biostatistics Unit</td>
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</table>

These coordinated laboratories and units have the broad goal of developing and applying computational methods in an interdisciplinary and quantitative approach to biotechnological and biomedical projects. The heavy reliance on computers required by our research projects is facilitated by the IT support unit.

Finally, through the Functional Genomics Unit our Department is part of the National Institute of Bioinformatics (INB) and the CIBERER (Center in Network for the Study of Rare Diseases).

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 GEPAS (the most cited web tool in its category, now part of the Babelomics, see [http://www.babelomics.org](http://www.babelomics.org)) for the analysis and functional profiling of genomic experiments, with an average of more than 1000 experiments analyzed daily, have been developed and maintained for more than seven years by the group. These developments allowed us to participate in the FDA initiative MAQCII to define best practices to build predictors using expression data (Shi, 2010 Nat Biotech) and now in the continuation, the SEQC initiative ([http://www.fda.gov/downloads/ScienceResearch/BioinformaticsTools/MicroarrayQualityControlProject/ucm122981.pdf](http://www.fda.gov/downloads/ScienceResearch/BioinformaticsTools/MicroarrayQualityControlProject/ucm122981.pdf)).

We are highly concerned with the fact that the potential of discovery that the new generation sequencing technologies has on biosciences 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 the facilitating these tasks.

Thus, different developments to accelerate the steps of primary data processing have been carried out (Salavert et al., IEEE, ACM, 2012; García-Alcalde et al., Bioinformatics 2012) or the step of characterization of the variants found (the VARIANT program; see Medina et al., NAR, 2012; and the SNPPEffect database, see De Baerts et al., NAR 2012 or the SYSNPs tool Lorente-Galdos et al., Int J Data Min Bioinform, 2012). A large set of computational tools of interest for genomic data analysis has recently been published as the webservices package CELLBASE (Bleda et al., 2012 Nucleic Acids Res).

Using all the developments for genomic data analysis, we have carried out the first exhaustive study of the map of variability of human miRNAs (Carbonell et al., 2012 Genome Medicine). In addition to re-sequencing experiments we have developed and applied tools to the study of epigenomes in a epigenetic disease, the ICF syndrome (Heyn et al., Epigenetics, 2012).

Beyond the data processing and the primary analysis, the interpretation of genomic data constitutes a major challenge. The use of regulatory, functional and physical interaction networks is a promising approach to understand the effects of mutations or expression deregulations on the phenotype. The Department has extensive experience in analyzing transcriptional networks as demonstrated by the recent publication of the algorithm RENATO to find the regulators (transcription factors or miRNAs) which are compatible with the genes activated or deactivated in a transcriptomics (or similar) experiment (See Bleda et al., NAR 2012). FIGURE 1 shows the transcription factors significantly associated to the expression in Fanconi Anemia patients.

We have also been intensely working on the potential of protein-protein interaction networks to detect gene associations to diseases (García-Alonso et al, NAR 2012). The use of networks allows detecting collective or synergistic associations of genes to diseases that otherwise cannot be detected by conventional gene-by-gene analysis, such as GWAS association tests.

FIGURE 1 - Transcription factors significantly associated to the expression in Fanconi Anemia patients.

FIGURE 2 shows a network of proteins significantly associated to bipolar disorder obtained using the same Wellcome Trust Case Control GWAS data by means of which only a few genes could be found as associated to the disease. This approach shows the increase of statistical power obtained just by changing the entity to be tested (from individual, independent proteins to subnetworks of proteins related among them).

Related projects in which the group has been involved grants BIO2011-27069, PROMETEO/2010/001 deal with functional aspects of diseases that harbor copy number alterations (using cancer as case study), and (BIO2011-27069) aim relate molecular defects or deregulations to the mechanism of disease using a systems biology perspective. The objectives of the projects were successfully completed, and all together resulted during 2012 in 22 papers published in international journals.

We have also addressed a number of collaborative works with other laboratories in the topic of genomic analysis in different fields such as stem cells (Carrero et al., Stem Cells Rev, 2012), cancer (Conesa-Zamora et al., International journal of cancer, 2012), rare
diseases (Fernandez et al., Orphanet J Rare Dis. 2012), development (Sundaran et al., BMC Evol. Biol. 2012), translation (Ventoso et al., PLoS ONE 2012), molecular mechanisms (Koziol et al., FASEB J. 2012), agrosciences (Rizza et al., Molecular plant pathology. 2012; Fernandez et al., PLoS ONE 2012; Jaime et al., BMC Genomics. 2012; Oppert et al., PLoS ONE 2012), and jointly we are developing large scale projects as FutureClinic, regarding the introduction of genomics data in the clinic personalized medicine (http://www.futureclinic.es/), or CITRUSEQ, a project for sequencing, genotyping and development of tools for genetic improvement of citric varieties (http://www.citruseq.es/).

Additionally, the head of the Department, Dr. Dopazo, is one of the promoters of the Medical Genome Project (http://www.medicalgenomoproject.com/), which aims to characterize rare diseases by genome sequencing. 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 - Network of proteins significantly associated to bipolar disorder.


CIPF Research in 2012

Courses

- Megasequencing Data Analysis: from reads to candidate genes, March 11-12, 2012, Centro de Investigación Príncipe Felipe, Valencia, Spain,
- SeqAhead Workshop on High Performance Computing for Next Generation Sequencing Analysis (HPCANGS), May 21-22, 2012, Centro de Investigacion Príncipe Felipe, Valencia, Spain,
- Megasequencing Data Analysis: from reads to candidate genes, July 11-12, 2012, Centro de Investigación Príncipe Felipe, Valencia, Spain
- Babelomics (Master medicina traslacional Cellae), March 12, 2012, Hospital Clínico de Barcelona

Conferences and meetings

- Nuevas estrategias, identificación y caracterización de genes responsables de enfermedades. 57 Curso de Genética Humana de la Sociedad Española de Genética. February 16, 2012, Madrid
- Poniendo las bases para la aplicación de la secuenciación genómica a la clínica. DNA day. April 25, 2012, Hospital La Paz, Madrid
- Visión de futuro, más allá de los objetivos discretos del Proyecto Future Clinic. III Jornada de Tecnologías para la Salud. Centro de Investigación Príncipe Felipe, June 5, 2012, Valencia
- Genome Sequencing and the new generation biomarkers. International Symposium on Neurehabilitation, October 18-19, 2012, CIPF, Valencia
- Buscando genes de enfermedad con técnicas genómicas. Seminarios Hospital Universitario Marqués de Valdecillas. December 20, 2012, Santander

Cellular Pathology

Group Leader
CONSUELO GUERRI CIRERA

- Researchers
  - Rosa Guasch Aguilar
  - María Pascual Mora
  - Maya Pascual Lucas
  - (until September 2012)
  - Silvia Alfonso Loeches
  - Sara Fernández Lizarbe
  - (until December 2012)

- Graduate Students
  - Antoni Pia Rodríguez
  - Juan Ramón Ureñ Peralta

- Technicians
  - María Gómez López
  - (until December 2012)
  - Jorge Montesinos Selfa
  - Mª José Morillo Bargues
  - Mª Amparo Pérez Aragón

- Collaborators
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  - Carmen Díez Fernández
  - José Miñarro López
  - Concepción Roger Sánchez
  - Vicente Rubio Zamora
  - Clara Mar Guarch Pérez
  - Fulgencio Ruso Julve
Overview

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

(DR. C. GUERRI)

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. We use neural cells in primary culture and animal models which mimic the alterations observed in alcohol-related pathologies, we attempt to: i) study the differential molecular mechanisms of the neurotoxicity of ethanol in the developing and in the adult brain, ii) elucidate the bases of the vulnerability of the developing neural cells to the action of ethanol, iii) assess the activation of the innate immune system in the brain damage and behaviour, iv) test the hypotheses that chronic ethanol treatment down-regulates proteins involved in myelination, including the proteolipid protein (PLP), myelin basic protein (MBP), myelin-oligo-dendrocyte, glycoprotein, 2,3-cyclic-nucleotide-3-phosphoesterase, and myelin-associated glycoprotein, in several brain regions of ethanol-treated WT mice. The immunohistochemistry analysis also revealed that ethanol-treatment altered myelin morphology reduced the number of MBP-positive fibres and caused oligodendrocyte death, as demonstrated by an increase in caspase-3-positive oligodendrocytes. The in vivo imaging system further confirmed that chronic ethanol intake markedly reduced the PLP in WT mice. Most myelin alterations were not observed in brains from ethanol-treated TLR4-KO mice. Electron microscopy studies revealed that although 41–47% of axons showed myelin sheath disarrangements in the cerebral cortex and corpus callosum of WT ethanol-treated mice, respectively, small focal fiber disruptions were noticed in these brain areas of ethanol-treated TLR4-KO mice (FIGURE 1). The results of this study may help improve our knowledge on the relationship between TLR4 and demyelination and could provide new insights into the treatment of cognitive dysfunctions caused by alcoholism.

Changes in histone acetylation in the prefrontal cortex of ethanol-exposed adolescent rats are associated with ethanol-induced platelet conditioning

Heavy binge drinking during adolescence have emerged after new evidences demonstrating that adolescence is a vulnerable brain maturation stage to alcohol toxicity, alcohol and substance use problems and psychiatric disorders. Abnormal plasticity in reward-related processes might contribute to the vulnerability of adolescents to drug addiction. Considering that histone acetylation regulates transcriptional activity and contributes to drug-induced alterations in gene expression and behavior, we addressed the hypothesis that ethanol is capable of inducing transcriptional changes by histone modifications in specific gene promoters in adolescent brain reward regions, and whether these events are associated with acquisition of place conditioning. We provide evidence that intermittent ethanol administration during adolescence upregulates histone acetyl transferase (HAT) activity in adolescent prefrontal cortex and increases histone (H3 or H4) acetylation and H3 (K4) di-methylation in the promoter region of cfos, cdk5 and fosB. Inhibition of histone deacetylase by sodium butyrate before ethanol injection enhances both up-regulation of HAT activity and histone acetylation of cfos, cdk5 and fosB. Co-administration of sodium butyrate with ethanol prolongs the extinction of place conditioning and increased the reinstatement effects of ethanol in ethanol-treated adolescents, but not in ethanol-treated adult rats. These results indicate that ethanol exposure during adolescence induces chromatin remodeling, changes in histone acetylation and methylation, and modify the effects of ethanol on place conditioning. The results suggest that epigenetic mechanisms might open up avenues to new treatments for binge drinking-induced drug addiction during adolescence.

Neuronal polarization is impaired in mice lacking RhoE expression

Rnd proteins are atypical Rho members, highly expressed in the brain, which alter several aspects of neuronal function. We have recently demonstrated that RhoE (RND3) is widely expressed in the central nervous system, especially in the early postnatal period and that mice lacking RhoE expression have neuromotor impairment and neuromuscular alterations, indicating an abnormal development of the nervous system. To get more insight into the specific role played by RhoE in neuronal development we have used cultured hippocampal neurons from mice lacking RhoE expression. We have shown that RhoE-deficient neurons exhibit a decrease in both neurite and axon outgrowth and also a delay in the process of neuronal polarization (FIGURE 2). In addition, we have found that the RhoA/Rock/Cofilin signaling pathway participates in the neuronal alterations induced by the lack of RhoE expression. We are also investigating the impact of this protein on the proliferation and differentiation of neural stem cells. Finally, we are collaborating with the CEU-Cardeal Herrera and Valencia University to study the function of RhoE in the neural progenitors migration from the subventricular zone of mice that lack RhoE expression.

Research results

Activation of the innate immune receptors, Toll-Like Receptor 4, participates in the Myelin Disruptions Associated with Chronic Alcohol Abuse

Alcohol abuse can cause brain damage, loss of white matter, myelination disruption, and even neuronal injury. Although the molecular mechanisms underlying ethanol induced brain damage and demyelination remain unknown, we have recently demonstrated that alcohol by activating TLR4 receptors in glial cells triggers neuroinflammation, brain damage and neurodegeneration. Using brains from wild-type (WT) and TLR4 knockout (KO, TLR4-/-) mice, this year we were able to demonstrate that chronic ethanol treatment down-regulates proteins involved in myelination, including the proteolipid protein (PLP), myelin basic protein (MBP), myelin-oligo-dendrocyte, glycoprotein, 2,3-cyclic-nucleotide-3-phosphoesterase, and myelin-associated glycoprotein, in several brain regions of ethanol-treated WT mice. The immunohistochemistry analysis also revealed that ethanol-treatment altered myelin morphology reduced the number of MBP-positive fibres and caused oligodendrocyte death, as demonstrated by an increase in caspase-3-positive oligodendrocytes. The in vivo imaging system further confirmed that chronic ethanol intake markedly reduced the PLP in WT mice. Most myelin alterations were not observed in brains from ethanol-treated TLR4-KO mice. Electron microscopy studies revealed that although 41–47% of axons showed myelin sheath disarrangements in the cerebral cortex and corpus callosum of WT ethanol-treated mice, respectively, small focal fiber disruptions were noticed in these brain areas of ethanol-treated TLR4-KO mice (FIGURE 1). The results of this study may help improve our knowledge on the relationship between TLR4 and demyelination and could provide new insights into the treatment of cognitive dysfunctions caused by alcoholism.

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Cytomics

Group Leader
ENRIQUE O’CONNOR BLASCO

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Laura Díez Vico

Collaborators
Teresa Palau Canós
Beatriz Pérez Benavente

Publications

- Garcia-Algar O, Black D, Guerri C, Pichini S. The effect of different alcohol drinking patterns in early to mid-pregnancy. BJOG, an international journal of obstetrics and gynaecology, Dec, 2012
- Guerri C. Adicción al Alcohol Revista de la SEBBM, Jun, 2012
- Guerri C. Bioquimica de las adicciones Revista de la SEBBM, Jun, 2012

Conferences and meetings

- Alcohol y adolescencia. Proyecto Les Valls Ciencia, CSIC, La Caixa (Charlas de Divulgación). IES La Vall de Segó, November 14, 2012

FIGURE 2 - Neurons lacking RhoE expression display a delay in neuronal polarization after 3,6,9 and 14 days in culture.
Overview

The laboratory is specialized in the development of advanced applications of Cytomics, including flow- and imaging cytometry. The three most relevant research lines are:

Prediction in vitro of acute and chronic toxicity of chemical compounds to humans.

Objective: To create and apply short-term assays based on cytomic technologies and cellular models in order to predict acute and chronic toxicity in vivo and to characterize toxicity pathways.

Translational approach: Some of our cytomicasays are performed in human blood cells ex vivo and predict hematotoxicity, immunotoxicity and hematotoxicity by drugs and xenobiotics.

Impact on Human Health Needs: We aim to characterize mechanisms of actions of drugs or drug candidates and to assess chemical risk in humans.

Toll-like Receptor (TLR) signaling in hematopoietic stem cell differentiation and homeostasis.

Objective: To investigate the role of TLR signaling pathways, an essential part of immune response against pathogens, in the regulation of hematopoietic stem cell differentiation and in the mechanisms controlling antifungal immune response.

Translational approach: Functional studies of TLR in murine models of stem cell differentiation in vitro and in vivo will be translated into in vitro models of human hematopoiesis and infection.

Impact on Human Health Needs: Our results might help to characterize the mechanisms of antifungal immune response, and to provide new therapeutic approaches in human infections, as well as to find new therapeutic approaches based on stem cell reprogramming in certain types of immunodeficiencies or leukemias.

Research results

Prediction in vitro of acute and chronic toxicity of chemical compounds to humans

On the basis of our strategy of designing multiparametric miniaturized cell-based assays predicting human toxicity, we have developed and validated a multiparametric cell-based protocol to screen and classify the hepatotoxicity potential of drugs. This protocol consisted of a high-content assay by fluorescence bioimaging of relevant markers of cell lesion (Collaboration with the Laboratory of Experimental Hepatology, University Hospital La Fe, Valencia). As a result of our participation in the recently concluded European Project ACuteTox, other series of in vitro toxicity multiparametric assays developed by our laboratory have been included by external biostatistical evaluation among the selected in vitro and in silico methods to predict acute oral toxicity in the regulatory context. These assays were integrated as two complementary panels of flow cytometry individual tests for general toxicity screening and oxidative stress screening, and resulted to be more predictive than other standard tests of cytotoxicity. Application of the recent technology of multispectral image in flow cytometry has allowed us to demonstrate for the first time that antiretroviral drugs, like atazanavir, used in modern treatment of HIV patients induce autophagy and mitophagy accompanied by oxidative stress in pre-adipocytes (Collaboration with the Department of Microbiology of the University of Valencia). They open a new way to consider immune response against pathogenic fungi, as TLR2-mediated defence against the fungus, and suggest that TLR mediates signaling may lead to reprogramming early progenitors to rapidly replenishing the innate immune system and generate the most necessary mature cells to deal with the pathogen.

Toll-like Receptor (TLR) signaling in hematopoietic stem cell differentiation and homeostasis

TLR-like receptors (TLRs) are expressed by hematopoietic stem and progenitor cells (HSPCs), and may play a role in hematopoiesis in response to pathogens during infection. We have purified lineage negative cells (Lin-), from bone marrow of C57BL/6 mice (CD45.2 alloantigen), and transplanted into B6Ly5.1 mice (CD45.1 alloantigen), which were then injected with viable or inactivated C. albicans yeasts. Three days after transplantation, bone marrow and spleen cells were enriched for CD45.2 cells and analysed by multicolour fluorescence and flow cytometry to detect donor-derived cells. Implanted cells were detected in the spleen and in the bone marrow of recipient mice, and they differentiate preferentially to macrophages, both in response to infection or to inactivated yeasts. The generation of macrophages was dependent on TLR2 but independent of TLR4, as mice generated macrophages similarly to control cells. These results were obtained during collaboration with the Department of Biomedical Sciences, University of Modena-Reggio Emilia, Modena, Italy).

These results show the value of our methodological approach to predict drug-induced toxicity and reveal a novel mechanism of antiretroviral-drug toxicity on adipose tissue which is likely involved in lipodystrophy associated to long-term treatment of HIV patients. This line of research will be pursued in the next future to implement high-throughput flow cytometry assays allowing screening large number of chemical compounds and, more specifically, to deepen in the mechanism and prevention of lipodystrophy associated to antiretroviral drugs.

Publications


The value of selected in vitro and in silico methods to predict acute oral toxicity in a regulatory context: Results from the European Project ACuteTox. Toxicol in Vitro 2012 Aug 16

Conferences and meetings


Courses

- Aplicaciones Clínicas de la Citometría de Flujo-6º Edición, Universidad de Valencia, November 2012

Gene Expression Coupled to RNA Transport

Group Leader
SUSANA RODRÍGUEZ-NAVARRO

- Researchers
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  MP Micaela Molina Navarro (until January 2012)
- Graduate Students
  Varcina García Molinero
  Encarnación García Oliver
- Technicians
  Eloisa Barber Caro
  Ana Llopis Moreno
  Ali Nabeel Abuqatam
- Collaborators
  Ali Nabeel Abuqatam
  Carla Fuster García
Overview

My laboratory is interested in understanding the functional crosstalk of distinct steps of the gene expression pathway. Gene expression is the most crucial cellular process as it is the fundamental mechanism for translating the static DNA information into life. It is a coordinated multistep process in which distinct protein complexes exert pivotal functions at different levels. Among them we are investigating the role of the transcriptional co-activator SAGA complex and the nuclear pore associated machinery TREX-2. SAGA participates in the regulation of stress-induced genes of distinct types of signaling pathways and TREX-2 is involved in mRNA metabolism, export and genome stability. Most of our efforts are directed to understand the role of Sus1, the shared factor between SAGA and TREX-2. Sus1 is a conserved factor through the eukaryotic kingdom. The research done to address the role of Sus1/ENY2 has provided in deep description of different mechanisms influencing gene expression. Although the role of Sus1 in human cells is largely unknown, preliminary results suggest interesting links to pathological states that range from cancer, rare diseases and diabetes.

Since SAGA and TREX-2 have key roles in fundamental physiological processes, it is unsurprising that they are involved in the etiology of human diseases. Our work will help us to understand how they impact/affict human health.

Research results

Describing new factors functioning at the interface between SAGA and TREX-2

Sus1 is part of two stable complexes, SAGA and TREX-2 and is crucial at different levels (FIGURE 1). However, it can transiently interact with other factors. In searching for unveiled Sus1, functional connections help understand how transcription is coupled to mRNA export; we re-investigated interacting partners of Sus1 via Sus1-TAP purification as part of our current project. The enriched calmodulin eluate from Sus1-TAP purification was analyzed by multidimensional protein identification technology (MudPIT) to determine the complete polypeptide mixture present in our affinity purification. This kind of analysis has been extensively used to identify new interactors of a known protein and to study the proteomic characterization of a pathway in more detail. Our MudPIT analysis revealed that apart from the histones and components of SAGA and TREX-2 complexes, peptides for several factors not previously found were identified. Among them we found Sem1, which is part of the proteasome. We have found very interesting links between Sem1 and TREX-2/SAGA that are under current research.

Molecular mechanisms of Sus1 regulation and its alternative splicing

In collaboration with Dr. José Gallego (Universidad Católica de Valencia) The structures formed by pre-mRNA molecules modulate splicing through a variety of mechanisms that need further characterization. Sus1 (ENY2 in mammals) is an evolutionary conserved protein involved in chromatin remodelling and mRNA biogenesis. Unlike most yeast genes, the Sus1 pre-mRNA of Saccharomyces cerevisiae contains two introns and is alternatively spliced2, retaining one or both introns in response to changes in environmental conditions. SUS1 splicing may allow the cell to control the expression of its mRNA export machinery, but the mechanisms that regulate this process remain unknown.

An in silico analysis of SUS1 pre-mRNA predicted the formation of two presumably stable RNA structures, one contained in the exon (E2) separating the two introns, and the other one in the downstream intron (I2)

A cellular assay based on a Cup1 reporter system revealed that SUS1 mutants containing altered I2 and E2 structures had significantly impaired splicing activity in vivo. Semi-quantitative RT-PCR experiments indicate that these mutants accumulate unspliced pre-mRNA. Nuclear magnetic resonance (NMR) spectroscopy and UV thermal denaturation experiments have confirmed that I2 forms a sub-stable 37-nucleotide A/U-rich stem-loop structure containing the branch site near its apical loop and the 3’ splice site at the 3’ terminus of the stem. E2 may form a more complex structure involving a 79-nucleotide four-helix junction and, up to now, NMR and UV melting experiments have confirmed that one of the E2 stem-loops forms an unusual and stable structure. RNA modification experiments (SHAPE) are currently underway to characterize the secondary structure of the entire E2 sequence. Investigating the role of pre-mRNA structure in SUS1 splicing may allow us to identify new functional RNA motifs and contribute to elucidate the mechanisms that regulate the splicing of an essential eukaryotic protein.

Metabolomics as a tool to study yeast mutant phenotype

In this study an optimized method for the extraction and analysis of S. cerevisiae metabolites has been established allowing the identification and comparison of a wide range of yeast metabolites. In addition, the analysis of intact S. cerevisiae cells by HRMAS as a complementary method has been developed. The optimized metabolomic protocols were applied to discriminate between the wildtype and several yeast mutant strains. Interestingly, the analysis revealed the importance of AIN4 for growth under fermentative conditions. Metabolic differences between wildtype and mutant cells suggest an alteration of lipid metabolism related to cell wall structure and synthesis. Moreover, metabolomics profiling from ailm42 cells show a delayed stress response suggesting that AIN4 might participate in stress adaptation in yeast (FIGURE 2). We will use similar approaches to investigate the phenotypes of yeast mutants that serve as a model for Rare diseases for instance Spinocerebellar Ataxia 7 (SCA7).

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Genomics of Gene Expression

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Publications


Conferences and meetings


Overview

The Genomics of Gene Expression Lab is interested in understanding the functional aspects of gene expression at the genome-wide level and its relationship with diseases and traits. For that we develop statistical methods and software tools that analyze the dynamics aspects of transcriptome data, integrate these with other types of molecular data and annotate them functionally, 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 (Chlamydia and Pseudomonas) and fungi (Aspergillus and Fusarium).
- Genomics and epigenomics in Lupus Eritomatoso Sistémico.
- Epigenomic and genomic markers in cancer 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), masSigPro and SEA (time series analysis), miniAS and ASCA-genes (gene expression analysis), SEA 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 data-sets, and DEANN, a Marie Curie IRESES for developing a Europe-South America NGS analysis network.

Research results

Molecular Biomarkers

Epigenomic markers in neuroblastoma. In collaboration with Dr. Castell from La Fe Hospital, we have developed a molecular maker of neuroblastoma progression based on gene promoter methylation data. This marker improves diagnosis of Risk 3 and Risk 4 neuroblastoma patients, where disease outcome is most variable, and predicts whether the disease will have unfavorable outcome. Manuscript in preparation.

We have identified new biomarkers of colorectal serrated adenocarcinoma by gene expression profiling and immunohistochemistry. Both fascin1 and hippoipalin were detected as significantly associated to the serrate state of colon carcinomas and can be used to predict the progression of primary adenomas. Results published in Int J Cancer. 2013 Jan 15;132(2):297-307. doi: 10.1002/ijc.27674.

Within the context of analyzing a molecular signature for tolipotency 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 8(4): e62135. doi:10.1371/journal.pone.0062135

Pathogenesis

Within the Pathomics ERA net project we have characterized the operon structure of pathogenic bacteria (Pseudomonas aeruginosa) and analyzed gene and operon expression under different nutritional conditions that tigm the expression of different bacterial membrane transport systems (S53 and S56) associated to pathogenicity. We obtained the operon composition from different clinic isolates and identified operon structure differences associated to different growth conditions. These results show that pathogenic bacteria might change their gene expression structure, not just the level of expression, in response to environmental conditions. This is a collaboration with Dr. Romé Voulhoux from Marseille University. Manuscript in preparation.

Within the Transplant ERA net project we have worked in the characterization of transcriptional networks in pathogenic fungi. We have characterized the gene expression signature of Aspergillus fumigatus, a facultative fungal pathogen, upon blood colonization and identified the iron assimilation pathway as a key regulatory process during infection. We have also characterized fungal invasion strategy differences in pathogenic Fusarium strains and the transcription factors that involved in these. This work has been in collaboration with Dr. Antonio di Pietro from University of Granada, Dr. Sven Krappman from Würzburg Research Center for Infectious Diseases and Dr. G. Baush from Gottingen University.

Bioinformatics

Within the SEQC project we have analyzed the quality of transcriptomics by sequencing (RNA-seq) and determined the level of reproducibility in transcript detection, as a function of the gene expression level, and the identification of spliced junctions by these technologies. Our results establish the range of accuracy by this technology and indicate the need of proper replication in experimental design to obtain confident measurements. Our results reveal the limitations of RNA-seq in the low expression range, where most to the novel transcript discoveries are taking place. Manuscript submitted to Nature Biotechnology.

We have also collaborated with Dr. Corey Nislow, from Toronto University in the characterization of the evolutionary mechanisms of adaptation to xenobiotic compounds by yeast. Three different chemogenomic fitness assays, haploinsufficiency (HIP), homoygous deletion (HOP), and multicopy suppression (MSP) profiling were combined with a transcriptomic analysis to gain insight in to the mode of action and mechanisms of resistance to chitosan oligosaccharides. Identified genes were involved in processes such as RNA biology (transcription, translation and regulatory mechanisms), membrane functions (e.g. signalling, transport and targeting), membrane structural components, cell division, and proteasome processes. Published in BMC Genomics. 2012 Jun 22;13:267. doi: 10.1186/1471-2164-13-267.


Others

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Publications

- Leida C, Conesa A, Llacer G, Badenes ML, Rios G. Histone modifications and expression of DAM6 gene in peach are modulated during bud dormancy release in a cultivar-dependent manner. The New phytologist 2012 Jan

Conferences and meetings

- Invited Seminar: Transcriptome analysis with RNAseq: promises and biases. Department of Genetics, Sanford University. Sanford, September 28, 2012
- Invited Conference: Next generation sequencing in transcriptomics: new views on the genome and much confusion. Langebio Institute, CINESTAV, Irapuato, Mexico, May 8, 2012
- Conference: Sequencing the Snake Venom Transcriptome for its Applications in Biomedicine. Plant and Animal Genome Conference XX. San Diego, California, January 15, 2012

Courses

- Automated Functional Annotation and Data Mining. Irapuato, Mexico. May 2012
- Automated Functional Annotation and Data Mining. Davis, California. July 2012
- Automated Functional Annotation and Data Mining. Valencia, Spain. October 2012
- BLAST2GO and Babelomics: suitable tools for data mining. Naples, Italy, November 2012

Intracellular Protein Degradation & Rare Diseases

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Overview

We are a Cell Biology laboratory mainly interested in two research areas: i) regulation of the major intracellular protein degradation mechanisms (autophagy and the ubiquitin-proteasome system (UPS)), and ii) relevance of their alterations in various rare diseases.

Autophagy and UPS, especially the former, can be induced or inhibited by environmental components, such as growth factors, nutrients and hormones, through a complex signaling network still poorly understood. Since both mechanisms play essential roles in cellular homeostasis and control many important cell processes, defects in their functions occur in several human pathologies. Therefore, a detailed knowledge of these signalling networks may allow indentifying new targets for their diagnosis and treatment.

Rare diseases are life-threatening or chronically debilitating diseases with low prevalence (with European standards, less than 1 in 2,000 people). They include about 5,000-7,000 different diseases and affect, approximately, 6-8% of the population. These diseases could also serve as models to understand more prevalent pathologies. We collaborate with other groups of the Spanish public consortium “Centre for Biomedical Network Research on Rare Diseases (CIBERER)” to analyse possible alterations in autophagy and/or the UPS and the consequences of restoring their normal function in those rare diseases in which undegraded proteins, polysaccharides or lipids accumulate within the cells.

Research results

Autophagy is a membrane trafficking pathway responsible for the breakdown of unwanted intracellular materials and crucial for the cell healthiness and survival. In the autophagic flux, various dynamic membrane rearrangements occur, starting with the elongation of the preautophagosomal structure, the phagophore, and its closure to build an autophagosome, and ending with its fusion with late endosomes and lysosomes to form an autolysosome. While investigating the regulation of the autophagic degradation of intracellular proteins, we carried out a shotgun proteome analysis of purified lysosomal membranes from mouse fibroblasts, to identify proteins whose levels change under conditions of high or low proteolysis. We found that three subunits of the vacuolar ATPase and three Ca2+-dependent phospholipid binding proteins (annexin A1, annexin A5 and copine 1) increased their levels on lysosomal membranes under high proteolysis. This suggested a role of these proteins in the regulation of starvation-induced autophagy. In the previous year, we have concentrated our work on one of these Ca2+-binding proteins, annexin A5, because it is an abundant annexin with a still unknown intracellular function, and analysed its possible role in lysosomal protein degradation. Based on different experimental approaches, including overexpression and silencing of the protein, our findings propose novel functions for annexin A5, promoting delivery of autophagosomes to lysosomes and inhibiting endocytosis. Therefore, annexin A5 (and possibly also annexin A1 and copine 1) emerges as a key positive and negative regulator of autophagy and of endocytosis, respectively, through Ca2+ and phospholipid signalling pathways that probably produce interactions with different lipid domains on lysosomal and late endosomal membranes.

Based on the implication of calcium in the binding of various proteins to phospholipids at the lysosomal membrane in starvation-induced autophagy found above, we also investigated the dependence of the increased autophagy produced by amino acid starvation on cellular calcium. This is a major second messenger regulating many physiological functions in the cells, such as secretion, contraction, metabolism, gene transcription, death, etc. In addition, calcium is also involved in some pathological processes, such as disorders of the nervous system, cardiac and vascular pathologies, diabetes and, possibly also, some rare diseases. In this work, we have demonstrated for the first time that amino acid deprivation provokes an increase of cytosolic calcium, which originates from both extracellular and, to a larger extent, from intracellular stores. In addition, we have proposed a signalling pathway by which withdrawal of amino acids could activate autophagy through calcineurin, calcium/calmodulin-dependent protein kinase kinase nase-beta (CaMKK-ß), 5’ AMP-activated protein kinase (AMPK), the serine/threonine protein kinase ULK1 and mammalian target of rapamycin complex 1 (mTORC1). Thus, intracellular calcium levels represent an important signal in the cell response to the availability of amino acids. As they have different metabolisms, it would be important to identify those that change intracellular calcium levels as well as the possible existence of an intracellular calcium-dependent sensor of amino acids. This work was carried out in collaboration with the group of Dr. Rosario Rizzuto of the University of Padua (Italy).

We work in collaboration with other groups of the CIBERER in several rare diseases, including neuronal ceroid lipofuscinoses, Danon disease, retinitis pigmentosa, X-adrenoleukodistrophy, MERRF, MELAS and Lafora disease. The main results published in 2012 concern the last one of these diseases. Lafora progressive myoclonic epilepsy, or Lafora disease (OMIM 254780) is an autosomal recessive neurodegenerative disorder resulting in severe epilepsy and death. It was first described in 1911 by the Spanish neurologist Gonzalo Rodríguez Lafora and a hallmark of this disorder is the presence in the cytoplasm of neurons of intracellular inclusions, called Lafora bodies. These inclusions are composed of insoluble, starch-like and poorly-branched glycogen molecules, called polyglucans. Lafora disease is caused in the vast majority of cases by recessive loss-of-function mutations in the genes encoding either laforin, a dual-specificity protein phosphatase with a carbohydrate-binding domain, or malin, an E3 ubiquitin-ligase. Previous studies suggested a role of these proteins, which organize into a complex, in regulating glycogen biosynthesis, in glycogen dephosphorylation and in the modulation of intracellular proteolytic systems. However, the contribution of each of these processes to the pathogenesis of Lafora disease is unclear. We found that dysfunction of autophagy is a common feature of both laforin and malin deficiency, preceding other pathological manifestations and, therefore, we have proposed that autophagy plays a primary role in the pathogenesis of Lafora disease and that it is a potential target for its treatment. This work was carried out in collaboration with some members of the Lafora consortium (CIBERER), which now includes the groups of Dr. Pascual Sanz and Vicente Rubio of the Instituto de Biomedicina (CSIC, Valencia), Dr. Federico Pallardó of the University of Valencia, Dr. Santiago Rodríguez de Córdovala of the Centro de Investigaciones Biológicas (CSIC, Madrid), Dr. Paola Bovolenta of the Centro de Biología Molecular (CSIC, Madrid) and Dr. José Serratos of the Fundacion Jiménez Díaz (Madrid).
CIPF Research in 2012

Molecular Neuroendocrinology

Publications

- Klionsky DJ et al. (2012). Guidelines for the use and interpretation of assays for monitoring autophagy. Autophagy 8, 455-544


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Klionsky DJ et al. (2012). Guidelines for the use and interpretation of assays for monitoring autophagy. Autophagy 8, 455-544


Overview

The incidence of diabetes and obesity is increasing at alarming rates throughout the world, creating a signifi-

cant social and economic burden in industrialised coun-

tries. Defective expression or function of insulin sign-

aling pathway components causes insulin resistance,

which occurs with normal ageing but is also a hallmark

disease states such as diabetes. The overall aim of our

research is to understand precisely how impaired insulin

signaling contributes to metabolic diseases. Through-

out 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 (dif-

ferentiation). 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 sig-
naling modulates the proliferation and differentiation of

glioblastoma multiforme (GBM) and neurogenic WAT failed to differentiate to adipocytes in vitro.

Inflammation. The population of adipocyte progenitor

cells located within the stroma-vascular fraction was in-

creased in visceral WAT of Irs2-deficient mice. Irs2-defi-

cient progenitor cells from both visceral and subcuta-

neous WAT failed to differentiate to adipocytes in vitro.

Impaired differentiation was associated with reduced

expression of key adipogenic transcription factors. Col-

lectively, these results implicate a role for Irs2 signals in

regulating the development and differentiation of adi-

pocyte progenitors. The results of this study suggest

that Irs2 null mice represent a physiologically relevant

model for studying the role of inflammation in the pro-

gression from pre-diabetes to diabetes.

Collaborations: Investigators of CIBERDEM; Dr. Thomas

Stulnig, University of Vienna; Herminia Gonzalez, INCLIVA.

GSK3β targets the tumor suppressor JunB for degradation

JunB, an activator protein-1 (AP-1) transcription factor

c component, acts either as a tumor suppressor or as an

c ongene depending on the cell context. In particular,

JunB is strongly upregulated in anaplastic lymphoma

kinase (ALK)-positive anaplastic large cell lymphoma

(ALCL) where it enhances cell proliferation. Although

its overexpression is linked to lymphomagenesis, the

mechanisms whereby JunB promotes neoplastic growth

are still largely obscure. Here, we show that JunB

undergoes coordinated phosphorylation-dependent

ubiquitylation during the G2 phase of the cell cycle.

Research results

Role of IRS2 in Adipocyte Stem Cells and Obesity

The recent study Diabetes performed by the national

network CIBERDEM revealed that at least 13.8% of the

Spanish population suffers from diabetes; the majority

of these cases are related to obesity. Reduced expres-

sion of IRS2 has been observed in islets of patients with

T2D, suggesting a direct link between failed insulin

signaling and human metabolic disease. Female Irs2-/-

animals develop moderate obesity but the role of IRS2 in

adipose tissue and obesity-related inflammation has

not yet been defined. Loss of Irs2 causes increased ac-

cumulation of both visceral and subcutaneous adipose

tissue in female mice. Serum levels of cytokines and ad-

ipokines are dysregulated in Irs2-deficient female mice,

reflecting a profile consistent with obesity-induced

inflammation. The population of adipocyte progenitor

cells located within the stroma-vascular fraction was in-

creased in visceral WAT of Irs2-deficient mice. Irs2-defi-

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mechanisms whereby JunB promotes neoplastic growth

are still largely obscure. Here, we show that JunB

undergoes coordinated phosphorylation-dependent

ubiquitylation during the G2 phase of the cell cycle.

We characterized a critical consensus phospho-degron

that controls JunB turnover and identified GSK3β and

SCF(FBXW7) as, respectively, the kinase and the E3

ubiquitin ligase responsible for its degradation in

G2. Pharmacological or genetic inactivation of the

GSK3β-FBXW7-JunB axis induced accumulation of JunB

in G2/M and entailed transcriptional repression of the

DNA helicase DDX11, leading to premature sister

chromatid separation. This abnormal phenotype due to
dysregulation of the GSK3β-JunB/DDX11 pathway

is phenocopied in ALK-positive ALCCL. Thus, our results

reveal a novel mechanism by which mitosis progression

and chromatic cohesion are regulated through GSK3β/

SCF(FBXW7)-mediated proteolysis of JunB, and suggest

that JunB proteolysis in G2 is an essential step in

maintaining genetic fidelity during mitosis.


Collaborations: Jaime Font de Fora, Departamento de

Oncologia, Hospital de la Fe; M. Piechaczek, Instituto de

Genéticu Médleulaire de Montpellier.

Irs2-deficiency reveals a molecular link between dia-

betes and cognitive impairment of Alzheimer’s disease

Various epidemiological studies have demonstrated

that patients with diabetes are at higher risk for devel-

oping Alzheimer’s, suggesting a link between metabolic
derangements and neurodegeneration. The beneficial

effects of insulin and insulin-like growth factor I on cog-
nition have been documented in humans and animal

models. Conversely, obesity, hyperinsulinemia, and
diabetes increase the risk for neurodegenerative disor-
ders including Alzheimer’s disease (AD). However, the

mechanisms by which insulin regulates synaptic plas-
ticity are not well understood. Complete disruption of

insulin receptor substrate 2 (Irs2) in mice impairs long-
term potentiation (LTP) of synaptic transmission in the

hippocampus. Basal synaptic transmission and paired-
pulse facilitation were similar between the 2 groups of

mice. Induction of LTP by high-frequency conditioning
tetanus did not activate postsynaptic N-methyl-D-as-

partate (NMDA) receptors in hippocampus slices from

Irs2(-/-) mice, although the expression of NR2A, NR2B,

and PSD95 was equivalent to wild-type controls. Acti-
vation of Fyn, AKT, and MAPK in response to tetanus

stimulation was defective in Irs2(-/-) mice. Interest-

ingly, IRS2 was phosphorylated during induction of LTP in

central mice, revealing a potential new component of

the signaling machinery which modulates synaptic plas-
ticity. Given that IRS2 expression is diminished in Type

2 diabetics as well as in AD patients, these data may

reveal an explanation for the prevalence of cognitive
decline in humans with metabolic disorders by provid-
ing a mechanistic link between insulin resistance and

impaired synaptic transmission.

Collaborations: Eduardo Martin, University of Castilla y

La Mancha; Jose Luis Trejo, Instituto Cajal (CSIC); Morris

White, Children’s Hospital Boston, Harvard University.

FIGURE 1 - (A) The human embryonic stem cell line VAL9 transduced to express

White, Children’s Hospital Boston, Harvard University.
Neurobiology

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Tiziano Balzano

Publications

Pérez-Benavente B, García JJ, Rodríguez MS, Pineda Lucena A, Piachatký M, Font de Mora J, Farrés R. GSK3-SCF(FBXW7) targets JunB for degradation in G2 to preserve chromatid cohesion before anaphase. Oncogene 2012 Jan 18
Murillo-Cuesta S, Camarero G, González-Rodríguez A, Du La Rosa LR, Burks DI, Avendaño C, Valverde AM, Varela Nieto I. Insulin receptor substrate 2 (IRS2) deficient mice show sensorineural hearing loss that is delayed by concomitant protein tyrosine phosphatase 1B (PTP1B) loss-of-function. Molecular medicine (Cambridge, Mass.) 2012 Mar 30


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 pathologic 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: 1) prevent death induced by acute ammonia intoxication; 2) prevent or delay death in rats with acute liver failure; 3) restore learning ability and, 4) reverse hypokinesia in rats with chronic HE.

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 coor-...

Research results

Differential effects of chronic hyperammonemia on modulation of the glutamate–nitric oxide-cGMP pathway by metabotropic glutamate receptor 5 and low and high affinity AMPA receptors in cerebellum in vivo

We have shown that chronic hyperammonemia impairs learning ability of rats by impairing the glutamate–nitric oxide (NO–cGMP pathway in cerebellum. Three types of glutamate receptors cooperate in modulating this pathway: metabotropic glutamate receptor 5 (mGlur5) and ionotropic AMPA and NMDA receptors. We assessed, by microdialysis in freely moving rats, whether hyperammonemia alters the modulation of the NO–cGMP pathway by mGlur5 and AMPA receptors in cerebellum in vivo. Activation of mGlur5 increases extracellular glutamate, which increases calcium in postsynaptic neurons by activating NMDA and AMPA receptors lacking the GluR2 subunit. Increased calcium leads to activation of neuronal nitric oxide synthase (nNOS), increasing NO and nitrite, NO activates guanylate cyclase, increasing cGMP (FIGURE 1).

We found that chronic hyperammonemia: (1) reduces glutamate release and activation of the glutamate–NO–cGMP pathway by activation of mGlur5; (2) strongly reduces the direct activation by AMPA receptors of the NO–cGMP pathway, likely due to reduced entry of Ca2+ through GluR2-lacking, high affinity AMPA receptors; (3) increases the indirect activation of the NO–cGMP pathway by high affinity AMPA receptors, likely due to increased entry of Na+ through GluR2-lacking AMPA receptors and NMDA receptors activation; (4) reduces the indirect activation of the NO–cGMP pathway by low affinity AMPA receptors, likely due to reduced activation of NMDA receptors.

The altered modulation by mGlur5 and AMPA receptors could be involved in the impairment of the glutamate–NO–cGMP pathway and cognitive impairment in hyperammonemia and hepatic encephalopathy.

Patients with minimal hepatic encephalopathy (MHE) show impaired mismatch negativity correlating with reduced performance in attention tests

Impairment of attention is an early event in cognitive deterioration of patients with liver disease and MHE which result in impairment in everyday functioning. The underlying mechanisms remain unclear. Mismatch negativity (MMN) is an auditory event-related potential which may reflect attentional trigger and seems modulated by NMDA receptors. We have shown that altered neurotransmission associated to NMDA receptors is a main contributor to cognitive impairment in animal models of HE.

We assessed whether: a) MMN is altered in cirrhotic patients with MHE compared to those without MHE or controls; b) alterations in MMN correlate performance in psychometric, including attention tests. Patients with MHE show reduced performance in tests of selective and sustained attention and in the visuo-motor and bimanual coordination tests. The MMN wave area was reduced in patients with MHE but not in those without MHE. Alterations in MMN correlate with alterations in tasks requiring attention. MMN is able to identify the attention deficits in patients with MHE. Understanding the mechanisms leading to attention deficits in MHE may help to design treatments to eliminate these deficits. This study was performed in collaboration with Instituto de Investigación Sanitaria INCLIVA and Hospital Clínico Universitario, Valencia.

Progressive reduction of sleep time and quality in rats with hepatic encephalopathy due to portacaval shunts

Patients with liver cirrhosis show sleep disturbances which affect their daily life. Sleep alterations have not been characterized in detail, the underlying mechanisms remain unclear and there are no effective treatments to improve sleep in these patients. Having an animal model reproducing these sleep alterations would allow studying the underlying mechanisms and assessing therapeutic treatments. We assessed: 1) whether rats with portacaval shunts (PCS), a model of HE, show alterations in sleep and if they are similar to those in patients with HE, 2) whether hyperammonemia plays a role in these sleep alterations, and 3) the time course of sleep alterations in these animal models. PCS rats show a significant reduction in REM and NREM sleep time and increased sleep fragmentation. Whereas reduced sleep occurs at 4 weeks and worsens at 7 and 11 weeks, sleep fragmentation appears at 7 weeks and worsens at 11 weeks. Hyperammonemic rats show decreased REM sleep, starting at 7 weeks and worsening at 11 weeks, with no changes in NREM sleep or sleep fragmentation. This study shows that PCS rats are a good model to study sleep alterations in HE, their mechanisms and potential treatment. This study was performed in collaboration with Universitario Pablo de Olavide, Seville, and Universitet Abdellal-

Príncipe Felipe Research Centre

Annual Report

CIPF Research in 2012

Neurobiology
### Publications


- **Cauli, O., Piedrafita, B., Llansola, M., and Felipo, V.** (2012) Gender differential effects of developmental exposure to methylmercury, polychlorinated biphenyls 126 or 153, or their combinations on motor activity and coordination. *Toxicology (in press)*
Patents

- Ex-Vivo Method For The Early Diagnosis Of Minimal Hepatic Encephalopathy By Means Of The Determination Of 3-Nitrotyrosine In Serum. P201000899, 12 de Julio de 2010. PCT/ES2011/070509 publicada el 19 de enero de 2012, con número WO2012007624

Memberships

- External Review Working Group of the European Food Safety Authority (EFSA)
- Executive Committee of the International Society for Hepatic Encephalopathy (ISHEN)

Conferences and meetings

- **Invited speaker**: International Symposium on Ionotropic Glutamate Receptors. Physiology, Pathology and Therapeutics, February 16-17, 2012 in Valencia, Spain
- **Invited speaker**: 15th ISHEN (International Society for Hepatic Encephalopathy and Nitrogen Metabolism) Symposium Grenaa, Denmark, May 29 to June 2, 2012
- **Invited speaker**: 48th EUROTOX Congress (European Societies of Toxicology). Stockholm, Sweden, June 17-20, 2012
- **Invited speaker**: Journey of Rare Diseases: El ciclo de la urea y sus patologías. Fundación Valenciana de Estudios Avanzados. 19 de Julio de 2012
- **Organizer**: International Symposium on Ionotropic Glutamate Receptors: Physiology, Pathology And Therapeutics. Valencia, Spain, February 16-17, 2012

Member of the Editorial Board of

- International Journal of Molecular Medicine
- World Journal of Gastroenterology
- Open Gastroenterology Journal
- Journal of Hepatology
- Review Editor de Frontiers in Neuroenergetics
- World Journal of Experimental Medicine

Awards

- Alberto Solis Prize to the best scientific work in Health Sciences

Neuronal & Tissue Regeneration

### Researchers

- Francisco Javier Rodríguez Jiménez

### Graduate Students

- Ana Alastrue Agudo

### Technicians

- Maravillas Mellado
- Teresa Valdés Sánchez (until March 2012)

### Collaborators

- Marta Cases Villar
- Viviana Bisbal
- Francesca De Giorgino
- Ana Belén Fernández
Overview

The long-term goal of the group is to improve the success of the therapeutic applications of stem cell-based approaches on the clinical practice. The lab is focused on the adult stem cells characterization and therapeutic applications into very 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 recently showed that acute transplantation of activated epidermal stem/progenitor cell (epSPC) can rescue lost neurological function after SCI in rodents (Stem Cells. 2009 Mar;27(3):733-43). In acute phase there is an axonal, neural and a glial massive cell death in the lesion epicenter provoked by apoptosis and necrosis that extends in a secondary phase, and affects all functional neurons and glial cell population including oligodendrocytes. In this severe disability the cell substitution-based therapies are well justified. The characterisation 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 of pharmacological tools favouring the applied cell-based therapeutic strategies and look for sinergistic effect within the pharmacological arsenal.

Osteoarticular pathologies very often require a regeneration process for bone, cartilage and/or tendon with de novo vascularisation. During the last couple of decades the mesenchymal stem cell population have been shown to be a very challenged option as a cell-based therapeutic approach. Because osteoarticular complications are very often occurring in dogs, it results an ideal model for our studies with direct traslational 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 osteoartritic associated pathology.

Research results

Spinal cord injury is one of the major challenged disabilities in which the scientific community has invested an exponential effort to disclose efficient therapeutic solutions. However, unfortunately not much plausible progress has been yet noticeable into the clinical practice. Our recent contribution, published in the prestigious international Journal Stem Cells (F Rodriguez et al, Stem Cells. 2012 Oct;30(10):2221-33) nicely offers a new therapeutic tool within an alternative mode of application with real translational potential. Our contribution associates, by its mechanism of action, the activity of a new pharmacological treatment, FM19G11, with the regenerative potential of endogenous adult neural precursors in an experimental model of a traumatic spinal cord injury that closely reproduce the human scenario. In the other hand, we have described that this new small molecule is able to induce both the self-renewal and proliferation of endogenous or exogenous neural precursors. FM19G11 induces in vitro self-renewal of the epidermal stem cells, the spinal cord neural precursors, either in neurosphere-like cultures or when are attached to a biocompatible biomaterial, alone or in co-culture with primary neurons (T Valdes et al, Tissue Eng Regen Med. 2013). The analysis of the mechanism of action revealed an early increment of mitochondrial uncoupling protein 2 and 1 with an early drop of ATP, followed by a subsequent compensatory recovery with activated mitochondrial metabolism and the induction of glucose uptake by upregulation of the glucose transporter GLUT-4. Here we show that phosphorylation of AKT and AMP-activated kinase (AMPK) is involved in FM19G11-dependent activation of GLUT-4, glucose influx, and consequently in stem cell self-renewal. Then, we also contribute for a better understand of the mechanism involved in this specific cell population for endogenous activation approaches.

The multipotency of adipose-derived mesenchymal stem cells (ASCs) can be maintained in vitro and they can be differentiated to osteocytes or chondrocytes offering a good tool for cell replacement therapies in human and veterinary medicine. Although ASCs can be easily obtained from adipose tissue, the amplification process is usually performed by a time consuming process of successive passages. In the lab, we use canine ASCs obtained under GMP cell culture conditions generating a minimum of 30 million cells within 2 weeks. This method provides a rapid and aseptic method for production of sufficient stem cells with potential further use in clinical applications. In this type of ASC cultures we have shown (F Rodríguez et al, Journal of Functional Biomaterial Vol: 3(3) pp: 556-568, 2012) that plasma rich in growth factors autologous (PRGF) treatment positively contributes to viability and proliferation of canine ASCs either alone or seeded into caprolactone 2-(methacryloyloxy) ethyl ester (CLMA) scaffolds (see illustration). This biomaterial does not need additional modifications for cASCs attachment and proliferation.

We propose a framework based on a combinatorial approach that may contribute to increase the therapeutical capability of stem cells by the use of PRGF and compatible biomaterials for bone and connective tissue regeneration.

FIGURE 1 - Proliferative activity of PRGF in Adipose-derived mesenchymal stem cells (ASCs). ASC were seeded into caprolactone 2-(methacryloyloxy) ethyl ester scaffolds as a suitable biomaterial for tissue engineering applications alone or treated with autologous PRGF which showed to improved the cell proliferation (quantified as double stain for green P-Value 0.03 (red) positive cells in the histogram).

FIGURE 2 - FM19G11 acute intrathecal administration improves locomotion recovery after Spinal Cord Injury. Upper panel illustrates the administration of FM19G11 delivered through the intrathecal space into the injured area and controlled by an osmotic pump. Lower left panel represents the functional analysis of the motor BBB test showing a significant difference the group FM19G11 treated group 1 month after spinal cord injury. Lower right panel shows the differential protein expression of stem cell related markers one month after injury and treatment on animals treated with vehicle (DMSO) or FM19G11.
Organic Molecules

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Overview

Medicinal Chemistry is the subject that refers to the discovery, identification and preparation of new biologically active chemical entities 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.

Research results

Our research group works as a Mixed Unit concerning University of Valencia and Prince Felipe Research Center (CIPF), where members of the group are distributed between the two institutions. In addition, collaborations with different groups from other institutions (Universitat de Valencia, Instituto de Salud Carlos III de Madrid) and pharmaceutical companies ( Lilly, Janssen Pharmaceuticals) provide us new lines of research in the area of drug discovery.

From a synthetic point of view, our work has been focused on the preparation of new fluorinated and non-fluorinated unnatural amino acids. Specifically, we have carried out the design and synthesis of cyclic and bicyclic quaternary amino acids by employing different stereoselective functionalization reactions of chiral iminolactones. The resulting compounds represent an attractive group of conformationally constrained peptidomimetics which have been used as building blocks in the synthesis of larger peptide chains. The analysis of those molecules using different experimental techniques (NMR, IR, X-ray) has demonstrated the formation of interesting conformations close to the turn-β substructure (Chem. Eur. J. 2012, 18, 3753. See also: Org. Lett., 2010, 12, 3014, J. Org. Chem 2009, 74, 4429).

In addition, in the context of peptidomimetics, and in collaboration with the group of Dr. José Gallego of the Catholic University of Valencia, we are working on the identification of inhibitors of HIV-1, through the design, synthesis and biological evaluation of a new generation of fluorinated unnatural amino acids. Specifically, we have 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 (Org. Biomol. Chem. 2012, 10, 6758, Chem. Eur. J. 2011, 17, 14772). It is well known that, in many cases, the introduction of fluorine into potentially bioactive compounds can modify their physical properties and chemical reactivity, thus affecting important factors such as the bioavailability or the molecular recognition. In fact, nowadays fluorinated organic compounds are present in a great number of natural products (Org. Lett. 2009, 11, 5527). In this context, we have developed a methodology for the stereoselective synthesis of fluorinated organic compounds represent approximately 25% of new 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.

Publications

Patents


Conferences and meetings

- Plenary Lecture: “Stereoselective synthesis of fluorinated building blocks: new synthetic strategies and applications” Johnson & Johnson, Beerse, Belgium, 2012
- Plenary Lecture: “Asymmetric tandem reactions: new synthetic strategies and applications” University of Barcelona, Barcelona, Spain, 2012

Peptide & Protein Chemistry

Group Leader
ENRIQUE PÉREZ PAYA

- Researchers
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  - Ainhoa Genovés Martínez
  - Tatiana Guevara Rozo
  - Andrés Herrera Aguilar


Plenary Lecture: “Síntesis estereoselectiva de building blocks funcionados: Nuevas estrategias sintéticas” CSIC (Instituto de Química Orgánica), Madrid, Spain, 2012


Overview

New insights and developments to open translational opportunities

Apoptosis (programmed cell death) is executed by strongly regulated pathways, and serve to remove infected, damaged or ectopic cells. The intrinsic pathway of apoptosis is initiated by many stresses inducing mitochondrial outer membrane permeabilisation (MOMP) mediated by proteins of the Bcl-2 family. Cytochrome c is then released into the cytosol and induces the formation of the apoptosome complex. Apoptosis dysregulation is at the root of a variety of diseases. While resistance has been linked to cancer and autoimmune diseases, excess apoptosis is connected to neurodegeneration and ischemia-reperfusion damage. Apoptosis can be modulated through links with other cell signaling pathways. Then, apoptosis proteins can participate and cross-talk to non-apoptotic processes, such as cell differentiation and proliferation, mitochondrial morphogenesis, autophagy and inflammation.

Apoptosis susceptibility is co-determined by a multifactorial interplay but the molecular mechanisms and controls remain poorly understood. We aim to investigate how apoptosis is integrated, interrelated and modulated. To this end, we develop chemical tools to gain quantitative molecular information to understand physiological (light) and pathophysiological (dark) modes of apoptosis modulation. We believe that our approach will facilitate the knowledge-driven identification and development of targeted pharmacological interventions that modulate cell death susceptibility.

Research results

Inhibitors of the tyrosine kinase activity of epidermal growth factor receptor, as erlotinib, have an established role in treating several cancer types. However, resistance to erlotinib, particularly in breast cancer cell lines, and erlotinib treatment-associated disorders have also been described. Then, methods and combination therapies that could reverse resistance and ameliorate non-desirable effects represent a clinical challenge. In this period we have evaluated drug-drug combinations to circumvent this problem. We showed that the ATP non-competitive CDK2/cyclin A inhibitor NBI1 sensitizes erlotinib-resistant tumor cells to the combination treatment (co-treatment) for apoptosis-mediated cell death. Furthermore, in erlotinib sensitive cells the effective dose of erlotinib was lower in the presence of NBI1. The analysis, in the breast cancer MDA-MB-468 erlotinib-resistant and in lung cancer A549 cell lines of the molecular mechanism underlying the apoptosis induced by co-treatment highlighted that the accumulation of DNA defects and depletion of cIAP and XIAP activates the ripoptosome that ultimately activates caspases-8 and -10 and apoptosis. This finding could have significant implications for future treatment strategies in clinical settings.

We have been also very active in the development and in cellulo analysis of nanodevices designed for controlled drug release. In collaboration with Prof Ramón Martínez-Máñez (Universidad Politécnica de Valencia) we have provided several evidences on how silica mesoporous-based nanostructures capped with different gate-like chemicals deliver cargo drugs in cells.

Publications


Villata-Romero, F; Gortat, A; Herrera, AE; Arguedas, R; Quesada, J; de Melo, RL; Calvete, JJ; Montero, M; Murillo, R; Rucavado, A; Gutiérrez, JM; Pérez-Paya, E. Identification of New Snake Venom, ACS MEDICINAL CHEMISTRY LETTERS, Volume: 3 Issue: 7 Pages: 540-543 2012

Martinez-Hoyer, S; Aranguren-Ibanez, A; Garcia-Garcia, J; Serrano-Candelas, E; Vilardell, J; Oliva, R; Orzaez, M; Perez-Paya, E; Itarte, E; Perez-Kibu, M. Phosphorylation of RCAN proteins by protein kinase. FEBS JOURNAL V: 279 Sl: SI St: 1 Pages: 160-160 SEP 2012
Polymer Therapeutics

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Overview

Clinical proof of concept for Polymer Therapeutics has already been achieved, however, many challenges and opportunities still lay ahead providing scope to further develop this technology platform. Delivery of new anti-cancer 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 well as molecular diagnostic tools. The development of polypeptide-based biodegradable carriers, the use of combination therapy or the design of nanoconjugates 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 2012 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. Herein, some of the main research results achieved are described.

A wide variety of polymers have been described as carriers for drug delivery or imaging probes. However, very few have been successfully transferred into patients (mainly polyethylene glycol) (PEG), N-2-Hydroxy-propylmethacrylamide (HPMA) and poly-L-glutamic acid (PGA). In our laboratory, synthetic pathways to a plethora of functional polyampholytes with well-defined structure, adjustable molecular weight and low dispersity (D = Mw/Mn<1.2) have been developed applying a controlled ring opening polymerization of N-carboxyanhydrides with novel initiators. Various functionalities such as alkyne, azides, reactive disulphides, etc. can be easily introduced by “post-polymerization modification” reactions yielding a set of orthogonal reactive attachment sites. Based on these, new polymeric libraries with different and sometimes unexpected properties as result of the contribution of the different architectures have been built, characterized and explored for different applications in the nanomedicine field.

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 molecular probes), in vivo monitoring or metabolomic approaches are being developed for this purpose. Novel cellu-
lar probes are being developed for this purpose.

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. Prostate cancer project is run in collaboration with Dr. Lopez (IVD, Valencia) and Dr. Schwartz I’r’ group (VHIR, Barcelona). We have achieved in vivo proof that the rational design of a combination conjugate bearing in the same mainchain a cocktail of bioactive agents could trigger an 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. This type of advanced therapies could provide significant clinical benefits, including: (i) the combination could be administered as a unique dose and consequently with better patient compliance and benefits in the production; (ii) after a passive target- getting by the EPR effect, this approach is the only one that secures the arrival of both drugs at the same time to the same tumour cell triggering therefore, drug synergy; (iii) chemoresistant advanced metastatic diseases are targeted due to the conjugate systemic and cellular trafficking properties; and finally, this approach (iv) offers the possibility to tune drug release kinetics by linker design allowing to move towards the design of ‘patient-individualised therapies’.

Focusing on the development of nanoconjugates for novel molecular targets, in collaboration with Prof. Saraiva’s group (IBMC, Porto) we have developed the first polymer conjugate targeting Familial Amyloidotic Polyneuropathy (FAP) a rare disease affecting the peripheral nervous system. The conjugates developed for FAP disease are currently being explored for the treatment of other amyloidosis, such as Alzheimer’s Disease. Within this context, drug carriers capable to cross the blood brain barrier and to provide drug delivery to the brain are also being explored with promising results. Enhancing brain delivery after a systemic administration is one of the main challenges within the nanomedicine field.

All conjugate design in our laboratory is always developed together with the use of advanced physico-chemical techniques for conjugate characterization that allow to define the structure-activity relationships in the context of proposed biological use. We pioneered the use of techniques, such as Small Angle Neutron Scattering (SANS) to explore conjugate solution conformation (in collaboration with Dr. Paul (Cardiff Univ., UK)) and the use of other techniques, such as NMR (in collaboration with Dr. Pineda Lucena Lab. (CIPF, Valencia)), including TOCSY, DOSY, 15N-HSQC or Pulsed-Gradient Spin-Echo NMR (PGSE-NMR) to elucidate conjugate solution conformation and rates of diffusion in the biological setting. Such techniques reinforce our ability to characterize novel complex structures and drug combinations as well as monitor conjugate interactions with their biological targets.

In the same context, we have also developed quantitative methods to study cell trafficking and nanoconjugate in vivo fate including subcellular fractionation, Amisseq Imaging Stream, in vivo monitoring or metabolomic approaches (in collaboration with Dr. Pineda Lucena). Novel cellular probes are being developed for this purpose.

During 2012 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. Herein, some of the main research results achieved are described. During 2012 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. Herein, some of the main research results achieved are described.
Publications

- **Controlled Release** 2012, 159(2) 290-301. Demonstrate the importance of polymer-conjugate conformation on its therapeutic output.
- **Invited Lecture:** M.J. Vicent. Well-defined polyglutamates as drug delivery carriers. 76th Prague Meeting on Macromolecules in Medicine 2012 IUPAC Prague, Czech Republic, 2012.

Conferences and meetings

- **Keynote Speaker:** M.J. Vicent. Polymer Conjugates as Nanomedicines: from single agents to combination Therapy. XXII International Symposiumon Medicinal Chemistry (EFMC-IUMSC), Berlin, Germany, 2012.
- **Invited Lecture:** M.J. Vicent. Well-defined polyglutamates as drug delivery carriers. 76th Prague Meeting on Macromolecules in Medicine 2012- IUPAC Prague, Czech Republic, 2012.

Patents

- **Invited Lecture:** M.J. Vicent. Well-defined polyglutamates as drug delivery carriers. 76th Prague Meeting on Macromolecules in Medicine 2012-IUPAC Prague, Czech Republic, 2012.

Memberships

- **Group Leader** Mª EUGENIA ARMENGOD
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  - Silvia Prado Martín
- **PhDs**
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- **Graduate Students**
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- **Technicians**
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  - Rafael Ruiz Partida
- **Collaborators**
  - Ana Martínez Zamora
Overview

Our research is focused on RNA modification and its relationships with mitochondrial and infectious diseases. Post-transcriptional modification of transfer and ribosomal RNAs (tRNAs and rRNAs, respectively) plays an important role in optimizing their functions as core molecules of the protein synthesis apparatus. Moreover, recent data support the notion that RNA modifications and RNA modifying enzymes may work as checkpoints for integrating protein synthesis with other cellular functions, and also as regulators of gene expression.

Defects in the modification of mitochondrial (mt) tRNAs have been associated with several neuromuscular diseases (e.g., MERRF and MELAS), acute infantile liver failure, and infantile hypertrophic cardiomyopathy. Our work contributes to the biochemical, structural and functional characterization of several families of evolutionarily conserved enzymes involved in the modification of both mitochondrial and bacterial tRNAs. We use a wide range of biological models (bacteria, yeast, nematodes, human cell lines) and approaches (Molecular Biology, Biochemistry, Microbiology, Cell Biology, Structural Biology and Bioinformatics) to obtain information on the mechanisms used by RNA-modifying enzymes to perform their functions and to control gene expression. Our research may help to gain a better understanding of the pathophysiological mechanisms leading to the aforementioned diseases and to develop new therapeutic treatments. We collaborate with CIBERER (Biomedical Research Networking Centre in Rare Diseases) groups to achieve these aims.

Modification of tRNA and rRNA is also closely related to bacterial virulence and resistance to antibiotics and stresses. Our group has identified and characterized several RNA modifying enzymes which are involved in translational fidelity, adaptability of bacteria to stress conditions, and activation of mechanisms promoting maintenance and spread of antibiotic-resistant bacteria. We believe it is possible to exploit this knowledge for the choice of therapeutic strategies and design of new drugs.

Research results

The presence of modified ribonucleotides derived from adenosine, guanosine, cytidine, and uridine is a hallmark of almost all tRNAs. So far, more than 100 different modified nucleotides have been described in tRNAs and rRNAs from organisms belonging to the three domains of life, Archaea, Bacteria and Eukarya. Modification enzymes specifically recognize the target RNA molecule and incorporate the chemical modifications at the precise position. The resulting modified nucleotides can alter RNA chemistry and structure, and they generally contribute to fine-tune protein synthesis.

tRNA exhibits a larger number and wider variety of modifications than rRNA. Modifications within the anticodon stem loop of tRNA, mainly at the wobble position and purine-37, collectively contribute to stabilize the codon-anticodon pairing, maintain the translational reading frame, facilitate the engagement of the ribosomal decoding site and enable translocation of tRNA from the A-site to the P-site of the ribosome. Modifications at the wobble uridine (U34) of tRNAs reading purine-ending codons of split boxes are complex. They result from the activity of two multi-enzyme pathways, the IsoS-MmmA and MnmEG pathways, which work on positions 2 and 5 of the U34 pyrimidine ring, respectively, and from a third pathway that modifies the 2’-hydroxyl group of the ribose (FIGURE 1). The latter is a single-step pathway controlled by the SP0UT methyltransferase TrmL (YibK), as recently demonstrated by our group. MnmEG is the only pathway common to all the aforementioned tRNAs. It involves the GTP- and FAD-dependent activity of the MnmEG complex (formed by proteins MmmE and MmmG) and, in some cases, the activity of the bifunctional enzyme MmmC. We have shown that the E. coli MmmE complex catalyzes the incorporation of an aminomethyl group into the C5 atom of U34 using methylene-tetrahydrofolate (MTHF) and glycine or ammonium as donors. The reaction requires GTP hydrolysis by MmmE (FIGURE 2), probably to assemble the active site of the complex or to carry out substrate recognition. Inactivation of the evolutionarily conserved MmmEG pathway produces a pleiotropic phenotype in bacteria and, in humans, mitochondrial dysfunction associated with infantile hypertrophic cardiomyopathy. Our group has actively participated in the characterization of bacterial proteins MmmG and MmmE and the human MmmE homolog (GTPBP3). Recently, we have shown that the tRNA-modifying function of MmmE is controlled by post-hydrolysis steps of its GTPase cycle. Thus, MmmE provides a new paradigm of how the ON/OFF cycling of GTPases may regulate a cellular process.

Furthermore, we have discovered that E. coli RimN catalyzes the methylation of purine-37 in a tRNA set. Modifying RNA enzymes are highly specific for substrate (rRNA or tRNA) and for the target position. We have shown that RimN is an exception because it works as a dual-specificity enzyme that catalyzes the methylation of both rRNA and tRNA. By using a chimeric tRNA, we have identified some tRNA identity determinants for RimN and other RNA modification enzymes. Our data suggest that RimN works in a late step of rRNA maturation by recognizing a precise 3D structure of tRNA, which shares sequence and structural similarity with 23S rRNA in the region including the target nucleoside. The rRNA nucleoside methylated by RimN (m2A2503 in 23S rRNA) is located at the entrance of the nascent peptide exit tunnel and has been involved in the ribosomal mechanism that promotes translation arrest in response to specific peptide sequences. We have found that RimN inactivation produces an error-prone phenotype since it increases the misreading of a UAG stop codon when tested in an in vivo assay. This phenotype is probably due to the lack of the modified nucleoside in 23S rRNA, which could facilitate the accommodation of an
incorrect tRNA in the peptidyl transferase center. RlmN inactivation has been reported to reduce susceptibility to linezolid, an antibiotic that targets 23S rRNA and is used for the treatment of serious infections. We are at present investigating how the selective advantage apparently conferred to antibiotic-resistant bacteria by mutations in rlmN and other genes can be counteracted.

RsmG is a methyltransferase responsible for the synthesis of m7G527 in the 530 loop of bacterial 16S rRNA. This loop is universally conserved, plays a key role in ribosomal accuracy, and is a target for streptomycin binding. Streptomycin has been a key antibiotic for the treatment of tuberculosis and today is still used in second-line therapy for patients suffering multidrug-resistant tuberculosis. Loss of RsmG activity confers low-level streptomycin resistance and inexplicably prompts the appearance of high-level streptomycin resistance mutations. As a first step in understanding the role of RsmG in the acquisition of these mutations, we have studied how the expression and activity of the E. coli RsmG methyltransferase are regulated. We have identified several regulatory mechanisms that control RsmG expression at both transcriptional and post-transcriptional levels, and adjust it to rRNA amounts. We have also demonstrated the critical importance of some residues in or around the active site of RsmG for the m7G modification process. Overall, our results contribute to the understanding of how cells adjust rRNA modification to different physiological conditions. At present, we are exploring the interplay between RsmG inactivation and acquisition of high-level streptomycin resistance.

Publications


Benítez-Páez A, Villarroya M, Armengod ME. The Escherichia coli RlmN methyltransferase is a dual-specificity enzyme that modifies both rRNA and tRNA and controls translational accuracy. RNA 18: 1793-1795 (2012)
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 2012, we have conducted several studies that could contribute to the development of molecularly targeted agents against several pharmacologically relevant targets. In this context, Nuclear Magnetic Resonance (NMR) has been 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, a relatively new approach that measures the metabolic profile of biological samples, has also been used to conduct comparative analysis of healthy and diseased individuals, information that can be used to identify biomarkers of disease and stratification of patients based on molecular subgroups. Furthermore, 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. The Structural Biochemistry Laboratory is interested in the development of novel molecularly targeted agents against several pharmacologically relevant targets. In this context, Nuclear Magnetic Resonance (NMR) has been 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, a relatively new approach that measures the metabolic profile of biological samples, has also been used to conduct comparative analysis of healthy and diseased individuals, information that can be used to identify biomarkers of disease and stratification of patients based on molecular subgroups. Furthermore, 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 these drugs. This methodology is useful to carry out a comparative analysis of healthy and diseased individuals, information that can be used in the identification of disease biomarkers and in the stratification of patients. We are applying this technology to evaluate the effect of molecularly targeted agents in the treatment of multiple myeloma (MM). The objective is to analyze the molecular basis of this disease and characterize the pharmacological effects of various combinations of drugs used in clinical practice for the treatment of MM. In close 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, MM at the time of diagnosis vs healthy individuals) that will facilitate the clinical monitoring of these patients.

Heparanase

This enzyme is capable of degrading the heparan sulphate, one of the main components of the extracellular matrix. Heparanase is involved in neoplastic processes such as tumor formation, angiogenesis and metastasis, which makes it a very attractive target for anticancer drug development. The inhibitors described to date have been identified by classical screening campaigns and could not be optimized following rational criteria because, until very recently, there was no structural information about this protein. Using a combination of biochemical and biophysical approaches, we have identified an heparanase subdomain of 29 kDa (Hep158-417) and have assigned both the backbone and side chain of this construct. In addition, we have conducted a screening campaign, using WaterLOGSY and STD experiments, that allowed us to identify a number of hits in a collection of fragments. Throughout 2012, we have put in place a new approach, combining computational techniques with other biophysical approaches (NMR, SPR), aimed at characterizing the structural determinants of these interactions. The final goal is to identify novel inhibitors of heparanase based on a rational design, which would improve the affinity and selectivity thereof. In particular, we have carried out an evaluation of various computational techniques (pharmacophore modeling and docking strategies) to identify potential inhibitors of heparanase using a chemical library consisting of already known drugs. The experimental validation was performed using NMR (WaterLOGSY and STD) and SPR. This work has allowed the identification of a compound with a better affinity in the low-micromolar range. It has also facilitated a SAR study that allowed a detailed characterization of the epitopes of this molecule playing a role in the interaction.

Metabolomics

We are also very interested in the application of metabolomics by NMR, an experimental strategy that measures the metabolic profile of biological samples, to assess the metabolic changes caused by antineoplastic agents, thus providing a way to evaluate the effectiveness (clinical validation) and selectivity/toxicity (mechanism of action) of these drugs. This methodology is useful to carry out a comparative analysis of healthy and diseased individuals, information that can be used in the identification of disease biomarkers and in the stratification of patients. We are applying this technology to evaluate the effect of molecularly targeted agents in the treatment of multiple myeloma (MM). The objective is to analyze the molecular basis of this disease and characterize the pharmacological effects of various combinations of drugs used in clinical practice for the treatment of MM. In close 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, MM at the time of diagnosis vs healthy individuals) that will facilitate the clinical monitoring of these patients.
Conferences and meetings

- Poster: Metabolomic profile assessment from umbilical cord blood sampling in early and late IUGR neonates. Sanz-Coletó, M., Carbajo, R., Crispi, F., Figueras, F., Pineda Lucena, A., Gratacós, E. World Congress 2012, International Society of Ultrasound in Obstetrics and Gynecology, Copenhagen, Denmark, 2012
- Poster: NMR metabolomics on fetal serum from early and late IUGR neonates. Sanz-Coletó, M., Crispi, F., Figueras, F., Pineda Lucena, A., Gratacós, E. World Congress 2012, International Society of Ultrasound in Obstetrics and Gynecology, Copenhagen, Denmark, 2012
- Memberships
  - Executive Board of Sociedad Española de Química Terapéutica
  - Collaborators
    - Javier Moncayo Arlandi
    - Pilar Sepúlveda Sanchis
Overview

The cardiac healing process is guided by intricate interactions between different components; following myocardial infarction (MI), injury, inflammation, regeneration, and repair are all interconnected processes. It is known that these processes are inadequate and over-complicated, since certain pathological or damaging factors, such as cardiomyocyte replacement cannot be repaired. To improve cardiac healing we are investigating:

First, how resident multipotent cell react after injury, in heart exist some resident cell with a role in cardiac healing these cells are able to differentiate into all three major cardiac cell lineages (endothelial, smooth muscle and cardiomyocyte cells). Since a role for complement anaphylatoxins (C3a and C5a) has been described in several regeneration/repair processes, we have examined the effects that C3a and C5a exert on these cells to improve cardiac healing after injury.

Second, how we can preserve cardiomyocyte vitality in absence of nutrients. Hydrogen sulfide has recently been identified as a potent cardioprotective signaling molecule, which is a highly effective pre- and post-conditioning agent. We are exploring the cardioprotective signaling pathways involved in hydrogen sulfide-based pre- and post-conditioning.

Third, how a genetic pathology (ARVD) modulates cardiomyocyte phenotype during disease progression. Arrhythmogenic right ventricular cardiomyopathy/dysplasia (ARVC/D) is a unique heart muscle disease, clinically characterized by non-ischemic ventricular arrhythmias originating from the right ventricle (RV), at risk of cardiac arrest. It is one of the major causes of sudden death in the young and in the athletes. We are generating preclinical models to find novel target with potential for clinical intervention in these patients.

Research results

Complement anaphylatoxins C3a and C5a induce a failing regenerative program in cardiac resident cells. Evidence of a role for cardiac resident stem cells other than cardiomyocyte renewal

This projet is being done in collaboration with Dr. Borja Ibañez (H. San Carlos, Dr. Ramon Brugada (H. Gerona). New preclinical models in ARVC Only mouse models of ARVC are available at the moment and the extent to which they recapitulate the human ARVC is questionable. A lack of suitable preclinical models is an important deficit in the field that precludes the development of therapeutic strategies and the understanding of its progression mechanisms. We are generating new knowledge in ARVC pathways. Taking advantage of the new data from patients, the in vitro models and animal models, we are addressing various hypotheses to understand ARVC: 1) CM death versus transdifferentiation, 2) Involvement of the Wnt pathway and others 3) involvement of the lipid biosynthesis pathways. The fulfillment of these objectives will provide new molecular pathways and paradigms for the design of new therapies. We have obtained 3 cardiomyocyte cell lines that carry a mutated PKP2 protein that has been found in a 8 families affected that mimics in part some of the molecular findings obtained in patients. In addition, we have 2 lines of transgenic mouse that also carry the PKP2 mutation found in patients. The expected developments are: - Diagnosis tools based on molecular markers, new animal and cellular disease models of ARVC for drug development. We will provide an unprecedented preclinical platform for understanding ARVC mechanisms, for validating candidate new mutations identified in and for identifying read outs for in vitro drug screening or probes for imaging. Mechanisms are not consider as an invention by patent law, they are discoveries, but some achievements related with them can be also studied for assessing their patentability.

Usefulness of hydrogen sulfide (H2S) protecting vascular cells from death by starvation

Coronary artery disease is a major cause of morbidity and mortality in the Western world. Acute myocardial infarction, resulting from coronary artery atherosclerosis, is a serious and often fatal consequence of coronary artery disease, resulting in cell death in the myocardium. Pre- and post-conditioning of the myocardium are two treatment strategies that reduce the amount of cell death significantly. Hydrogen sulfide has recently been identified as a potent cardioprotective signaling molecule, which is a highly effective pre- and post-conditioning agent. We are investigating cardioprotective signaling pathways involved in hydrogen sulfide-based pre- and post-conditioning like antioxidant signaling mechanisms, antiapoptotic signaling mechanisms & mitochondrial preservation and activation of autophagic pathways. We have identified hydrogen sulfide as a protector against death by glucose starvation. H2S induce autophagy and pretreatment with H2S simulates starvation. Activation of different signals during H2S pretreatment protects cardiomyocyte from death.

Hydrogen sulfide donors may play an important role in cardiac surgery, where damage to the heart and ischemia happen at a highly predictable time. H2S donors could be administered preoperatively to invoke delayed or late preconditioning cytoprotective signaling cascades if the surgery was not time critical, or right before or during ischemia to invoke the early pre- and post-conditioning signaling cascades. In addition in organ transplantation, controlled delivery of H2S throughout the heart will induce protective effects and a state of hibernation that will prolong heart viability and reduce ischemia-reperfusion injury and radical oxidative species and improving heart function in transplants.

But will also be easily applicable to today’s organ transport methods.

Publications

2. Cerrada I, Ruiz-Saúl A, Carreno R, Trigueros C, Dorronsoro A, Sanchez-Puelles IM, Diaz-Juan A, Montero JA, Sepulveda P. Hypoxia-inducible Factor-1 Alpha Contributes to Cardiac Healing in Mesenchymal Stem Cells-Mediated cardiac Repair. Stem cells and development 2012 Sep 14
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.
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 antigens, 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, 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 have 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

During 2012 the CIPF proteomics core facility concluded the project entitled “Desarrollo de una metodología de análisis para anticuerpos monoclonales en suero humano. Aproximación novel para su cuantificación” in collaboration with AINIA. The project was focused on mass spectrometric detection of therapeutic antibodies. It also participated 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. It is designed to map the entire human proteome in a systematic effort using currently available and emerging techniques. Completion of this project will enhance understanding of human biology at the cellular level and lay a foundation for development of diagnostic, prognostic, therapeutic and preventive medical applications.

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.
Screening

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

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

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

Other services and facilities

GMP facilities

360m² surface
4 classified manufacturing rooms
1 cryoegeny 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**

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**Ministry of Education**

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Carlos III Institute of Health

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<td>Antonio Díez</td>
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<td>Ana Conesa</td>
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<td>Becas Santiago Grisolia para la formación</td>
<td>Enrique Peréz-Payá</td>
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<td>Christian De Ford</td>
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Education Cencillary (GVA)

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Travel grants

Ministry of Economy and Competitiveness

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<td>Mª Jusó Garzón</td>
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<td>Patricia Sebastián</td>
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<td>Mª Dolores Oliver</td>
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Ministry of Education

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<tr>
<td>Ana Conesa</td>
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Research projects grants

7th Framework Programme

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Ministry of Economy and Competitiveness

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<tr>
<td>Programa Consolider</td>
<td>The Spanish Ion Channel Initiative (SICI)</td>
<td>Vicente Felipo, Enrique Pérez-Payá, Victoria Moreno</td>
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<tr>
<td>Proyectos de proyecto fundamental no orientada</td>
<td>Selección natural en genomas completos de especies de mamíferos y drosophila. Una aproximación desde la biología de sistemas</td>
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<td>Papel de las anafilatoxinas del complemento en la recuperación del daño cardíaco</td>
<td>Antonio Díez</td>
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<td>Proyectos de proyecto fundamental no orientada</td>
<td>Papel de los receptores LTs y el inflammasoma en el daño que induce el etanol en el cerebro</td>
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<td>Proyectos de proyecto fundamental no orientada</td>
<td>Estudio de nuevas relaciones genoma-transcriptoma mediante técnicas de ultrasecuenciación</td>
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<td>Proyectos de proyecto fundamental no orientada</td>
<td>Rutas de modificación de tRNAs que descodifican codones de cajas mixtas terminados en purinas</td>
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<td>Proyectos de proyecto fundamental no orientada</td>
<td>Mecanismos moleculares moduladores de apoptosis</td>
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<td>Proyectos de proyecto fundamental no orientada</td>
<td>Nuevas tecnologías para toxicología in vitro: Diseño y validación de una Plataforma integrada Admetox de ensayos celulares para predicción de riesgo químico en humanos</td>
<td>Enrique O’Connor</td>
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<tr>
<td>Proyectos de proyecto fundamental no orientada</td>
<td>Nanofármacos polímericos utilizados como agentes simples y en terapia de combinación. Plataforma tecnológica versátil para regeneración tisular y tratamientos anticancerígenos</td>
<td>Mª Jesús Vicent</td>
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<tr>
<td>Proyectos de proyecto fundamental no orientada</td>
<td>Bases moleculares de las alteraciones neurológicas en hiperamonemia y encefalopatía hepática. Implicaciones terapéuticas</td>
<td>Vicente Felipo</td>
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### Carlos III Institute of Health

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<td>Proyectos de investigación en salud</td>
<td>Papel de rho en el crecimiento axonal y en la mielinización. Implicación en las enfermedades neurodegenerativas</td>
<td>Rosa Guasch</td>
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<td>Proyectos de investigación en salud</td>
<td>Regeneración de la función motora tras lesión medular traumática: activación del potencial regenerador endógeno</td>
<td>Victoria Moreno</td>
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<td>Proyectos de investigación en salud</td>
<td>Biología celular y función de ANKK1 en el cerebro: Relación con el sistema dopaminérgico y la neurogénesis</td>
<td>Janet Hoenicka</td>
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<td>Proyectos de investigación en salud</td>
<td>Caracterización molecular de rutas de señalización oncogénicas en células madre tumoresales de cáncer de pulmón no microcítico. Implicación en el desarrollo de nuevas estrategias terapéuticas</td>
<td>Rosa Farràs</td>
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### Spanish Agency for International Cooperation

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<tr>
<td>Acción integrada para el fortalecimiento científico e institucional</td>
<td>Acción para el fortalecimiento científico tecnológico en áreas relacionadas con la genómica y bioinformática aplicadas</td>
<td>Joaquín Dopazo</td>
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### Plan of Action on Drugs

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<td>Proyecto de Investigación</td>
<td>Papel de la activación del sistema inmunitario y de la glia en el daño cerebral inducido por el consumo de alcohol</td>
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### Education Canecilliary (GVA)

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<tr>
<td>Prometeo</td>
<td>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</td>
<td>Vicente Felipo</td>
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<tr>
<td>Prometeo</td>
<td>Identificación de nuevas dianas terapéuticas en angiogénesis y apoptosis basadas en interacciones proteína-proteína</td>
<td>Enrique Pérez-Payá</td>
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<td>Prometeo</td>
<td>Papel de las anafilatoxinas del complemento en la recuperación del daño cardíaco</td>
<td>Joaquín Dopazo</td>
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<tr>
<td>Acciones complementarias</td>
<td>The Spanish Ion Channel Initiative (SICI)</td>
<td>Vicente Felipo</td>
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<td>Acciones complementarias</td>
<td>Rutas de modificación de mRNA que descodifican codones de cajas mixtas terminados en purinas</td>
<td>Mª Eugenia Armengod</td>
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<td>Acciones complementarias</td>
<td>Nanofármacos poliméricos utilizados como agentes simples y en terapia de combinación. Plataforma Tecnológica versátil para regeneración tisular y tratamientos anticancerígenos</td>
<td>Mª Jesús Vicent</td>
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<td>Acciones complementarias</td>
<td>Pathogenomics: Metabolómica e interactomic de la relación huésped-patógeno</td>
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<td>Ayudas a congresos</td>
<td>International symposium on Ionotropic Glutamat. Physiology, pathology and therapeutics</td>
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<tr>
<td>Ayudas a congresos</td>
<td>9º International Symposium on polymer therapeutics: from laboratory to clinical practice</td>
<td>Mª Jesús Vicent</td>
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### Fundación Española para la Ciencia y la Tecnología

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<td>Ayudas para el programa de cultura científica y de la innovación</td>
<td>Semana de la Ciencia en el CIPF</td>
<td>Vanesa Pérez</td>
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### Foundations and other Private Entities

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<td>Fundación Renal Tomás de Osma</td>
<td>Validación de inhibidores de apoptosis como agentes preservantes de órganos en trasplante renal</td>
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<td>Fundación Gent per Gent</td>
<td>Análisis de gemelosdiscordantes para investigar la correlación entre alteraciones en expresión y metilación de DNA en Lupus eritematoso</td>
<td>Ana Conesa</td>
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<td>Epilepsia progresiva mioclónica de Lafora: Bases fisiopatológicas de la enfermedad y aproximaciones terapéuticas</td>
<td>Erwin Knecht</td>
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<td>Fundación La Marató TV3</td>
<td>Study of cohesion functions in Cornelia de Lange Syndrome</td>
<td>Susana Rodríguez-Navarro</td>
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### Contracts Research

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<td>DEMETER</td>
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<td>Europath Bioscience</td>
<td>Prototipo de sistema experto de tratamiento y diagnóstico oncológico personalizado para enfermedades de cáncer</td>
<td>Joaquín Dopazo</td>
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<td>Caracterización celular y molecular de células troncales derivadas de tejido adiposo de biopsias subcutáneas de perro adulto</td>
<td>Victoria Moreno</td>
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<td>Fundación Cugat</td>
<td>Proyecto de Investigación en factores de crecimiento 2012-2013</td>
<td>Victoria Moreno</td>
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<td>FIIDS</td>
<td>Dispositivo para la eliminación del platino en el flujo sanguíneo</td>
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<td>Europath Bioscience</td>
<td>Dispositivo para la eliminación del platino en el flujo sanguíneo</td>
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<td>SALVAT</td>
<td>Diseño de sistema de transporte adecuado para aplicaciones oftalmológicas</td>
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### Collaborative Research

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<td>Joaquín Dopazo</td>
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<tr>
<td>Kutxa</td>
<td>Red de Investigación en neurogénesis en enfermedades de Parkinson en la CV</td>
<td>Jose Manuel García Verdugo</td>
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### Innovation & Technology Transfer

#### Patents Portfolio

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<td>Beta-lactam compounds that inhibits APAF1</td>
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<td>Novel conjugates of polymers having a therapeutically active agent</td>
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<td>and an angiogenesis targeting moiety attached thereto and uses thereof in the treatment of angiogenesis related diseases</td>
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<td>Ex-vivo method for the early diagnosis of Minimal Hepatic Encephalopathy by means of the determination of 3-nitrotyrosine in serum</td>
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<td>Difluorobenzyl ethanolamine derivatives with antimicrobial activity</td>
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<td>Triazine derivatives and their uses as TRPV1 inhibitors</td>
<td>WO/2012/136873</td>
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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 2012, the CIPF has set up collaboration agreements with 3 new spin-off companies:

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: Ana Conesa, Stefan Götz
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: MP Jesús Vicent, Richard England
Website: http://polythers.com/index.html

Scientific collaboration

During 2012, 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 with the University of Helsinki.
- Collaboration Agreement with the García Cugat Foundation.
- Collaboration Agreement with the UPV.
- Collaboration Agreement with the Foundation for Research and Innovation for Social Development.
- Collaboration Agreement with the University of Catania.
- Collaboration Agreement with the Ministry of Education, Culture and Sports to manage aid to promote the mobility of Human Resources in Research.
- Collaboration Agreement with the University of Genoa.
- Collaboration Agreement with the Ministry for conducting student placements.
- Collaboration Agreement with the University Complutense of Madrid for hosting students in the fields of Bioinformatics and Computational Biology.
- Collaboration Agreement with the CSIC to carry out a research project in Platelet Rich Plasma.
- Collaboration Agreement with Bull to create the Chair of Computational Genomics.
Facts & figures
Table 1 - Research & Support Staff

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<td>Technical</td>
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<td>Researchers</td>
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<td>Students</td>
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Table 2 - Research & Support Staff by academic studies

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<tr>
<td>Computer Scientists</td>
<td>2</td>
</tr>
<tr>
<td>Industrial Engineers</td>
<td>1</td>
</tr>
<tr>
<td>Journalists/Communication</td>
<td>2</td>
</tr>
<tr>
<td>Telecommunications</td>
<td>1</td>
</tr>
<tr>
<td>Others</td>
<td>12</td>
</tr>
<tr>
<td>Students</td>
<td>85</td>
</tr>
<tr>
<td>TOTAL</td>
<td>317</td>
</tr>
</tbody>
</table>

Personnel

- 59% Research Staff
- 26% Support Staff: Technical
- 15% Support Staff: Management

CHARTER 1 - Research and support staff
Table 3 - Research Personnel (staff and collaborator) with PhD

<table>
<thead>
<tr>
<th>Category</th>
<th>Nr. of persons</th>
</tr>
</thead>
<tbody>
<tr>
<td>PhD Research Personnel</td>
<td>94</td>
</tr>
<tr>
<td>Non PhD Research Personnel</td>
<td>64</td>
</tr>
<tr>
<td>TOTAL</td>
<td>158</td>
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</table>

Table 4 - Classification by labor relationship

<table>
<thead>
<tr>
<th>Staff</th>
<th>Nr. of persons</th>
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</thead>
<tbody>
<tr>
<td>Structure positions</td>
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</tr>
<tr>
<td>Positions vinculated to research projects</td>
<td>87</td>
</tr>
<tr>
<td>PhD Fellowships</td>
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<tr>
<td>FPI</td>
<td>9</td>
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<tr>
<td>FPU</td>
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<tr>
<td>Becas Pre del ISCIII</td>
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<tr>
<td>Val i+d C. Educación</td>
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<tr>
<td>Becas CIPF</td>
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</tr>
<tr>
<td>External Research Staff</td>
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<tr>
<td>Collaborator Researchers</td>
<td>46</td>
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<tr>
<td>CIBERDEM - Diabetes y Enfermedades Metabólicas Asociadas</td>
<td>3</td>
</tr>
<tr>
<td>CIBERER - Enfermedades Raras</td>
<td>6</td>
</tr>
<tr>
<td>Joint Units Personnel</td>
<td>6</td>
</tr>
<tr>
<td>Students</td>
<td>85</td>
</tr>
<tr>
<td>TOTAL</td>
<td>317</td>
</tr>
</tbody>
</table>

Funding

Table 1 - Income in 2012

<table>
<thead>
<tr>
<th>Source</th>
<th>Amount (in KE)</th>
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<tbody>
<tr>
<td>Privately-funded fellowships</td>
<td>84</td>
</tr>
<tr>
<td>Conselleria de Sanidad</td>
<td>4400</td>
</tr>
<tr>
<td>Contract and Privately-funded research</td>
<td>537</td>
</tr>
<tr>
<td>Donations</td>
<td>51</td>
</tr>
<tr>
<td>Regional Competitive Funding</td>
<td>510</td>
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<tr>
<td>Instituto de Salud Carlos III</td>
<td>948</td>
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<tr>
<td>National Competitive Funding</td>
<td>1940</td>
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<tr>
<td>European and International Funding</td>
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Table 2 - Funding Agencies in 2012

<table>
<thead>
<tr>
<th>Funding Entities</th>
<th>New grants awarded in 2012</th>
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<tbody>
<tr>
<td>Regional</td>
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<tr>
<td>Generalitat Valenciana (GVA) Conselleria de Educación</td>
<td>9</td>
</tr>
<tr>
<td>National</td>
<td></td>
</tr>
<tr>
<td>Ministerio de Economía y Competitividad</td>
<td>6</td>
</tr>
<tr>
<td>Instituto de Salud Carlos III</td>
<td>1</td>
</tr>
<tr>
<td>FECYT</td>
<td>1</td>
</tr>
<tr>
<td>International</td>
<td></td>
</tr>
<tr>
<td>CE - Dirección General de Investigación e Innovación (DG RESEARCH), Cooperación - Salud</td>
<td>3</td>
</tr>
<tr>
<td>Private</td>
<td></td>
</tr>
<tr>
<td>Fundación Gent per Gent</td>
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</tr>
<tr>
<td>TOTAL</td>
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</tbody>
</table>
## Events

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

<table>
<thead>
<tr>
<th></th>
<th>Nr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seminars</td>
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<tr>
<td>Congresses</td>
<td>3</td>
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<tr>
<td>Meetings</td>
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<td>Symposia</td>
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<td>Workshops</td>
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<tr>
<td><strong>TOTAL</strong></td>
<td><strong>29</strong></td>
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</tbody>
</table>

### Table 3 - Active Grants by Agency in 2012

<table>
<thead>
<tr>
<th>Funding Entities</th>
<th>Active Grants in 2012</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Regional</strong></td>
<td></td>
</tr>
<tr>
<td>Generalitat Valenciana (GVA) - Conselleria de Educación</td>
<td>11</td>
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<tr>
<td><strong>National</strong></td>
<td></td>
</tr>
<tr>
<td>Ministerio de Economía y Competitividad</td>
<td>43</td>
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<tr>
<td>Instituto de Salud Carlos III</td>
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<tr>
<td>Ministerio de Sanidad y Política Social</td>
<td>1</td>
</tr>
<tr>
<td>Ministerio de Educación</td>
<td>3</td>
</tr>
<tr>
<td>Agencia Española de Cooperación Internacional y Desarrollo (AECID)</td>
<td>2</td>
</tr>
<tr>
<td>FECYT</td>
<td>1</td>
</tr>
<tr>
<td>CDTI</td>
<td>1</td>
</tr>
<tr>
<td><strong>International</strong></td>
<td></td>
</tr>
<tr>
<td>CE - Dirección General de Investigación e Innovación (DG RESEARCH), Cooperación - Salud</td>
<td>6</td>
</tr>
<tr>
<td>CE - Dirección General de Investigación e Innovación (DG RESEARCH), Cooperación - Environnement</td>
<td>1</td>
</tr>
<tr>
<td><strong>Private</strong></td>
<td></td>
</tr>
<tr>
<td>Fundación La Marató de TV3</td>
<td>2</td>
</tr>
<tr>
<td>Fundación Gent per Gent</td>
<td>3</td>
</tr>
<tr>
<td>Fundación Renal Tomás de Osma</td>
<td>1</td>
</tr>
<tr>
<td>KUTXA</td>
<td>2</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>91</strong></td>
</tr>
</tbody>
</table>
**Thesis**

**Title:** Inhibición de complejos CDK/ciclina en modelos celulares de patología  
**PhD Student:** Tatiana Guevara  
**Director:** Dr. Enrique Pérez Payá, Universidad de Valencia

**Title:** Estudio del papel de la proteína activadora de apoptosis Apaf-1 en modelos celulares de la enfermedad de Huntington  
**PhD Student:** Andrés Herrera  
**Director:** Dr. Enrique Pérez Payá, Universidad de Valencia

**Title:** Caracterización estructural de Heparanasa por RMN. Nuevas estrategias en la búsqueda de fármacos antitumoral  
**PhD Student:** Silvia Mosulén Machuca  
**Director:** Dr. Antonio Pineda, Dr. Rodrigo Carbajo, Universidad de Valencia

**Title:** Funcionalizaciones estereoselectivas de iminolactonasquirales: Aplicación a la síntesis de peptidomiméticos  
**PhD Student:** Natalia Mateu Sanchis  
**Director:** Prof. Santos Fustero, Dr. José Luis Aceña, Universidad de Valencia

**Title:** Papel de los receptores TLR4 en el daño cerebral causado por el consumo de alcohol  
**PhD Student:** Silvia Alonso Loeches  
**Director:** Dr. Consuelo Guerri, Universidad de Valencia

**Title:** Differentiation of human Embryonic Stem Cells (hESC) into neural progenitors as a tool to study both the pathways during early brain development and the neuro-teratogenic effects of ethanol  
**PhD Student:** Jelena Kostic  
**Director:** Dr. Consuelo Guerri, Universidad de Valencia

**Title:** The Role of IRS2 in Testicular Development  
**PhD Student:** Richard Griffeth  
**Director:** Dr. Deborah Burks, Universidad de Valencia

**Title:** Polyacetalic systems as novel nanoconjugates for the treatment of prostate cancer  
**PhD Student:** Vanessa Giménez Navarro  
**Director:** Dr. Mª Jesús Vicent, Universidad de Valencia

**Title:** Nuevos sistemas alílicos y propargílicos fluorados: preparación y aplicaciones sintéticas  
**PhD Student:** Paula Bello García  
**Director:** Prof. Santos Fustero, Dr. Carlos del Pozo, Universidad de Valencia

**Title:** Nuevos sistemas alílicos y propargílicos fluorados: preparación y aplicaciones sintéticas  
**PhD Student:** Paula Bello García  
**Director:** Prof. Santos Fustero, Dr. Carlos del Pozo, Universidad de Valencia

**Publications**

**Table 1 - Thesis**

<table>
<thead>
<tr>
<th>Thesis in progress</th>
<th>Nr.</th>
<th>PhDs</th>
<th>Nr.</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>13</td>
<td>11</td>
<td></td>
</tr>
</tbody>
</table>

**Table 2 - Nr. of publications by type of journal**

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>103</td>
<td></td>
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<td></td>
<td>1</td>
<td></td>
<td>105</td>
<td></td>
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</tbody>
</table>

**Table 3 - Nr. of publications included in JCR**

<table>
<thead>
<tr>
<th>Included in JCR</th>
<th>Nr.</th>
<th>Not included in JCR</th>
<th>Nr.</th>
<th>TOTAL</th>
<th>Nr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>90</td>
<td></td>
<td>15</td>
<td></td>
<td>105</td>
<td></td>
</tr>
</tbody>
</table>

**Chart 1 - Publications by type of journal**

- Journals: 98%
- Books: 14%
- Books chapters: 1%

**Chart 2 - Publications included in JCR**

- Included in JCR: 98%
- Not included in JCR: 14%
### Table 4 - Publications by Laboratory

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Nr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systems Biology</td>
<td>18</td>
</tr>
<tr>
<td>Neurobiology</td>
<td>12</td>
</tr>
<tr>
<td>Genomics of Gene Expression</td>
<td>10</td>
</tr>
<tr>
<td>Cellular Pathology</td>
<td>7</td>
</tr>
<tr>
<td>Cytomics</td>
<td>7</td>
</tr>
<tr>
<td>Molecular Endocrinology</td>
<td>7</td>
</tr>
<tr>
<td>Structural Biochemistry</td>
<td>7</td>
</tr>
<tr>
<td>Intracellular Protein Degradation &amp; Rare Diseases</td>
<td>6</td>
</tr>
<tr>
<td>Neuronal &amp; Tissue Regeneration</td>
<td>6</td>
</tr>
<tr>
<td>Organic Molecules</td>
<td>5</td>
</tr>
<tr>
<td>Peptide &amp; Protein Chemistry</td>
<td>5</td>
</tr>
<tr>
<td>Polymer Therapeutics</td>
<td>5</td>
</tr>
<tr>
<td>Gene Expression Coupled to RNA Transport</td>
<td>3</td>
</tr>
<tr>
<td>RNA Modification &amp; Mitochondrial Diseases</td>
<td>3</td>
</tr>
<tr>
<td>Vascular Repair &amp; Regeneration</td>
<td>3</td>
</tr>
</tbody>
</table>

![Pie Chart - Publications by Laboratory](chart.png)