The history of our young center has been dominated by our effort to install infrastructure and consolidate the base for our future development. In recent years we have seen the fruits of this effort in our ever stronger publication record, the organization of important clinical studies and the recruitment of top flight researchers. A key to our success is the social commitment from the private sector through the ProCNIC Foundation. The additional support for the Center from this public-private partnership liberates our ambition to aim for excellence, and provides us with invaluable business and organizational expertise. Looking at the achievements of 2011, no one could doubt that the CNIC now ranks as one of the leading biomedical research centers on the world stage.

In a year filled with important landmarks for the CNIC, the crowning achievement was undoubtedly receiving the Premio Severo Ochoa in November. The Severo Ochoa Centers of Excellence Program is a new government initiative that celebrates and rewards world-class excellence in Spanish research. The CNIC is one of eight garlanded centers, including Life and non Life Sciences centers, chosen from a cohort of seventy-five applicants in a highly competitive selection procedure. Twenty-two shortlisted candidates were assessed by panels of international experts led by nobel laureates in their respective fields. The Life Sciences and Medicine panel, chaired by Robert Huber of the Max-Planck-Institut für Biochemie, selected the CNIC among just three research institutes in this area.

In the selection procedure, we presented a basic and translational research program based on the development of advanced imaging technologies for early diagnosis and prevention of cardiovascular disease. We will develop new fusion imaging technologies and magnetic particle imaging, and generate probes to detect and characterize atherosclerotic lesions. These advances will potentiate our clinical studies exploring new frontiers in the detection of atherosclerosis burden and inflammation. Imaging results from patients will be correlated in population studies with genetic and molecular parameters such as mitochondrial DNA sequence, the link between telomere length and atherosclerosis, and the implication of aging pathways in cardiovascular disease, including age-related brain degeneration. The CNIC’s Severo Ochoa program will have a major positive impact, not only because improved non-invasive imaging techniques will allow rapid, non-disruptive diagnosis, but also because these methods are increasingly valuable for basic and preclinical studies, clinical research, and population studies.

In 2011, Simón Mendez Ferrer, who joined the Center in 2010, received a prestigious International Early Career Scientist award from the Howard Hughes medical Institute, to take effect in January 2012. Simón, one of 28 recipients of the award in its inaugural year, will be supported by €625,000 and will join a network of other world-class scientists across the globe. Another momentous achievement was the selection of Francisco Sanchez Madrid’s GENTRIS project for a European Research Council Advanced Grant. This award recognizes Francisco’s status as a world authority in the field of immune and inflammatory cell interactions, and is a testament to the originality of his ground-breaking research into the exchange of genetic information between cells during immune interactions. And one of us (Valentin Fuster) received, under the aegis of the French Academy of Sciences, the Grand Prix Scientifique from the Lefoulon-Delalande Foundation, considered the highest award in the cardiovascular field.

The CNIC continued its strong publication record last year, increasing the number of citation-indexed papers to 150, up from 111 in 2010. Since 2008, the number of publications with an index above 10 has increased sevenfold. Among many outstanding papers in 2011 were two landmark basic science publications, one in Science, on fundamental mechanisms of tissue specification in the developing embryo (Miguel Torres’s group), and another in Cell, on key cell interactions that determine cell shape and motility (Miguel Ángel del Pozo’s group). The Center’s commercial activity also continues to strengthen, with work underway on the commercialization of 12 patent families.

The total competitive funding raised by the CNIC between 2008 and 2011 is approximately €40 m. The receipt of this investment, based on merit, brings an additional level of security to the CNIC project, leaving the Center better placed to navigate difficult economic waters. External funding is also powering our search for new talent. The European Commission financed COFUND Programme for the recruitment of young group leaders, which activated in 2011, provides €2.4 m, ensuring that the CNIC continues to attract the brightest young group leaders. Five shortlisted candidates presented their research proposals to the CNIC community in October, and their applications are now being evaluated by the members of the Scientific Advisory Board (SAB), the CNIC’s external panel of international experts.

Linked to our mission to foster the career development of talented scientists, the career structure at the CNIC was overhauled last year with the definition of three tiers for group leaders: Assistant, Associate and Full Professor. Important new incorporations during 2011 included Jesús Vázquez, who leads the new Cardiovascular Proteomics group in the Vascular Biology and Inflammation Department. Jesús, who joins as a Full Professor, is a highly creative scientist working at the frontiers of proteomics technology; his incorporation will strengthen both basic research and the technical capabilities at the CNIC. Also joining the VBI Department, as an Associate Professor, is Almudena Ramiro. Her research into B cell biology is supported by an ERC starting grant, bringing the number of ERC grants in the VBI Department to four: three Starting Grants and one Advanced Grant.
The Department of Cardiovascular Development and Repair was formed last year by the union of the former departments of Regenerative Cardiology and Developmental Biology, streamlining our research into how the cardiovascular system is built, maintained and repaired. Another important change was the creation of the Translational Platform. The Platform, which replaces the Translational Cardiovascular Research Department, links CNIC research to the science and technology sector and coordinates our commercial and intellectual property activity.

Our major investment in infrastructure culminated last year in the formation of the Advanced Imaging Unit. The AIU spearheads our program to identify people at risk of cardiovascular disease, understand the early steps of the disease, and design preventive strategies. A fundamental element of this program is the Cardiovascular Imaging Laboratory, inaugurated last September in the Carlos III Hospital. The laboratory, situated a short walk from the CNIC building, is dedicated to pioneering non-invasive imaging studies in humans. With these facilities up and running, the AIU will allow us to rapidly transfer advances from animal studies to the clinic, and is already contributing to our clinical projects.

This contribution is evident in the PESA project, supported by Grupo Santander and the Funcación Botín. The capacity for non-invasive imaging established in the AIU, together with the funding from the Premio Severo Ochoa, is giving additional impulse to this study of subclinical atherosclerosis in middle-aged participants, and plans are underway to expand the study to include a greater range of high-throughput analyses of imaging data and molecular parameters. In the METOCARD-CNIC study, exploring the benefit of early β-blocker treatment after myocardial infarction, myocardial infarction, imaging analyses are being carried out in the Cardiovascular Imaging Laboratory.

The CNIC-FERRER Polypill is now marketed in Guatemala, and commercialization is planned soon in Argentina and Mexico. Last year saw the launch of the European Commission funded FOCUS study, which examines the efficacy of the polypill and explores the factors that determine poor treatment adherence in a cohort of 4000 patients across 80 centers and five countries.

Progress also continued in the IMJOVEN study, which examines the risk of myocardial infarction in young women, and the Aragon Workers Health Study (AWHS) into cardiovascular risk factors in car-plant workers at General Motors (Zaragoza). The number of participants recruited to the CNIC’s major clinical projects now stands at more than 8000.

Through our partnership with the Mount Sinai School of Medicine, four scientists were selected last year to receive training in molecular-level imaging in New York. And we are beginning to see the return on this investment in people, with five returnees from the CARDIOJOVEN and CARDIOIMAGE programs actively collaborating in CNIC projects. Our agreements with centers in the Spanish National Health system are also helping to create a close-knit web of translational researchers. Over 900 people have participated in CNIC workshops, and 25 medical professionals have enrolled on our training programs. Furthermore, the CNIC’s open human resources program has brought around 30 professionals from the Spanish National Health System to work on translational projects at the CNIC through stable part-time positions and collaboration agreements. Through these programs, the CNIC is creating a strong base of talented and expertly trained researchers, capable of leading high-quality translational projects.

Last year also saw the launch of our new cycle of international CNIC Conferences. These 1.5-2 day meetings bring together scientists with common interests in a particular field of cardiovascular research. The inaugural conference, held in October, examined advances our understanding of cardiac gene expression and regulation. A second conference, on inflammation, aging and imaging, is planned for March 2012. These conferences provide opportunities for discussion and exchange of ideas at the frontiers of cardiovascular research, and help raise the CNIC’s profile within the international scientific community.

The core of the CNIC team is the 300 plus scientists and support staff based at the CNIC site and elsewhere, but our project would be impossible without the contribution of our many partners. The SAB gives essential critical underpinning to our scientific program, and the long term vision of the ProCNIC Foundation in straitened times provides invaluable financial support and business knowhow. Our collegial relationships with Johns Hopkins University, Mount Sinai Medical Center and other centers around the world integrate our work with that of our peers and provide unique opportunities for training and the exchange of knowledge. Through our collaborations with industrial partners and with hospitals and emergency services we can undertake clinical projects that bring knowledge advances to real-world applications of social benefit. And above all our work is built on the continued commitment of the Spanish government and people. It is this recognition by society of the value of scientific enterprise that will ensure real improvements in public health and quality of life.
Research Departments

Cardiovascular Development and Repair

A. Cardiovascular Developmental Biology Program

Genetic control of organ development and regeneration
Miguel Torres

Intercellular signaling in cardiac development, disease and tissue homeostasis
José Luis de la Pompa Mínguez

Stem cells in organ generation, regeneration and aging
Ignacio Flores Hernández

Development of the epicardium and its role during regeneration
Nadia Mercader

Role of new genes in cardiovascular development
Juan José Sanz Ezquerro

B. Stem Cell Biology Program

Functional genomics of embryonic pluripotency and heart development
Miguel Manzanares

Gene expression and genetic stability in adult stem cells
Antonio Bernad

Stem cell niche pathophysiology
Simón Méndez Ferrer

Cardiovascular related risks of obesity
Beatriz González Gálvez

Cellular signaling
Kenneth Mc Creath

C. Tissue Homeostasis and Repair Program

Functional genetics of the oxidative phosphorylation system
José Antonio Enriquez

Stem cell aging
Susana González

Nuclear receptor signaling
Mercedes Ricote

Molecular regulation of heart development and disease
Enrique Lara Pezzi

Vascular Biology and Inflammation

Gene regulation in cardiovascular and inflammatory diseases
Juan Miguel Redondo

Intercellular communication in the inflammatory response
Francisco Sánchez-Madrid

Integrin signaling
Miguel Ángel del Pozo

Cardiovascular proteomics
Jesús María Vázquez Cobos

Matrix metalloproteinases in angiogenesis and inflammation
Alicia García Arroyo

B cell biology
Almudena Ramiro

Immunobiology of inflammation
David Sancho

Epidemiology, Atherothrombosis and Imaging

Cardiovascular imaging
Valentín Fuster

Molecular and genetic cardiovascular pathophysiology
Vicente Andrés

Imaging in experimental cardiology
Borja Ibañez

Imaging cardiovascular inflammation and the immune response
Andrés Hidalgo

Vascular wall remodeling and cardiovascular disease
Carlos Zaragoza

Cardiovascular epidemiology and population genetics
Valentín Fuster

Multi-Departamental Clinical Projects

IMJOVEN

AWHS

PESA CNIC- GRUPO SANTANDER AND FUNDACIÓN BOTÍN

POLYPILL CNIC-FERRER

METOCARD-CNIC

Translational Platform

Transgenics

Genomics

Pluripotent Cell Technology

Proteomics

Bioinformatics

Cellomics

Viral Vectors

Comparative Medicine

Appendix

Publications 2011

Training Programs and Courses

Seminars, Events and Awards

Strategic Alliances

Funding

Staff Figures
Research Departments

1. Cardiovascular Development and Repair
Research Departments

1 Cardiovascular Development and Repair

The Department of Cardiovascular Development and Repair seeks to understand how the cardiovascular system is built, maintained and repaired. Our research programs examine the molecular and cellular basis of cardiovascular development, cardiovascular homeostasis and repair, and the role of stem-cell biology in these processes.

DIRECTOR: Miguel Torres

PROGRAM COORDINATORS: José Luis de la Pompa, Miguel Manzanares and José Antonio Enríquez

DEPARTMENT MANAGERS: Beatriz Ferreiro (coordinator), Ángel Ciprés and Isabel Barthelemy

DEPARTMENT LOGISTICS: Teresa Casaseca and Mª Ángeles Oliva

ADMINISTRATIVE SUPPORT: Sandra Cillero and Marta Ramón

A. Cardiovascular Developmental Biology

We study how cardiac lineage specification occurs and the signaling mechanisms that regulate cellular proliferation and patterning of the different cardiac regions that will form the mature heart. We want to unravel how alterations in these mechanisms lead to cardiovascular disease and how they can be manipulated to repair a diseased heart.

Program Coordinator: José Luis de la Pompa

B. Stem Cell Biology

Our aim is to understand the role of stem and progenitor cells in the development and maintenance of the cardiovascular system, as well as their contribution to the repair of the diseased state. We study different stem-cell populations—including embryonic, mesenchymal, cardiac and hematopoietic populations—in order to understand common and type-specific aspects of stem-cell biology that can be translated to the cardiovascular setting.

Program Coordinator: Miguel Manzanares

C. Tissue Homeostasis and Repair

We aim to understand the molecular and cellular processes that control the response of the cardiovascular system to acute and chronic damage resulting from large and small scale injury. We are interested in how cells and tissues adapt to and regulate oxygen availability, how the cardiovascular system communicates with other body systems, and how innate cardiovascular repairing mechanisms function and could be enhanced to treat disease.

Program Coordinator: José Antonio Enríquez
Research Departments

1 Cardiovascular Development and Repair

A. Cardiovascular Developmental Biology

Genetic control of organ development and regeneration

Head of Laboratory: Miguel Torres
Research Scientists: Laura Carramolino
Silvia Martín Puig
Postdoctoral Researchers: Cristina Clavería
Ricardo Costa
Daniel A. Felix
Mónica González Lázaro
Laura Padrón de Vaumas
Alberto Roselló-Díez
Predoctoral Researchers: Daniel Mateos
Cristina Villa
Master Student: Covadonga Díaz
Technicians: Beatriz Escobar
Joana Fuentes
Lucía Muñoz
Rocío Sierra
Susana Temiño
Visiting Scientists: Bouke de Boer, AMC, Amsterdam, The Netherlands (22-27 Jan)
Maria Kaffe, German Research Center for Environmental Health, Munchen, Germany (20 Jul – 5 Aug)

RESEARCH INTEREST

Our work focuses on three areas: the role of transcription factors and the environment in cardiovascular and limb pattern formation, the use of new genetic mosaic approaches to study the cellular basis of organ morphogenesis and homeostasis, and the role of hypoxia during cardiogenesis.

Our work on pattern formation has identified a novel mechanism, through which antagonistic diffusible signals, not autonomous mechanisms, control a network of transcription factors (Hox-TALE) to form the distinct structures of the vertebrate limb. This study has contributed to the understanding of how embryonic cells obtain and interpret instructions to produce body structures and organs in the correct spatio-temporal order. We are now studying the relevance of this mechanism to heart development and characterizing the role of TALE transcription factors in angiogenesis.

In our work on genetic mosaics, we have developed two new strategies to analyze the cellular basis of organ development and homeostasis. In one, an in vivo clonal analysis is used to define cell lineage and topological relationships among cardiovascular lineages during embryonic development and adult homeostasis. The second strategy allows the generation of random genetic mosaics, and has enabled us to demonstrate that cell competition in the early mouse embryo is a driving force for the maintenance of cell quality in stem cell pools. These findings have been submitted for publication and we are now analyzing the role of cell competition in cardiac development, homeostasis and regeneration.

For the study of hypoxia, we have generated conditional gain- and loss-of-function lines for the canonical hypoxia regulators HIF and VHL. These mice are being used to analyze the consequences of altering physiological embryonic hypoxia on the behavior of cardiovascular progenitors. We are also determining the relative oxygen levels in different regions of the embryonic heart in order to understand the distribution of cardiac populations within hypoxic niches.

![Cell-resolution 3D reconstruction from confocal microscopy data of a mosaic E10.5 mouse heart. The image shows a lateral view of the heart tube, comprising the atrioventricular canal, outflow tract and the left and right ventricles. Nuclei are depicted in red.](image-url)
Details of vascular development in live zebrafish. CD41-positive cells (green) can be observed intimately associated with the developing vasculature. The main axial vessels, the aorta and cardinal vein, run along the bottom of the image, with intersomitic vessels sprouting upward.

Oxygen distribution in the E9.0 mouse heart. Transverse confocal section of an E9.0 mouse embryo showing the expression of the LIM-homeodomain transcription factor Isl1 (red) and hypoxic regions labeled with pimonidazole (green). Note the high degree of hypoxia in the neural tube and pharyngeal mesoderm area, while a lower level of hypoxia is detected within the heart tube. Nuclei are revealed by Dapi staining (blue).

MAJOR GRANTS

- COST – European Cooperation in the field of Scientific and Technical Research (EU RTD FP7, Ref. BM0805). PI and Action Chair: M. Torres
- Ministerio de Ciencia e Innovación. FIS RETICS (TERCEL: RD06/0010/0008)
- Ministerio de Ciencia e Innovación (BFU2009-08331)
- European Commission FP7, Initial Training Network (28600)
- Comunidad de Madrid (S2010/BMD-2315)
- Comunidad de Madrid (S2010/BMD-2542). PI: S Martín Puig
- Ministerio de Ciencia e Innovación. FIS (CP09/00100). PI S. Martin Puig
- European Commission FP7. Marie Curie European Reintegration Grant (276891). PI S. Martin Puig
- European Commission FP7 Marie Curie (IEF-GA-2009-251226). PI: R. Costa

SELECTED PUBLICATIONS

Kovacic J, Mercader N, Torres M, Boehm M, Fuster V. Epithelial- and endothelial- to mesenchymal transition: from cardiovascular development to disease. Circulation (accepted)


A Roselló-Diez, Torres M. Regulative patterning in limb bud transplants is induced by distalizing activity of apical ectodermal ridge signals on host limb cells. Dev Dyn (2011) 240: 1203-11

We are interested in the signals that regulate cardiac development and homeostasis and how these are altered in disease. Last year we continued our work on the role of the Notch pathway, which signals between adjacent tissues to direct cell fates. Our work examines Notch function in cardiac-valve, ventricular-chamber and coronary-vasculature development, modified Notch function in aortic stenosis models, and the implication of Notch and other factors in zebrafish heart and fin regeneration. To address these issues, we use a combination of state-of-the-art mouse and zebrafish genetics, cell biology, biochemistry and whole-genome and image analysis.

Notch is active in epicardial progenitors, and is crucial for coronary artery differentiation and the generation of signals involved in compact myocardium development. Spatial expression reconstruction and analysis of ventricular chamber development indicates that Notch is sequentially activated by the ligands Dll4 and Jag1, with Dll4 activating Notch in the early chamber and Jag1 taking over as development proceeds. Signaling by both ligands depends on their modification by the ubiquitin ligase Mind bomb1 (Mib1). Myocardium-specific Mib1 inactivation affects ventricular chamber maturation and function, producing a phenotype reminiscent of certain human cardiomyopathies. Gain-of-function analysis supports a regulatory role for Notch in this process. Our studies of the role of Notch in aortic valve disease center on the influence of endothelial inflammation—using mice doubly deficient for ApoE and Notch—and the role of other Notch-interacting signals in the onset of valve disease.

In zebrafish, Notch signals are reactivated after cardiac damage, and are required for repair. Ectopic Notch activation impairs fin repair, suggesting that Notch maintains blastema cells in an undifferentiated, progenitor-like state and that it must be deactivated to allow differentiation of the repaired tissue.
MAJOR GRANTS

- Fundació La Marató de TV3 (081731)
- European Commission FP7. Initial Training Network (215761 and 28600)
- Centro Nacional de Investigaciones Cardiovasculares (CNIC-09)
- Ministerio de Ciencia e Innovación. FIS RETICS (TERCEL: RD06/0010/1013 and RECAVA II: RD06/0014/0038)
- Ministerio de Ciencia e Innovación (SAF2010-17555)
- Ministerio de Ciencia e Innovación. FIS (CD08/00257). PI: B. Prados
- Ministerio de Ciencia e Innovación. FIS (CD09/00452). PI: M. Nus
- Ministerio de Ciencia e Innovación (JCI-2010-06343). PI: B. Martínez Poveda

SELECTED PUBLICATIONS


The promise of regenerative medicine is now a reality. Successful cases of enhanced repair with stem cells have been achieved in tissues with high turnover rates, such as the skin or the hematopoietic system. However, for tissues with limited regeneration capacity, like the heart, progress to the clinic is more challenging. Nevertheless, the fact that a subpopulation of cardiomyocytes can divide after infarction or pressure overload suggests that the heart may contain an internal self-healing mechanism. Stimulation of such a mechanism could be used to partially replenish those cells that are lost after a heart attack or during normal aging. Achieving this goal requires deeper understanding of the nature of the replicating cells, their putative progenitors and the pathways that control their fate.

We are interested in the location, prevalence and status of different stem cell populations and their progeny during organogenesis and aging, focusing primarily on cardiac cells. Our experimental approach exploits our recent finding that longer telomeres are a general feature of adult stem cell compartments. We are also interested in characterizing potential regulators of telomere length during the course of stem cell differentiation, with the aim of defining their contribution to cell fate determination. Finally, we are also interested in how cells sense different amounts of telomerase and telomeres during organogenesis and tissue maintenance. Through these efforts, we hope to achieve a more complete picture of the role of stem cells in organ formation and maintenance, which could lead to the development of improved regeneration therapies.

**RESEARCH INTEREST**

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**A. Cardiovascular Developmental Biology**

**Stem cells in organ generation, regeneration and aging**

**Head of Laboratory:** Ignacio Flores

**Postdoctoral Researchers:**
- Tania Aguado
- Cristina González Estévez

**Predoctoral Researchers:**
- Esther Aix
- Dorothea Bednarek
- Maria del Mar de Miguel

**Technician:**
- Irene de Diego

**Quantitative analysis of cardiac proliferation in zebrafish heart after infarction.**

A specifically tailored image analysis program was used to segment, classify and quantify proliferation of different subtypes of cardiac cells after infarction. This work was done in collaboration with Hind Azegrouz of the Cellomics Unit.
Apoptotic cell death in co-cultures of cells of different genotypes. One population expresses the marker act-GFP. Apoptotic cells of one genotype are detected when the cells are in close proximity to the other genotype, suggesting cell competition.

MAJOR GRANTS
- Ministerio de Ciencia e Innovación (SAF2009-10480)
- Ministerio de Ciencia e Innovación (RYC-2006-3067)
- Asociación Española contra el Cáncer. PI: Tania Aguado

SELECTED PUBLICATIONS


Our work is aimed at understanding the morphogenesis of the epicardium and its role as a source of cells and signals during development and regeneration. A second main goal is to elucidate the molecular mechanisms of fibrotic tissue degradation during heart regeneration.

The epicardium is a unicellular epithelial layer that envelops the myocardium. It derives from the proepicardium (PE), a group of cells that arises at the inflow tract of the forming heart. During development, epicardial derived cells (EPDCs) delaminate from the embryonic epicardium, undergo epithelial-mesenchymal transition (EMT), and differentiate into the smooth muscle and vascular endothelial cells of the coronary vasculature and cardiac fibroblasts. Differentiation of EPDCs into cardiomyocytes has been proposed but has not yet been confirmed. The epicardium also promotes the development of the myocardium through a paracrine action.

In the adult, myocardial damage leads to the rapid reexpression of epicardial genes such as Wilms tumour 1 encoding gene (Wt1). The early response to cardiac damage also includes the formation of a thickened epicardial cap over the injured area. These observations suggest a role for the epicardium as a source of signals and progenitor cells during cardiac regeneration.

We use the zebrafish as a model system to analyze the molecular mechanisms of epicardium formation in vivo. The zebrafish has the capacity to regenerate cardiac tissue after injury. We recently described the use of cryoinjury for inducing cardiac injury in zebrafish. Heart cryoinjury induces massive cell death and the formation of a fibrotic scar, resembling the outcome of myocardial infarction in mammals. However, unlike mammals, the zebrafish is able to remove these lesions and regenerate the lost tissue, indicating the existence of endogenous mechanisms to degrade fibrotic tissue and replace it with newly-formed cells.
Epicardial EMT during cardiac regeneration. Myosin heavy chain (red) and GFP (green) immunostaining on a sagittal section of a Tg(wt1b:GFP) cryoinjured heart at 3 days postinjury. Nuclei are stained with DAPI (blue). The epicardial layer is several cell layers thick and covers the injured area (asterisk).

Complete regeneration and scar removal after cryoinjury of the adult zebrafish ventricle. Picro-Mallory stained sagittal sections of adult zebrafish heart fixed at the indicated days after cryoinjury of 24% of the ventricle. Collagen is stained blue, damaged tissue red and myocardium brown. At 7 days postinjury (dpi) a massive collagen deposition can be observed, which subsequently regresses. At 100 dpi regeneration of the heart is almost complete. Scale bar, 100 µm.

### MAJOR GRANTS

- Ministerio de Ciencia e Innovación (BFU2008-00212)
- Ministerio de Ciencia e Innovación (RYC-2006-001694)
- Comunidad de Madrid (S2010/BMD-2321)

### SELECTED PUBLICATIONS

Neto A, Mercader N, Gómez-Skarmeta JL. The osr1 and osr2 genes act in the pronephric anlage downstream of retinoic acid signaling and upstream of wnt2b to maintain pectoral fin development. *Development* (accepted)


Our group investigates the molecular and cellular basis of organogenesis during embryonic development. We use gain- and loss-of-function approaches in chick and mouse embryos combined with in vitro cell culture models to dissect the role of new genes in the morphogenesis of the heart and other aspects of cardiovascular development.

Our main interest is the role of Arid3b, a transcription factor of the highly-conserved ARID family. Embryos of Arid3b-knockout (KO) mice die early in development and have severe craniofacial, limb and heart defects, but the precise functions of Arid3b in development remain unclear. We have shown that Arid3b is required for correct maturation of the apical ectodermal ridge in the growing limb, regulating cell motility. Our analysis of Arid3b expression during heart development shows that it is expressed from early stages in the myocardium of the tubular heart, as well as in the cardiac precursors in the pharyngeal mesoderm. Later, its expression gets restricted to the heart poles, structures derived from the second heart field.

A detailed anatomical and histological characterization of Arid3b KO embryos has revealed three main cardiac defects: a marked shortening of the outflow tract; a reduction in the size of the inflow region, with abnormal atria formation; and altered development of the atrio-ventricular (AV) canal, including defective formation of the AV cushions due to failed endothelial to mesenchymal transition. The expression of several molecular markers of the secondary heart field and the heart chambers is altered in mutant embryos. RNA microarray analysis of these embryos identified a set of differentially-expressed genes, which we are now validating for putative mediators of Arid3b functions.

We have also developed a conditional KO mouse line, in which loxP sites flank Arid3b. By crossing these animals with Cre-driver lines, we can remove Arid3b at different times and in specific tissues, avoiding the embryonic lethality of the full KO and allowing analysis of Arid3b function in particular organs and at later stages.
MAJOR GRANTS
- Fundació La Marató TV3 (082031)
- Ministerio de Ciencia e Innovación. FIS (CP07/00251)

SELECTION PUBLICATIONS
The central aim of our research is to understand how genome activity is regulated during development, and how this can contribute to human disease. For our approach, we identify regulatory sequences and study how they act on their target genes, organizing them into regulatory networks. This work is conducted through a combination of bioinformatics, comparative genomics, genome-wide analysis, and functional assays in transgenic mouse embryos, chick embryos, and stem cells.

We have shown that the pluripotency of embryonic cells is an evolutionary novelty in mammals. Using bioinformatics tools we found that the regulatory elements through which core factors control their downstream targets appeared de novo in the mammalian lineage. We have also analyzed the role of miRNAs in stem cells by deleting Dicer, finding that embryonic and extra-embryonic stem cells have different requirements for miRNAs. We also find that miRNAs do not have critical patterning or lineage-specification roles in the early embryo, but rather act as modulators of signaling pathways that ensure proper growth and proliferation.

In an effort to understand how regulatory elements interact with target genes, we have studied the genomic architecture of the Irx gene clusters, a family of homeobox transcription factors with crucial roles in heart development and function. We find that the chromatin factor CTCF acts to partition the regulatory landscape of the clusters, allowing differential expression of Irx genes in the heart. We have also participated in a genome-wide screen analyzing the evolutionary conservation of CTCF bound regions among vertebrates, which has established the importance of these regions in maintaining proper regulation of adjacent genes. Future studies will address how general this role of CTCF and chromatin domains is in regulating cardiac gene expression, and how it is linked to disease.
MAJOR GRANTS

- Ministerio de Ciencia e Innovación (BFU2008-00838)
- Ministerio de Ciencia e Innovación CONSOLIDER Project (CSD2007-00008)
- CNIC Translational Grants (08-2009)
- Comunidad de Madrid (S2010/BMD-2315)
- Ministerio de Ciencia e Innovación (JCI-2008-2980). PI: C. Arias

SELECTED PUBLICATIONS


An organism’s health and fitness depend on the preservation and functional maintenance of adult stem cells (aSCs). To investigate how stem cells balance the processes of self-renewal and differentiation, we work with cardiac progenitor cells (CPCs) isolated from adult mammalian heart and with mouse and human mesenchymal stem cells (MSCs).

A precise definition of cardiac precursor and stem cells is still lacking. We have continued with the molecular characterization of mouse, pig and human CPCs, characterized as MSC-like populations. Our recently submitted results indicate that Bmi-1 is a potential marker of mouse CPCs (mCPCs) and that two muscle-specific microRNAs, miRNA-1 and miRNA-133a, modulate the ability of several adult and embryonic stem cell populations to respond to cardiomyogenic signals. In another submitted study, transplantation experiments revealed that mCPCs genetically manipulated to overexpress miRNA-133a protect against the deleterious effect of acute experimental infarct. We are now investigating the mechanism underlying this activity.

Our differential analysis of miRNA expression has established that miR-335 is required to maintain hMSCs in the undifferentiated state, supporting the hypothesis that miR-335 downregulation is critical for the acquisition of reparative MSC phenotypes. In parallel we have investigated the influence of cell culture conditions on the ex vivo genomic stability and senescence of hMSCs. Our results show that the genetic instability of hMSCs is increased by culture at high oxygen tension (21%), which substantially alters intracellular metabolic parameters and renders the cells highly dependent on oxidative phosphorylation.
Transplantation of miR-133a-modified mCPCs protects the heart against myocardial infarction. A, B: Cardiac function analyzed by echocardiography before intervention (Baseline), and at 2 and 4 weeks after infarction and transplant, expressed in terms of fractional area change (A) and fractional shortening (B). The data reveal improved cardiac function in animals transplanted with miR-133a-modified mCPCs. C: Masson’s tricrome staining showing the infarcted area (blue) and healthy myocardium (red/pink) at 4 weeks post infarction. D: Quantification of scar area (% left ventricle area); n= 7.

Fluorescence in situ hybridization analysis of fixed nuclei from hMSCs, using specific centromere probes for chromosome 8 (red), chromosome 11 (green) and chromosome 17 (pale blue). Nuclei are stained with DAPI (blue). In normal cells (diploid) there are two signals for each color.

MAJOR GRANTS

- Ministerio de Ciencia e Innovación (INNPACTO-01-2011). Sub-project coordinator: A. Bernad
- Ministerio de Ciencia e Innovación (PLE2009-0147). Sub-project coordinator: A. Bernad
- Ministerio de Ciencia e Innovación (PLE2009-0100). Sub-project coordinator: A. Bernad
- Ministerio de Ciencia e Innovación (PLE2009-0112). PI: M. A. González de la Peña
- Ministerio de Ciencia e Innovación (PSE-010000-2009-3). Sub-project coordinator: A. Bernad
- Ministerio de Ciencia e Innovación (PIFNONEC08). PI: A. Bernad
- CNIC Translational Grants (13-2007). Sub-project coordinator: A. Bernad
- Comunidad de Madrid (S2010/BMD-2402)

SELECTED PUBLICATIONS


Stem cells reside in specialized niches that allow them to self-renew, proliferate, differentiate and migrate according to the organism’s requirements. Our group studies the mechanisms by which the stem cell niche fulfills these complex functions and how its deregulation contributes to disease.

Our earlier work described a tight regulation of the bone marrow stem cell niche by circadian oscillations of sympathetic activity. Light onset induces noradrenaline release from nerve terminals in the bone marrow, leading to downregulation of CXCL12/SDF-1, the only chemokine known to direct hematopoietic stem cell (HSC) migration. Our recent studies indicate that the stromal cells targeted by the sympathetic nervous system and that regulate this HSC traffic are Nestin+ mesenchymal stem cells (MSCs). Collaborative studies have recently shown that deregulation of this pathway contributes to poor HSC mobilization in diabetic subjects. An increased number of sympathetic fibers in the bone marrow of diabetic mice correlates with the inability of MSCs to down-modulate the production of CXCL12. HSC attraction to MSCs is also affected by other cells of the bone marrow microenvironment. A subset of monocytes promotes the retention of HSCs by MSCs in the bone marrow. MSCs regulate not only HSC traffic but also the egress of inflammatory monocytes from the bone marrow. How peripheral infections or inflammation promote monocyte egress from the bone marrow was not clear before. MSCs respond to pro-inflammatory cytokines by producing the chemokine CCL2/MCP1, which directs the egress of these monocytes from the bone marrow compartment toward the peripheral circulation.
Deficient hematopoietic stem cell mobilization in diabetes. Diabetic bone marrow shows several alterations compared with the healthy state: the content of hematopoietic stem cells (HSCs) is increased, and these cells are more proliferative; there are fewer osteoblasts; and there are more sympathetic nerve terminals, leading to impaired responsiveness of β3-adrenergic receptors (β3-AR) expressed on nestin+ MSCs, the major source of CXCL12. In healthy bone marrow, granulocyte colony-stimulating factor (G-CSF) decreases osteoblast numbers, releases norepinephrine (NE), which binds to β3-AR, and reduces CXCL12 expression in nestin+ MSCs, resulting in transmigration of hematopoietic stem cells to the peripheral circulation. In diabetic bone marrow, G-CSF induces a similar reduction of osteoblasts and CXCL12 expression in osteoblasts but no reduction of CXCL12 expression in nestin+ MSCs, thereby impeding HSC mobilization toward the peripheral circulation.

Model of the regulation of HSC and monocyte traffic in the bone marrow. Nestin+ MSCs, which can generate mesenchymal lineages in the bone marrow, regulate the egress of monocytes in response to Toll-like receptor ligands and also the traffic of hematopoietic stem cells (HSC). Both the production of CXCL12 by nestin+ MSCs and these cells’ attraction to HSCs are inhibited by sympathetic nerve fibers and stimulated by soluble factors produced by monocytes. CLP, common lymphoid progenitor; CMP, common myeloid progenitor; GMP, granulocyte-macrophage progenitor; MEP, megakaryocyte-erythroid progenitor; MPP, multipotential progenitor.

**MAJOR GRANTS**

- Howard Hughes Medical Institute. International Early Career Scientist. PI: Simón Méndez
- Ministerio de Ciencia e Innovación (RYC-2009-04703) PI: Simón Méndez
- Ministerio de Ciencia e Innovación (RYC-2011-09726) PI: Abel Sánchez-Aguilera
- Ministerio de Ciencia e Innovación (RYC-2011-09209) PI: Joan Isern
- Ministerio de Ciencia e Innovación (SAF-2011-30308) PI: Simón Méndez
- Comunidad de Madrid. (P2010-BMD-2342) PI: Simón Méndez
- European Commission FP7. Marie Curie Career Integration Grant (294262) PI: Simón Méndez
- European Commission FP7. Marie Curie Career Integration Grant (294096) PI: Abel Sánchez-Aguilera

**SELECTED PUBLICATIONS**


Understanding the biology of cardiac progenitor cells is an essential step toward their therapeutic use for cardiomyocyte restoration and functional heart repair. Our previous studies identified cardiac mesoangioblasts as precommitted progenitor cells in the postnatal heart, which can be expanded in vitro and efficiently differentiated in vitro and in vivo to contribute new myocardium after injury. Based on their proliferation potential in culture, we recently discovered that two mesoangioblast populations can be isolated from explant cultures of mouse and human heart. Although both populations express similar surface markers, together with a panel of instructive cardiac transcription factors, they differ significantly in their division rates and cellular mitochondria content. One population is composed of slow dividing (SD) cells containing many mitochondria, and can be efficiently differentiated with 5-azacytidine (5-aza) to generate cardiomyocytes expressing mature structural markers. The second population is composed of fast dividing (FD) mesoangioblasts that contain fewer mitochondria and do not respond to 5-aza.

We are exploring the relationship between mitochondrial content and differentiation potential in these mesangioblast populations by pharmacological manipulation of the number of mitochondria. Nitric oxide (NO) donors, by increasing mitochondrial load, reverse the differentiation block on FD mesoangioblasts and lead to a progressive maturation to cardiomyocytes. Conversely, to arrest cardiomyocyte differentiation in SD populations, we decrease mitochondrial content by administering respiratory chain inhibitors and chloramphenicol. We have also isolated cardiac mesangioblasts from the hearts of aged mice and human patients, and we are characterizing the number of mitochondria in these cells and their potential for differentiation into mature cardiomyocytes. Our findings illustrate a central role for mitochondria in cardiac mesangioblast differentiation and raise the interesting possibility that treatments that increase cellular mitochondrial content may have applications in cardiac stem cell therapy.
Growth curves for fast (black) and slow (white) dividing cardiac precursors.

Electron micrograph showing mitochondrial morphology in slow dividing cardiac precursors.

- Ministerio de Ciencia e Innovación (RYC2009-04669)
- Ministerio de Ciencia e Innovación (SAF2010-15239)


Impaired cellular energy metabolism can be considered a hallmark of many metabolic disease states such as heart failure and obesity, and can affect homeostatic signaling processes. G-protein coupled receptors (GPCRs) are a family of cell-surface signaling proteins involved in mediating many biological actions of the cardiovascular system, and encompass numerous therapeutically-attractive pharmacological targets. The succinate receptor, SUCNR1, is a recently discovered GPCR which is activated by binding of its natural ligand succinate, a tricarboxylic acid metabolite. Extracellular concentrations of succinate are known to increase after dysregulated energy metabolism and thus SUCNR1 can be described as a metabolic sensor of cellular injury, or loss of cellular homeostasis.

SUCNR1 is expressed in many metabolically-active tissues, such as myocardium and adipose tissue, and could potentially orchestrate cellular responses to micro-environmental stimuli such as hypoxia and dysregulated mitochondrial metabolism, two processes of pathological relevance in metabolic disease. To address the roles of SUCNR1 our laboratory has recently constructed both whole animal and also novel and specific loss-of-function murine models, using Cre-lox technology. We are currently using these models to study the roles of SUCNR1 in a variety of acute and chronic metabolic disorders.

**Research Departments**

**1 Cardiovascular Development and Repair**

**B. Stem Cell Biology**

**Cellular signaling**

**Head of Laboratory:** Kenneth J. McCreath  
**Research Scientist:** Ana M. Cervera  
**Postdoctoral Researcher:** Sandra Espada Serrano  
**Masters Student:** Enrique Gallego

**RESEARCH INTEREST**

Impaired cellular energy metabolism can be considered a hallmark of many metabolic disease states such as heart failure and obesity, and can affect homeostatic signaling processes. G-protein coupled receptors (GPCRs) are a family of cell-surface signaling proteins involved in mediating many biological actions of the cardiovascular system, and encompass numerous therapeutically-attractive pharmacological targets. The succinate receptor, SUCNR1, is a recently discovered GPCR which is activated by binding of its natural ligand succinate, a tricarboxylic acid metabolite. Extracellular concentrations of succinate are known to increase after dysregulated energy metabolism and thus SUCNR1 can be described as a metabolic sensor of cellular injury, or loss of cellular homeostasis.

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**MAJOR GRANTS**

- Ministerio de Ciencia e Innovacion (SAF2009-07965)

**SELECTED PUBLICATIONS**


Our group studies the biogenesis, structural organization and functional regulation of the OXPHOS system. Our main goal is to gain a molecular understanding of the role of the OXPHOS system in health and disease. We are especially interested in the role of mitochondria in the pathological consequences of ischemia/reperfusion and in how mitochondrial dysfunction impacts longevity and the progression of cardiovascular and neurodegenerative diseases. A longer term aim is to identify potential therapies for these conditions. Our approach involves functional genetic studies of genes encoded by the mitochondrial genome (mtDNA) and others encoded by the nuclear genome (nDNA). We are currently conducting a series of high-throughput screens based on a genome-wide lentiviral siRNA library, genome trap technologies, and mitochondrial proteomics. The purpose of this program is to identify and characterize genes required for the correct biogenesis and performance of the OXPHOS system. We are also studying the functional consequences of allelic variants of mtDNA and their influence on the protection from or development of disease. For this project, the group works with human and mouse cell lines and mouse disease models, and studies the disease association of common human mtDNA haplotypes.


* Joint 1st authors


MAJOR GRANTS

- Ministerio de Ciencia e Innovación (SAF2009-08007)
- Ministerio de Ciencia e Innovación CONSOLIDER project (CSD2007-00020)
- Comunidad de Madrid (S2010/BMD-2402)
- European Commission, FP7. Marie Curie Reintegration Grant. PI R Acín
- Ministerio de Ciencia e Innovación (RYC-2011-07826) PI: R Acín
- Ministerio de Ciencia e Innovación. FIS (CD10/00173) PI: C Colás

Model of hypoxia-induced stability of OXPHOS complexes and supercomplexes.

Model showing the involvement of NDUF4A4L2 induction by HIF-1α in hypoxic adaptation.
The INK4b-ARF-INK4a locus encodes three tumor suppressors, p15INK4b, ARF, and p16INK4a. Together, these factors constitute one of the most important sources of cancer protection in mammals, equalled in importance only by p53. These tumor suppressors have taken on additional importance in the light of recent evidence that at least one product of the locus, p16INK4a, also contributes to the decline in the replicative potential of self-renewing cells with age. Thus, on the one hand, p16INK4a promotes longevity through its action as a potent tumor suppressor, while on the other hand the increased expression of p16INK4a with age reduces stem and progenitor cell proliferation, ultimately reducing longevity. In other words, p16INK4a appears to balance the need to prevent cancer against the need to sustain regenerative capacity throughout life. These observations suggest the provocative but unproven notion that mammalian aging results in part from the effectiveness of tumor suppressor proteins at preventing cancer.

Our group is investigating the role and molecular regulation of the INK4b-ARF-INK4a locus in the context of self-renewal, proliferation and aging of hematopoietic stem cells in vitro and in vivo, with planned extension of these studies to cardiac stem cells. In parallel, we are developing tools for the study of the genetic and epigenetic mechanisms that regulate stem cells, and how these unique cells differentiate from a pluripotent to a more restricted state.

**Head of Laboratory:** Susana González  
**Postdoctoral Researcher:** Lorena Arranz  
**Predoctoral Researchers:** Antonio Herrera, Isabel Hidalgo  
**Visiting Scientist:** Ana Branco

### MAJOR GRANTS
- Human Frontier Science Program Organization (HFSPO). Career Development Award (CDA 0026/2006-C)  
- Ministerio de Ciencia e Innovación (SAF2010-15386)  
- Ministerio de Ciencia e Innovación. FIS (PI060627)

### SELECTED PUBLICATIONS


Nuclear hormone receptors constitute a superfamily of ligand activated transcription factors with diverse roles in development and homeostasis. Work by our group is contributing to the definition of a role for nuclear receptors in lipid metabolism and inflammatory responses in macrophages. We are interested in the roles of PPARs (peroxisome proliferator-activated receptors) and RXRs (retinoid X receptors) in two areas: chronic inflammatory disease and the homeostasis of adult stem cells.

We recently found that myeloid-specific PPARγ or RXRα knockout mice develop chronic renal inflammation and autoantibodies to nuclear antigens, a phenotype that resembles the nephritis seen in human systemic lupus erythematosus. This phenotype is caused by the impaired clearance of apoptotic cells by the knockout macrophages. These defects eventually lead to the development of cardiac hypertrophy, and we are currently trying to understand how the lack of PPARs and RXRs leads to this condition.

We are also exploring the role of PPARs and RXRs in the promotion and control of inflammation during cardiac repair and regeneration. Myocardial infarction is followed by an acute inflammatory response, leading to cell death and scar formation in the infarcted area and the development of fibrosis in non-infected myocardial regions. We are trying to understand how PPARs and RXRs might modulate cardiac repair and regeneration.

Our research into adult stem cells addresses the roles of PPARs and RXRs in the differentiation, proliferation and self-renewal of hematopoietic stem cells. We have generated hematopoietic-specific PPARγ and RXRα,β knockout mice, and have embarked on a research program to define the role of these nuclear receptors in the differentiation of bone marrow stem cells into diverse cell populations, including adipocytes and osteoclasts.

**RESEARCH INTEREST**

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Myocardial macrophage infiltration.
Immunofluorescence analysis of a mouse heart subjected to cryoinjury. Seven days after cryoinjury, sections were stained for the macrophage marker CD68 (red) and the endothelial marker CD31 (green), and with the nuclear stain Dapi (blue). Extensive infiltration of macrophages and endothelial cells can be seen throughout the cryolesion.

MAJOR GRANTS
- Ministerio de Ciencia e Innovación (SAF 2009-07466)
- Ministerio de Ciencia e Innovación. CDTI (Programa CENIT-2008 1004)
- Fundación la Mataró TV3 (MTV308)
- Fundación Genoma España. MEICA Project (PICPPFGE08)
- European Commission FP7. Marie Curie European Reintegration Grant (FP7-PEOPLE-2009-RG) PI L.Fuentes
- European Commission FP7. Marie Curie Intra-European Fellowships for Career Development (FP7-PEOPLE-IEF-2008) PI T Röszer

SELECTED PUBLICATIONS


*Ricote M, Vidal-Puig A. Myocardial macrophage infiltration.


Our lab is interested in the molecular mechanisms that regulate cardiac development and heart disease. One of our major goals is to understand the role of alternative splicing (AS) in these processes. AS is the molecular process that removes introns from immature pre-mRNAs and links exons together in different combinations. AS affects 86% of all human genes and is in part responsible for the great diversity of proteins that are generated from the relatively small number of genes found in the human genome.

Together with the Genomics and Bioinformatics Units at the CNIC, we have used RNA-Seq and exon microarrays to analyze the splicing pattern in heart failure. Using these data we have been able to identify cis-regulatory sequences and trans-regulatory splicing factors associated with AS. We will now analyze the roles of these factors in the heart through gain- and loss-of-function strategies.

A prime example of how alternative splicing can dramatically change protein function is the calcineurin variant CnAβ1. Calcineurin regulates a wide variety of physiological and pathological processes, including cardiac development and hypertrophy. CnAβ1 is a naturally occurring splice variant of the calcineurin Aβ gene which contains a unique C-terminal region, different from the autoinhibitory domain present in all other CnA isoforms. We previously showed that CnAβ1 regulates cell proliferation and enhances skeletal muscle regeneration. Our recent results show that CnAβ1 protects the heart from the effects of myocardial infarction by improving cardiac function and reducing inflammation and scar formation. This is achieved through the activation of the Akt signaling pathway and the transcription factor ATF4. We are now exploring the role of CnAβ1 in stem cells and in the developing embryo, where it is strongly expressed.

Histological analysis of an infarcted mouse heart. To induce a myocardial infarction, the left coronary artery was occluded for 30 minutes and then reperfused. After 28 days the heart was fixed and analyzed by the Masson’s Trichrome method. Cardiomyocytes and collagen fibers are stained in red and blue, respectively.
Alternative splicing of the β-myosin heavy chain gene in the infarcted myocardium. RNA was extracted from myocardial tissue 28 days after ligation of the left coronary artery and sequenced using SOLiD technology. Reads were mapped and then visualized using the Integrative Genomics Viewer. The figure shows the distributions of reads along the β-myosin heavy chain gene (Myh7). Reads mapped to Myh7 are shown in the central box. All exons and introns in the Myh7 gene are shown at the bottom. The upper part of the figure depicts the chromosomal region analyzed.

**MAJOR GRANTS**

- European Commission FP7. Marie Curie Reintegration Grant (CARDEB1-239158)
- Ministerio de Ciencia e Innovación (BFU2009-10016)
- Ministerio de Ciencia e Innovación. FIS (CP08/00144)
- BritishHeart Foundation (PG/08/084/25827). Co-PI Lara-Pezzi. Funds held at Imperial College London, UK
- European Commission FP7, Initial Training Network (28600). Coordinator: E. Lara-Pezzi
- Comunidad de Madrid (S2010/BMD-2321)

**SELECTED PUBLICATIONS**


Research Departments

2

Vascular Biology and Inflammation
Research Departments

2 Vascular Biology and Inflammation

The Department of Vascular Biology and Inflammation investigates interactions between the cells of the vascular system. Specific research lines address signaling by adhesion receptors and inflammatory mediators, autoimmunity and inflammation, physiological and pathological angiogenesis, and vascular wall remodeling. Groups within the department use a range of animal, tissue, cellular and molecular models to investigate normal vascular function and the key steps in the vascular alterations that underlie cardiovascular diseases.

DEPARTMENT DIRECTOR: Juan Miguel Redondo
DEPARTMENT MANAGER: Antonio Jesús Quesada
TECHNICIANS: Andrea Quintana
Juan José Lazcano
María José Gómez
ADMINISTRATIVE SUPPORT: Almudena Fernández
Eduardo Bieger
Many important biological processes, including the regulation and development of the immune and cardiovascular systems, are regulated by the calcineurin-NFAT (CN-NFAT) pathway. Much of our previous work relates to molecular interactions of CN with substrates. We are now studying the regulation and function of this pathway in inflammatory settings and cardiovascular disease.

Our work on angiogenesis addresses the regulation of CN in endothelial cells by VEGF. We use retinopathy of prematurity as a model of the mechanisms of neovessel formation in ischemic retinopathies, and are using lentiviral vectors to identify potential therapeutic targets.

We are also analyzing gene expression triggered by angiotensin II (AngII) in cardiomyocytes and vascular smooth muscle (VSM). This work is aimed at identifying molecular mediators of cardiac hypertrophy. We have found several CN-regulated genes in two mouse models of cardiac hypertrophy, and plan to characterize their roles in this pathology.

Through in vivo infection with lentiviral vectors encoding motifs important for CN-NFAT interactions, we can prevent or retard the development of arthritis in mice. In our system, inflammation is curtailed by infection of macrophages at distinct locations and the subsequent migration of these cells to inflammation sites.

We are also dissecting signaling pathways involved in vascular wall remodeling, a major feature of vascular diseases such as atherosclerosis, aneurysm, and restenosis. We have set up animal models of these pathologies, and have generated mice deficient for AngII-target molecules that are regulated by CN. Some of these animals are totally resistant to these diseases and we are working to elucidate the molecular and cellular mechanisms underlying this protection.
Cross-sections of uninjured and injured mouse femoral arteries stained with hematoxilin-eosin (H&E) and Van Gieson's stain (VG).
The group’s present work focuses on key cell-to-cell communication events during cognate immune interactions. A key goal is to define how the microtubule organizing complex (MTOC), by controlling cytoskeletal rearrangements at the immune synapse (IS), provides a mechanism for macromolecular transport and the concentration of signaling molecules during synaptic contact. This research program has the potential to reveal how transfer of miRNA between the T cell and the cognate antigen presenting cell (APC) regulates the early initiation of immunity. We are also developing methodologies for the in vivo imaging of immune cell infiltration, the inflammatory response and the role of immunoregulatory molecules (galectins and tetraspanins) in animal models of inflammation and human diseases.

Our current specific objectives are the following:

1. To assess the role of MTOC polarization as a signaling and structural platform for the control of secretion during IS formation.

2. To investigate the mechanisms and functional consequences of intercellular transfer of miRNA via the IS.

3. To image immune-inflammatory responses in vivo in order to define the role of immunoregulatory molecules in autoimmune inflammatory diseases.

**RESEARCH INTEREST**

T cells transfer microRNA-loaded exosomes to antigen presenting cells. The image shows confocal microscopy detection of the exosomal marker CD63-GFP (green) on the surface of recipient APCs (Raji) after incubation with J77-CD63-GFP exosomes. CD45 is stained red and nuclei are blue.

P-selectin glycoprotein ligand-1 modulates immune inflammatory responses in the enteric lamina propria. J Pathol (2011) 224; 212-21

The mitochondrial fission factor Drp1 modulates T-cell receptor signaling, regulating mitochondria translocation toward the immune synapse. Left: A T cell conjugated with an APC (blue cell). The intense tubulin staining reveals the localization of the T cell MTOC at the IS, at the center of the concentration of translocated mitochondria. Right: Detail of IS structures showing mitochondria (red) relocated toward the IS. Depletion of the mitochondrial fission protein Drp1 impairs this process and disrupts T cell receptor clustering (green) and T cell activation.

MAJOR GRANTS
- ERC Advanced Investigators Grant (ERC-2011-AdG 20110310) (GENTRIS)
- Ministerio de Ciencia e Innovación (SAF2008-02635)
- Ministerio de Ciencia e Innovación. FIS RETICS (RECAVA: RD06/0014/0030)
- Fundación Genoma España. MEICA Project. Coordinator, F. Sanchez Madrid

SELECTED PUBLICATIONS


Our interest is in the mechanisms through which integrins, Rho/Rac GTPases and caveolin-1 (Cav1) cooperate to regulate gene expression, cell cycle progression, migration, polarization, vesicle trafficking and epithelial-mesenchymal transition (EMT), key processes in the pathogenesis of cancer and inflammatory and cardiovascular diseases.

A growing body of work supports a role for caveolae and Cav1 in mechanosensing and mechanotransduction. We have shown that Cav1 can modulate cell shape and responses via force-dependent remodeling of the 3D microenvironment. Stromal fibroblast cells surrounding many human cancers express high levels of Cav1, which activate the enzyme Rho, causing cells to stretch out. In three-dimensional gel matrices in vitro and in vivo, the elongated Cav1-fibroblasts form stiff, parallel-fiber networks through which cancer cells move rapidly, promoting local invasion and subsequently distant metastasis.

Loss of integrin-mediated adhesion triggers an inward traffic of Cav1-rich membranes, which regulates Rac1 plasma membrane (PM) targeting and hence directs cell migration and controls cell proliferation. We have now found that Rac1 can be palmitoylated, and identified palmitoylation as a mechanism of Rac1 function in actin cytoskeleton remodeling by controlling its membrane partitioning, which in turn regulates membrane organization.

Other recent work has delineated how filamin A regulates actin-linked caveolae dynamics at the PM, and shows that Cav1-membrane inward trafficking depends on the actin polymerization machinery, microtubules (MT), dynamin2, and phosphorylation of filamin A by PKCα. Upon loss of tension caused by loss of adhesion, Cav1-rich membranes internalize in the form of complex multilobed “rosettes” in an actin-dependent manner. Caveolar domains are then transferred to an MT-dependent system that targets them to a Rab11-recycling endosome. In response to cell adhesion, Cav1 recycles back to the PM via a mechanism involving actin polymerization. Cav1 forms caveolae as stress fibers are formed, but caveolae are flattened by high PM tension induced by excessive actin-mediated force. To fully understand the molecular mechanisms by which the interplay between adhesion, mechanical tension and actin cytoskeleton regulate Cav1 trafficking, we are conducting an RNAi-based high-content image analysis screen in collaboration with the Cellomics Unit.
2 Vascular Biology and Inflammation

**MAJOR GRANTS**

- Ministerio de Ciencia e Innovación (SAF2008-02100)
- Ministerio de Ciencia e Innovación. Consolider COAT (CSD2009-00016)
- Instituto de Salud Carlos III. Red RTICC (RD06/0020/1033)

**SELECTED PUBLICATIONS**


Our group works on the development of high-throughput quantitative approaches for the dynamic analysis of the deep proteome. We have developed a comprehensive technology that includes advanced peptide identification algorithms and a novel, multi-layered statistical model for the analysis of quantitative data. Our approach also includes a universally applicable method for stable-isotope labeling that allows full control of variance sources. We are working on the generalization of the statistical model and on the integration with systems biology algorithms to improve interpretation of results from a proteome-wide perspective. We have also developed a novel method for simultaneous analysis of relative protein abundance and dynamic alterations in the thiol redoxome.

We are applying these developments to the study of key aspects of cardiovascular disease, with the aim of defining molecular mechanisms and identifying specific protein factors for use as pharmacological targets or biomarkers. One area of interest is the dynamic expression changes to the secretome and other subcellular fractions of vascular smooth muscle cells in models of hypertension and hypertrophy, including the role of the calcineurin-NFAT pathway. In addition, we are analyzing dynamic alterations to the mitochondrial proteome and the targets of oxidative damage that occur upon ischemia-reperfusion and the mechanisms of ischemic preconditioning in animal models of deletion or overexpression of several protein factors. We are also studying protein interactions during T-cell activation by APCs and during leukocyte recruitment to the activated endothelium. This work has recently characterized the interactome of tetraspanins in T-lymphocytes and derived exosomes from human patients, as well as from KO mouse models lacking specific tetraspanin components.

### RESEARCH INTEREST

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### RESEARCH INTEREST

**Head of Laboratory:** Jesús María Vázquez Cobos

**Postdoctoral Researchers:**
- Estefanía Núñez Sánchez
- Elena Bonzón Kulichenko
- Inmaculada Jorge Cerrudo

**Predoctoral Researchers:**
- Pilar Caro Chinchilla
- Pablo Martínez Acedo
- Daniel Pérez Hernández
- Marco Trevisan Herraz
- Fernando García Marqués

**Technicians:**
- Raquel Mesa Carrasco
- Juan Carlos Silla Castro

**Visiting Scientist:**
- Mariano Ortega Muñoz
Determination of changes in the redox state of cysteine-containing peptides in high-throughput proteomics experiments using GELSILOX technology. The figure shows the effect of a thiol-specific oxidative agent on vascular endothelial cells. The abundance of peptides containing cysteines in the oxidized state (red points) tends to increase (toward the left), that of peptides containing reduced cysteines (blue points) tends to decrease (toward the right), while non-cysteine containing peptides remain unaltered (green curve). The effect is more evident when the standardized peptide log-ratio distributions are analyzed separately (red and blue curves).

Left: Characterization of the intracellular tetraspanin interactome in human T-cells. Lower right: The tetraspanin interactome encompasses a large proportion of the composition of T-cell exosomes. Upper right: Quantitative high-throughput proteomics demonstrates that elimination of tetraspanin CD81 in KO mice diminishes the abundance in exosomes of some of its specific interaction partners, suggesting a role in the sorting machinery.

MAJOR GRANTS
- Ministerio de Ciencia e Innovación (BIO2009-07990)
- Ministerio de Ciencia e Innovación. FIS RETICS (RECAVA: RD06/0014/0030)
- Proyecto FEC de Investigación Básica 2010. PI: Raquel Yotti Álvarez (Sociedad Española de Cardiología)
- Instituto Madrileño para el Desarrollo (IMADE): Programa PIE-IMADE (MAXPRO)

SELECTED PUBLICATIONS


Angiogenesis in adults is often coupled to inflammation, and its deregulation can contribute to the development and progression of chronic inflammatory disorders such as atherosclerosis, rheumatoid arthritis, inflammatory bowel disease or psoriasis. Our previous work showed the contribution of the matrix metalloproteinase MT1-MMP to inflammation and angiogenesis and the cell context-dependence of MT1-MMP functions in inflammation. To explore this in more depth we have conducted proteomic analyses (SILAC) to identify the collection of cellular substrates (degradome) processed by MT1-MMP in endothelial cells and leukocytes. We have also used a similar approach to identify the substrates of MT4-MMP, a poorly characterized GPI-anchored MMP, in macrophages. Our proteomics analysis points to specific and unexpected functions for these proteases in the interplay between inflammation and angiogenesis, in particular the induction of endothelial tip cells and the decision between stabilization and regression of the new vasculature, and how these processes are linked to the phenotype of macrophages and other components of the inflammatory infiltrate. We are currently exploring these functions in cell-based systems, genetically-modified mouse models of angiogenesis and inflammation, and samples from patients affected by inflammatory disease. We are also characterizing the role of recently identified MT-MMP substrates and other related molecules such as extracellular matrix metalloproteinase inducer (EMMPRIN) in the regulation of vascular integrity and stability.

Through these efforts we aim to extend our knowledge of where, when and how MT-MMPs and their substrates modulate endothelial, smooth-muscle cell and leukocyte behavior during the establishment and progression of chronic inflammatory disorders.
Research Departments

2 Vascular Biology and Inflammation

MAJOR GRANTS

- Ministerio de Ciencia e Innovación (SAF2008-0214)
- Ministerio de Ciencia e Innovación. FIS RETICS (RECAVA; RD/06/0014/1016)
- Fundación Genoma España. MEICA Project
- Ministerio de Ciencia e Innovación (SAF2011-25619)

SELECTED PUBLICATIONS


B lymphocytes are central players in the immune response, mostly through the generation of a hugely diverse repertoire of protective antibodies. However, misregulation of B lymphocyte function is associated with multiple health conditions, including immune deficiencies, autoimmunity and cancer. Our lab is interested in various aspects of B cell biology, in particular the regulatory and diversification events that take place in germinal centers. Diversification in germinal centers entails the remodeling of immunoglobulin genes through two mechanisms—called somatic hypermutation (SHM) and class switch recombination (CSR)—that allow the generation of high-affinity, specialized antibodies. SHM and CSR are initiated by the same enzyme, activation-induced deaminase (AID), whose activity can also promote deliterious lesions in DNA, such as mutations and chromosome translocations.

Over the last several years we have focused on understanding AID function and microRNA-regulatory mechanisms in germinal centers. Diversification in germinal centers entails the remodeling of immunoglobulin genes through two mechanisms—called somatic hypermutation (SHM) and class switch recombination (CSR)—that allow the generation of high-affinity, specialized antibodies. SHM and CSR are initiated by the same enzyme, activation-induced deaminase (AID), whose activity can also promote deliterious lesions in DNA, such as mutations and chromosome translocations.

Over the last several years we have focused on understanding AID function and microRNA-regulatory mechanisms in germinal centers. We addressed the overall function of microRNAs in late B cell differentiation in CD19-Cre^{+/loz} Dicer^{loz/loz} mice, finding that in the absence of Dicer, late B cell differentiation is compromised and B cells produce self-reactive antibodies that lead to autoimmune disease. These results reveal a crucial role of microRNAs in the establishment of tolerance during late B cell differentiation.

In addition, we showed that the microRNA miR181b negatively regulates AID expression. We are currently investigating various aspects of AID function, including sequence specificity and its contribution to autoimmune disease and cancer development.

**RESEARCH INTEREST**

B lymphocytes are central players in the immune response, mostly through the generation of a hugely diverse repertoire of protective antibodies. However, misregulation of B lymphocyte function is associated with multiple health conditions, including immune deficiencies, autoimmunity and cancer. Our lab is interested in various aspects of B cell biology, in particular the regulatory and diversification events that take place in germinal centers. Diversification in germinal centers entails the remodeling of immunoglobulin genes through two mechanisms—called somatic hypermutation (SHM) and class switch recombination (CSR)—that allow the generation of high-affinity, specialized antibodies. SHM and CSR are initiated by the same enzyme, activation-induced deaminase (AID), whose activity can also promote deliterious lesions in DNA, such as mutations and chromosome translocations.
MicroRNA depletion in CD19-Cre \textsuperscript{ki/+} Dicer \textsuperscript{fl/fl} mice promotes immunocomplex deposition and kidney damage. Kidney sections from 40-60 week old CD19-Cre \textsuperscript{ki/+} Dicer \textsuperscript{fl/+} and CD19-Cre \textsuperscript{ki/+} Dicer \textsuperscript{fl/fl} animals were stained with anti-IgG antibodies (A) or were subjected to Silver-PAS staining (B).

Uracil-N-glycosylase (UNG) shapes the specificity of AID-induced somatic hypermutation. UNG, an enzyme that typically repairs U:G lesions, is involved in their pro-mutagenic processing during antibody diversification. Making use of high-depth next generation sequencing and loss- and gain- of function approaches, we found that UNG can promote either error-free and error-prone resolution of AID-generated U:G mismatches. Importantly, the choice between these two pathways depends on the sequence context. This provides the first evidence of a molecular player, other than AID itself, that shapes the specificity of SHM.

Selected Publications


Major Grants

- Ministerio de Ciencia e Innovación (SAF2010-21394)
- European Commission. European Research Council Starting Independent Researcher Grant (ERC-BCLYM 2007)
Impaired clearance of apoptotic cells results in the accumulation of secondary necrotic corpses, with profound immune consequences. Cell death triggers the macrophage inflammatory response, which normally contributes to tissue repair but under certain conditions can induce a state of chronic inflammation that is the basis of many diseases. Necrosis sensing by dendritic cells (DCs) might explain adaptive immunity in seemingly infection-free situations such as autoimmunity. Myeloid C-type lectin receptors (CLRs), such as Mincle in macrophages and CLEC9A (DNGR-1) in DCs, have been identified as receptors for necrotic cells that couple to the tyrosine kinase Syk, which in turn can trigger innate and adaptive immune responses. Our hypothesis is that recognition of cell death by Syk-coupled CLRs in myeloid cells might lie at the root of immune pathologies associated with an accumulation of dead cells. We are characterizing signaling and gene induction via CLEC9A as a model of innate sensing of necrotic cells by DCs. We are also investigating the role of Syk signaling and Syk-coupled receptors in myeloid cells in models of autoimmunity and of immune responses to dead tumor cells after chemotherapy. CLEC9A and Mincle are prime candidate mediators of the response to dead cells in DCs and macrophages, but our preliminary findings indicate that Syk deficiency has a more profound effect than CLEC9A deficiency on the sensing of necrosis by DCs, suggesting that additional receptors are involved. The third strand of our research is thus focused on the identification of new Syk-coupled receptors that recognize necrosis in myeloid cells.
MAJOR GRANTS

- European Commission. European Research Council Starting Independent Researcher Grant (ERC-StG-260414)
- Ministerio de Ciencia e Innovación (RYC2009-04235)
- Research cooperation agreement with MedImmune (Cambridge, UK)

SELECTED PUBLICATIONS


Metabolic syndrome is a medical disorder defined by the co-occurrence of obesity, impaired glucose tolerance, dyslipidemia and hypertension. The condition is associated with proinflammatory and prothrombotic states, and the major clinical outcomes are cardiovascular disease and type 2 diabetes. Moreover, metabolic syndrome may be a predisposing factor for the development of some types of cancer, such as hepatocellular carcinoma.

The high cardiovascular risk associated with metabolic syndrome and type 2 diabetes suggests that common mechanisms are involved in the etiology of these conditions, and that disease parameters in both might be improved by agents acting on the same therapeutic targets. Research suggests that one such target might be the stress activated protein kinases (SAPKs), an important family of kinases implicated in the transduction of stress signals into the cell. Our recently formed group investigates the involvement of SAPKs in the development of cancer and atherosclerosis induced by obesity. Our research is conducted with a number of disease models in combination with whole-body and tissue-specific knockout mice, and has shown that the SAPK JNK regulates fat metabolism, obesity, dyslipidemia and glucose intolerance through its actions in various tissues.

Hematoxylin and eosin (H&E)-stained section of liver from C57Bl6/J mice fed a normal diet (ND) or a high-fat diet (HFD) for 16 weeks.


**MAJOR GRANTS**

- European Commission. European Research Council Starting Independent Researcher Grant (ERC-StG-260464)
- European Foundation for the Study of Diabetes (EFSD 0203)
- Comunidad de Madrid. INMUNOTHERCAN (S2011/BMD-2326)
- Ministerio de Ciencia e innovación (SAF2010-19347)
- Ministerio de Ciencia e innovación (RYC-2009-04972)
Understanding peripheral mechanisms operating in autoimmune and chronic inflammatory diseases is critical for the design and development of novel treatments. Autoimmune diseases, which include conditions such as arthritis, asthma, contact dermatitis and myocarditis, are characterized by a breakdown in the mechanisms of tolerance to self antigens, and there is no definitive treatment for their eradication. Our group seeks to identify new regulatory cells and molecules involved in the control of these diseases.

The early leukocyte activation antigen CD69 is a membrane receptor of the family of type II C-type lectins. CD69 is rapidly induced after cell activation in all bone marrow derived cells except erythrocytes. Expression in vivo is restricted to positively selected thymocytes and leukocytes undergoing activation, particularly at inflammation sites. Engagement of CD69 with monoclonal antibodies in the presence of phorbol esters induces a Ca\textsuperscript{2+} influx that activates ERK, induces IL-2 and IFN-\textgreek{g} gene expression, and promotes T cell proliferation. Our recent work shows that the cytoplasmic tail of CD69 interacts with Jak3/Stat5 proteins, which regulate the transcription of ROR\textgreek{t} in human and mouse Th17 cells, thus establishing a mechanistic link between CD69 and the regulation of Th17 differentiation. The balance between Th17 cells and regulatory T cells determines the net balance between pro- and anti-inflammatory cytokines at inflammatory foci, and is thus critical for the regulation of the immune response. CD69 might also regulate the function or differentiation of regulatory T cells, thus affecting the outcome of Th17 responses indirectly. This is supported by the finding that mice lacking CD69 develop exacerbated forms of contact dermatitis, allergic asthma and autoimmune myocarditis. Our data demonstrate that CD69, by regulating Th17 effector responses, limits myocardial inflammation and subsequent heart failure. A similar process is likely to occur in humans with myocarditis and related dilated cardiomyopathy.

**RESEARCH INTEREST**

Understanding peripheral mechanisms operating in autoimmune and chronic inflammatory diseases is critical for the design and development of novel treatments. Autoimmune diseases, which include conditions such as arthritis, asthma, contact dermatitis and myocarditis, are characterized by a breakdown in the mechanisms of tolerance to self antigens, and there is no definitive treatment for their eradication. Our group seeks to identify new regulatory cells and molecules involved in the control of these diseases.

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**CD69 receptors are expressed on the membrane of T cells following activation. The cytoplasmic tail of CD69 associates with Jak3 and Stat5 proteins, triggering phosphorylation of Stat5 and its translocation to the nucleus where it can activate the transcription factor FoxP3, stimulating the differentiation of regulatory T cells. CD69 engagement can also induce expression of IL-2 and TGF\textgreek{β}. These cytokines may act in an autocrine manner to induce the differentiation of regulatory T cells. CD69 can inhibit the Th17 differentiation pathway through at least two mechanisms: CD69-activated Stat5 directly inhibits the translocation of Stat3 to the nucleus and indirectly, via FoxP3 activation, antagonizes Stat3-mediated ROR\textgreek{t} activation.**
CD69 acts as a brake on the progression and severity of autoimmune myocarditis and the development of dilated cardiomyopathy (DCM). Our study paves the way to investigations into whether defects in CD69 expression or function influence the development of DCM in humans. These findings increase our knowledge of the development of myocarditis, providing a cellular and molecular basis for the development of novel therapies.

Analysis of heart inflammation and fibrosis in experimental autoimmune myocarditis (EAM). (A) Mice lacking CD69 (CD69−/−) show a larger increase in heart-weight/body-weight (HW/BW) ratio upon treatment with myosin heavy chain peptide α (MyHCα). Representative myocardial cross sections are shown. LV, left ventricle; RV, right ventricle. (B) Masson’s trichrome staining reveals enhanced fibrosis in heart tissue from CD69−/− mice in the chronic phase of EAM. (C) Fluorescence molecular tomography (FMT) imaging of control or MyHC-peptide injected mice. The graph shows quantitative analysis of heart inflammation after injection of the protease-activated fluorescence agent ProSense 750 (Perkin Elmer).

MAJOR GRANTS

- Ministerio de Ciencia e Innovación (SAF2008-02719)
- Ministerio de Ciencia e Innovación (RYC2006-2966)

SELECTED PUBLICATIONS


Research Departments

Epidemiology, Atherothrombosis and Imaging
Research Departments

3 Epidemiology, Atherothrombosis and Imaging

The EAI department investigates several aspects of cardiovascular disorders by combining approaches spanning the range from the molecular basis of disease to clinical and population studies. Our studies include the identification of molecular and cellular mechanisms involved in atherosclerosis, restenosis, and aging; the role of neutrophils and other myeloid leukocytes in various aspects of the inflammatory response; the actions of vasoactive factors and proteolytic enzymes during the early steps of vascular remodeling; and cardioprotection during myocardial infarction, including studies in animal models and humans using latest-generation advanced imaging techniques. The combination of molecular, animal and human studies with large-scale clinical and epidemiologic analyses greatly strengthens the translational potential of the Department’s work.

DEPARTMENT DIRECTOR: Valentín Fuster
DEPARTMENT MANAGER: Ana Isabel Castillo
TECHNICIANS: Javier Mateos
Inés Ortega
Virginia Zorita
Gonzalo Javier López
Angel Maclías
Ana Vanessa Alonso
STUDY NURSE: Maite Dubraska Rodríguez Cabrera
ADMINISTRATIVE SUPPORT: Eeva Inari Soininen
Ana Gutiérrez
Our group conducts research into the development and application of non-invasive, high-resolution imaging technologies. Sophisticated imaging technologies play an ever more important role in research into cardiovascular disease, yielding novel information about the origin and development of disease, and through this providing means for early diagnosis of asymptomatic disease and monitoring treatment outcomes.

We are directly involved in the development of two large cohort studies (PESA and AWHS, see multidepartmental projects), where we are evaluating the application of non-invasive imaging for tracking atherosclerosis development and stratifying risk in asymptomatic populations. Last year we established several collaborations with renowned international groups conducting population studies, and we are developing a multinational, transatlantic network to evaluate the role of imaging in different populations.

2011 also saw important developments in other clinical projects. New on the scene is FOCUS, a multinational study testing the efficacy of a novel polypill for primary prevention. Several hundred patients have already been recruited across the globe. With the end of the EU Hyperimage initiative last year, we are now planning to follow this study with an international endeavor in collaboration with several groups and with our industrial partner, Philips. We also collaborate with other CNIC groups and centers throughout Spain in the METOCARD-CNIC trial, which compares the effect of early and delayed β-blocker treatment on infarct size and clinical outcome in patients with acute myocardial infarction. Our group is performing the advanced imaging in these trials.

Last year we launched our ambitious Advanced Imaging Program, where we are already using novel imaging modalities in a wide varieties of models, including small animals (high field 7T MR, nano PET/CT, optical imaging, and echocardiography), large animals (3T MR Tx, PET/MDCT, intravascular OCT, and 3D echocardiography), and humans (256 row MDCT, PET/MR system, 3D echocardiography, and 3D vascular ultrasonography). We recently incorporated experts in novel imaging technology development (novel sequences, etc.) and also a team of chemists, who are generating cutting-edge nanoparticles for use in our preclinical models. Our preliminary studies have already yielded exciting data that should bring the CNIC to the forefront of this field.
Sample $^{18}$FDG PET-MDCT chest images in a rabbit. Left: Axial MDCT image at the level of the ascending (red cross) and descending aorta. Right: Hybrid image of MDCT and $^{18}$FDG PET acquisitions in the same plane.

MAJOR GRANTS

- European Commission FP7 (201651 HyperImage)
- European Commission FP7 (241559 FOCUS)
- Ministerio de Sanidad y Política Social (EC10-042 Metocard, CNIC Translational Projects)
- Departamento de Salud y Consumo of the regional government of Aragon, General Motors Spain and CNIC (AWHS)
- NIH Grant (U01 HL-071988-01A1)
- NIH Grant (R01 HL-092989)
- NIH Grant (NHLBI-BAA-10-08)

SELECTED PUBLICATIONS


Accumulation of blood-borne leukocytes and their proliferation within the atherosclerotic plaque is a hallmark of atherosclerosis. During disease progression, inflammatory mediators produced by activated neointimal macrophages and lymphocytes induce the proliferation of vascular smooth muscle cells (VSMCs) and their migration toward the growing lesion. An additional process contributing to atheroma growth is the accumulation of non-cellular material, such as modified lipids and extracellular matrix components produced by activated VSMCs, which undergo de-differentiation from a ‘contractile’ to a ‘synthetic’ phenotype. Excessive cellular hyperplasia is also a feature of restenosis, the major limitation to the long-term success of revascularization via stent placement.

We investigate cellular, molecular and genetic mechanisms that underlie the development of atherosclerosis and restenosis. Our main interest is in regulatory circuits that control gene transcription and cell proliferation, and our long-term goal is to identify novel therapeutic targets and provide the basis for the development of new tools for the early diagnosis of individuals at high risk of atherosclerosis and restenosis. We also investigate the role of telomeres and A-type lamins in the regulation of signal transduction, gene expression and cell-cycle activity in pathophysiological processes, including aging and cardiovascular disease (CVD). Our multifaceted approach combines in vitro, cellular, animal and human studies and a variety of technologies, including mouse genetic engineering, proteomics, transcriptomics, FRET, confocal microscopy, and yeast 2-hybrid screens. We have a special interest in the use of cre/lox strategies combined with studies of VSMC and macrophage primary cultures to manipulate genes of interest and examine their role in CVD and in normal and premature aging.

**RESEARCH INTEREST**

**Head of Laboratory:** Vicente Andrés García  
**PhD Researchers:**  
- Raphaël Chèvre  
- José Javier Fuster Ortuño  
- José María González Granado  
- Oscar Muñiz Pello  
- José Rivera Torres  
- Laia Trigueros Motos  
- Ricardo Villa Bellota  
**PhD Researchers:**  
- Pedro Molina Sánchez  
- Ana Navarro Puche  
- Carlos Silvestre Roig  
- Magda Rita Hamczyk  
**Technicians:**  
- María Jesús Andrés Manzano  
- Cristina González Gómez  
**Undergraduate Student:** Alba de Juan Guillén

**Research Departments**

3 Epidemiology, Atherothrombosis and Imaging

**Molecular and genetic cardiovascular pathophysiology**
Deficiency of p27 phosphorylation at serine 10 increases atherosclerosis burden in apoE-null mice.
Our laboratory focuses on the study of myocardial diseases, ranging from ischemia/reperfusion to heart failure. Our studies span the molecular origins of disease and their manifestations at the macro-anatomical and physiological levels, and our group includes experts in molecular biology, clinical cardiology and cardiovascular imaging. Our evaluation of experimental animal models makes use of advanced imaging techniques that can also be applied to humans, strengthening the translational potential of our research. To exploit this potential, we work on multidisciplinary programs in close collaboration with hospitals and clinical researchers.

A major interest of the group is cardioprotection during myocardial infarction (MI). We have established models of MI in rodents and large animals, and we are using these to study the mechanisms underlying the beneficial effects of various cardioprotective strategies, mainly related to modulation of the adrenergic system. We also investigate the relationship between circadian oscillations and spontaneous cardioprotection, and another program examines the potential of gene therapy for myocardial diseases in swine models of cardiomyopathy. Our group participates in the European Commission funded HYPERImage project for the development of new imaging technologies.

In the clinical setting, our team is a key participant in the METOCARD-CNIC trial, which uses magnetic resonance imaging to evaluate the effectiveness of a cardioprotective strategy based on beta adrenergic modulation in patients with a previous myocardial infarction. We have already launched the metocard-CLOCK study, which prospectively evaluates circadian oscillations of infarct size in humans with ST-segment elevation MI (STEMI).

**Ischemia/reperfusion injury.** Death of myocardium during ischemia follows an exponential course. (a) If there is no reperfusion, the entire ischemic area becomes necrotic. (b) When reperfusion is limited to antegrade flow restoration, necrosis is significantly reduced; however the reperfusion itself induces additional damage to the myocardium (reperfusion injury). (c) Minimizing reperfusion injury further reduces infarct size. (d) Non infarcted myocardium. Panels a to d show representative axial slices of left ventricles of mice. Coronary occlusion was applied for 45 minutes, and reperfusion for 24 h. Red staining (TTC positive) represents live myocardium, and the pale area (TTC negative) indicates necrosis.
Local delivery of probes to swine myocardium. Local delivery of different probes to the endocardium is achieved by in vivo percutaneous injection via the femoral artery. This technique allows the direct injection of selected probes in a chosen area of the swine heart. In this case, Evans blue was injected into the outflow tract of the right ventricle.

Axial slices of swine heart (LV=left ventricle, RV=right ventricle). Top panels are axial slices obtained by in vivo magnetic resonance imaging (MRI) immediately after RV Evans blue injection. Arrows mark areas of edema on T2W sequences. Bottom panels show corresponding post-mortem axial slices. Arrows mark the blue/black areas of RV Evans blue staining, which were visualized in vivo as edemonic areas by MRI.

MAJOR GRANTS
- Fundación Mutua Madrileña (AP8695-2011)
- CNIC Translational Grants (01-2009)
- Ministerio de Sanidad y Política Social. FICI (EC10-042)
- Ministerio de Ciencia e innovación. FIS (PI10/02268).

SELECTED PUBLICATIONS


Our group is interested in the roles that neutrophils and other leukocytes play in the body. Part of their functions relate to their well-known functions during inflammation, as the first and critical cellular response to injury. Our current program includes studies of the ability of neutrophils to interact with platelets and the consequences of these interactions for inflammatory injury, as well as the recruitment of neutrophils and other myeloid leukocytes to atherosclerotic plaques. We are also interested in the molecules involved in these interactions and in the infiltration of inflamed tissues by neutrophils and other inflammatory leukocytes. Other functions of neutrophils are less expected, and relate to their modulation of fundamental homeostatic processes in the body. One example of these processes is the modulation of hematopoietic stem cell niches in the bone marrow by a population of old neutrophils, which we are currently characterizing. Our studies make use of genetically-modified mice, inflammatory models of disease and in vivo imaging at subcellular resolution. Our ultimate goal is to uncover processes at work during situations of health and disease in which myeloid cells are involved. In our work, we try to combine the excitement of discovering basic physiological phenomena with the mission of identifying therapeutic targets that promote human health.
Research Departments

3 Epidemiology, Atherothrombosis and Imaging

Section image of bone marrow showing the location near the bone of a neutrophil (PMN, white) that has homed to this organ, localizing close to hematopoietic niche cells (green) and in contact with a CD169+ macrophage (red). Scale bar, 10 \( \mu \)m.

MAJOR GRANTS

- Ministerio de Ciencia e Innovación (SAF2009-11037)
- European Commission FP7 (246655 LEMPIT)
- National Institutes of Health (1RC1HL099545-01). co-PI, A. Hidalgo. Funds held at the Albert Einstein Institute, New York

SELECTED PUBLICATIONS


Our research is focused on the effect of vasoactive factors and proteolytic enzymes during the early steps of vascular wall remodeling, a process fundamental to the development and progression of atherosclerosis, aneurysm, myocardial infarction, and arterial hypertension, four of the most prevalent diseases worldwide. We recently showed that nitric oxide-mediated inhibition of proteolysis is a potent inhibitor of neointimal hyperplasia of denuded arteries. Other recent work established the contribution of eNOS partner molecules to the maintenance of vascular tone and the efficiency of NO in cardiac protection of mice subjected to ischemia/reperfusion. Our results open lines of research toward the use of new strategies for early visualization and treatment of cardiovascular disease. Based on our previous findings, we are now designing synthetic reagents for early and multimodal non-invasive detection of selected targets of myocardial infarction, with potential therapeutic implications.

Our group participates in the European Commission funded HYPERImage project for the development of new imaging technologies.
Research Departments

3 Epidemiology, Atherothrombosis and Imaging

Magnetic resonance images of mouse hearts injected with gadolinium enriched nanoparticles containing EMMPRIN-specific binding peptide AP9 (right), or scramble peptide (left). EMMPRIN is detected as an enhanced signal in the wall of the AP9-injected heart (arrow). Insets show magnified views.

En face detection of M1 macrophages in aortas from wild-type mice (A) and eNOS knockout mice (B). Mouse aortas were subjected to endothelial denudation, and seven days after surgery were stained with anti-CD68 antibodies (green) and anti-iNOS antibodies (red) as a marker of M1 macrophages.

MAJOR GRANTS
- Ministerio de Ciencia e Innovación (SAF2008-04629)
- Ministerio de Ciencia e Innovación (SAF2011-28375)
- European Commission FP7 (TD1007 COST). Work package leader: C. Zaragoza

SELECTED PUBLICATIONS


*Kорresponding authors


The group conducts high-quality and high-impact population research studies into the environmental, individual and genetic risk factors that are causally related to cardiovascular disease. The group works closely with the Translational Platform on the design and coordination of the CNIC’s population studies, such as the Aragon Workers Health Study (AWHS), PESA (Progression of Subclinical Atherosclerosis), and IMJOVEN.

Our multidisciplinary group pursues highly innovative research that covers the major risk factors for cardiovascular disease, including diet, genetics and epigenetics, metabolic factors, the environment, and psychosocial factors. We are also developing expertise in the analysis of high throughput data and in the evaluation of novel and established cardiovascular risk factors in studies of populations with subclinical measures of atherosclerosis. Through these approaches, the group is making significant contributions to the understanding and control of the current epidemic of cardiovascular diseases.

The members of the group also continue to make significant contributions to leading international studies such as the Framingham Heart Study, the Atherosclerosis Risk in Communities (ARIC) Study, the Multiethnic Study of Atherosclerosis (MESA), the Strong Heart Study, the US National Health and Nutrition Examination Survey, and the UK National Diet and Nutrition Survey.

**RESEARCH INTEREST**

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**Research Departments**

3 Epidemiology, Atherothrombosis and Imaging

**Cardiovascular epidemiology and population genetics**

**Head of Laboratory:** Valentín Fuster (CNIC, Mt. Sinai Medical Center, New York)

**Research Scientists:**
- Manuel Franco
- Martín Laclaustra
- José Luis Peñalvo

**Visiting Scientists:**
- Eliseo Guallar (CNIC, Johns Hopkins Bloomberg School of Public Health, Baltimore)
- José Mª Ordovás (CNIC, Tufts University, Boston, IMDEA-FOOD, Madrid)

**Post-residency Researcher:** María Téllez

**Predoctoral Researchers:**
- Usama Bilal
- Marta Ledesma
- Belén Moreno

**Biostatistician:** Pedro López

**Technicians:**
- Raquel Langarita
- Esther Rovira
- Alicia Usón
- Rosa Villa

Adjusted differences (95% CI) in serum lipids by urinary enterolignan excretion in US adults (n=1492). Enterolignan values were modeled as restricted quadratic splines with nodes at the 5th, 50th, and 95th percentiles. The multivariable linear regression models were adjusted for sex, age, race/ethnicity, education level, income, and creatinine (log transformed).
A and G allelic variants of the miR-522 PLIN4 3 UTR sequence

MAJOR GRANTS

- Centro Nacional de Investigaciones Cardiovasculares (FPIT CNIC-08). PI: E Guallar
- Instituto de Salud Carlos III (CP08/112). PI: M Laclaustra
- Ministerio de Ciencia e Innovación (SAF2008-01995). PI: JL Peñalvo
- Instituto de Salud Carlos III (CM08/0037). PI: M Tellez
- Ministerio de Ciencia e Innovación (RYC-2010-07554). PI: M Franco
- Instituto de Salud Carlos III (PI10/21). PI: M Laclaustra
- FP7 Marie Curie Reintegration Grant (GA-249302). PI: M

SELECTED PUBLICATIONS


Peñalvo JL, López-Romero P. Urinary enterolignan concentrations are positively associated with serum HDL cholesterol and negatively with serum triglycerides in US adults. *J Nutr* (accepted)


Multi-departmental Clinical Projects
Although heart disease in young women causes many deaths, it has been virtually ignored by the medical profession because it represents only a small fraction of the total incidence of atherosclerotic heart disease. However, young women who suffer an acute myocardial infarction (AMI) have a mortality risk markedly higher than that of young men, and the limited data on young women from minority groups in the USA suggest that this population may have the highest risk of any young subgroup. There have been no large prospective studies of ischemic heart disease in young women, even though the death toll is comparable to that due to breast cancer. Findings from the small number of studies that have been published suggest that the biology, epidemiology, care, and outcomes of heart disease in women differ from those of men. The IMJOVEN study is the Spanish counterpart of the VIRGO study, an NIH-sponsored investigation led by Harlan Krumholz of Yale University into the excess risk in young women with AMI.

The specific aims of VIRGO and IMJOVEN are as follows. 1) To characterize sex differences after hospitalization for AMI for a broad range of outcomes including mortality, all-cause readmission, rehospitalization for cardiovascular causes, and adverse health status. 2) To evaluate the influence of demographic, clinical, metabolic, biochemical, genetic, psychosocial, and lifestyle factors on outcomes for young women and men with AMI and to examine whether sex-based variation in these factors is associated with variation in outcomes. 3) To compare the clinical treatment of young men and women who present at hospital with AMI and determine whether differences in quality of care are associated with differences in outcome. 4) To describe the relationship of female-specific factors—including genetic variants, sex hormones, reproductive history, prior use of estrogens and menstrual cycle history—with disease outcomes for women. 5) To develop comprehensive prognostic scores to stratify risk in this young population and identify predictors of early (within 1 month of discharge) and longer-term (1 year) outcomes. 6) To create a blood and DNA repository as a resource for future studies. 7) To partner with national and international organizations to disseminate study findings in order to improve the prevention, care, and outcomes for young patients with AMI.

Our aim with IMJOVEN was to study 450 patients (300 women and 150 men) with a previous history of AMI, using the same protocol as the VIRGO study. We have finally recruited 529 patients (359 women and 170 men) in 24 hospitals in Spain, and recruitment was completed in October 2011. IMJOVEN is coordinated by the Translational Platform at the CNIC, the Spanish Society of Cardiology and the RECAVA and Heracles networks. Funding comes from a FIS grant, the NIH and the CNIC.
The Aragon Workers Health Study (AWHS) is being conducted in collaboration with the Instituto Aragonés de Ciencias de la Salud (IACS) and the General Motors factory in Zaragoza. The study examines the development of cardiovascular disease and its risk factors by monitoring factory workers at their annual medical checkups. AWHS is an open cohort study including more than 5000 workers. During 2011, study participants underwent a standardized clinical exam, and laboratory assays were conducted on collected biological samples including serum, plasma, whole blood, urine and DNA. At the medical imaging facility of the study, participants are examined for the presence of subclinical atherosclerosis. Several hundred participants have already undergone this process. Over the coming years, all participants will be examined for TC calcium score, 3D ultrasound of carotid arteries and abdominal aorta, and ankle-brachial index. All laboratory procedures have been reviewed and improved to meet the ISO 9001:2008 standard, verified by an external audit. The study is financed by the Departamento de Salud y Consumo of the Aragon regional government and the CNIC.

Additional external funding has been raised for the following sub-studies on the cohort, which are being conducted by CNIC-based researchers:

- Insulin resistance and inflammatory response to oxidative stress: Study of determinants and interactions (ISCIII CP08/112)
- Identification of the genetic determinants of mitochondrial DNA content in a working population, and its relationship with oxidative stress and subclinical atherosclerosis (ISCIII PI10/21)
- Cadmium exposure, metallothionein levels, and kidney disease in a General Motors company assembly plant (Johns Hopkins NIOSH Education and Research Center Research Project Award)
- DNA methylation and the association of cadmium exposure with chronic kidney disease in a population-based occupational study (Johns Hopkins NIEHS Center in Urban Environmental Health Award).
Multi-departmental Clinical Projects

PESA CNIC- GRUPO SANTANDER AND FUNDACIÓN BOTÍN

(Progression of Early Subclinical Atherosclerosis)

The PESA CNIC- Grupo Santander and Fundación Botín study will help to identify risk factors and daily habits that influence the development of atherosclerosis, and will improve the prevention of atherosclerotic disease by achieving early diagnosis before the appearance of symptoms.

Strategies to identify individuals with subclinical alterations indicating increased risk of cardiovascular disease have been boosted by the development of basic imaging techniques (3D ultrasound) and advanced non-invasive imaging techniques (magnetic resonance imaging, positron emission tomography, and computerized tomography) that can be applied to large populations. Several studies currently underway, such as the High-Risk Population (HRP) study, led by Valentín Fuster in the USA, are pioneering the application of these techniques to population studies. However, most studies to date have examined populations composed of individuals above the age of 60. Atherosclerotic disease in this group has already had several decades of evolution and may not be fully reversible. To assess the early onset of atherosclerosis, longitudinal vascular imaging studies are needed to provide information about middle-aged populations.

PESA is a longitudinal study, run in partnership with Banco Santander and the Marcelino Botín Foundation, into the use of imaging techniques to detect the prevalence and rate of progression of subclinical vascular lesions in a population of 4500 male and female workers aged between 40 and 54 years. The study examines the association of these clinical parameters with the presence of genetic, epigenetic, metabolomic, proteomic and environmental factors, including dietary habits, physical activity, biorhythms, psychosocial characteristics and exposure to environmental pollutants.

Detection of subclinical atherosclerosis is first assessed in participants by basic imaging techniques, including CT imaging to estimate coronary calcium, 3D and 2D ultrasound of the carotid, iliac and femoral arteries, and 2D ultrasound measurement of the abdominal aorta and the ankle-brachial blood pressure index, assessed by the pulse Doppler method. These techniques are used to identify individuals with subclinical atherosclerosis, and the identified participants are invited to participate in an advanced imaging study to characterize the atherosclerosis. A new PET-MRI system used at the CNIC allows advanced sequential acquisition of positron emission tomography (PET) and magnetic resonance imaging (MRI) data for the atheroma plaques. Together, these imaging techniques enable early detection of subclinical atherosclerosis, the characterization of the atherosclerotic burden, and the monitoring of disease progression.

The study will also provide important information about the prevalence of unrecognized myocardial infarction in this population, and will assess the prevalence and progression of subclinical atherosclerosis in women during perimenopause and its relation to cardiovascular risk factors and hormonal changes.

In the 2010-2011 period, the PESA study received more than 2000 applications for participation. PESA technical staff and Santander Group Medical Service staff have been coordinated, trained and certified in accordance with the study procedures, and quality control procedures have been established. Anonymized data are recorded in the PESA study database. Samples of serum, plasma, whole blood, urine and DNA from all study participants are stored in a biobank for further analysis. All participants receive a report with their tests results and healthy lifestyle recommendations.
The prevention of cardiovascular disease is hampered by several factors, including wide variability in the pattern of prescription among physicians, limited access to expensive drugs in emerging countries, and poor adherence to medication. The use of a fixed dose drug combination (polypill) has been recommended to improve accessibility and adherence to treatment. The CNIC, working in a private-public partnership with Ferrer International, has devised a fixed dose polypill for secondary prevention, comprising aspirin, simvastatin and ramipril. The CNIC-Ferrer polypill project is led by Valentín Fuster and is coordinated by the CNIC Translational Platform.

During the last year we have conducted several clinical trials to ensure the quality and safety of the polypill. The Spanish arm of a study exploring potential pharmacodynamic interactions with simvastatin in 100 patients was completed last year, and more than 400 patients have been recruited to parallel trials in other countries. To date no serious adverse event has been reported, and the number of adverse events recorded with polypill treatment is not significantly different from that for participants receiving aspirin, simvastatin and ramipril separately.

Last year also saw the launch of the European Commission funded FOCUS study. This multinational trial examines the efficacy of the CNIC-FERRER polypill and explores the factors that determine poor treatment adherence in a cohort of 4000 patients across 80 centers and five countries. The Consortium 2011 Annual General Assembly of the FOCUS project was held in Milan last October, and patient recruitment is scheduled for completion during 2012.

FOCUS will establish recommendations for better use of medication in patients with ischemic heart disease, and after its successful completion, secondary prevention medication will be available and affordable for large numbers of patients in developed and developing countries. The CNIC’s partners in the FOCUS Consortium are the Mario Negri Institute (Milan), the Fundación Ruscalleda (Buenos Aires), the Fundació Clinic (Barcelona), Ferrer Internacional (Barcelona), the Agencia Española de Evaluación de Tecnologías Sanitarias, the Instituto de Salud Carlos III (Madrid), the World Heart Federation (Geneva) and the Federación Argentina de Cardiología (Buenos Aires).
Acute myocardial infarction (AMI) is the main cause of death in western countries. The best strategy to limit myocardial damage is to perform an early coronary reperfusion. However, reperfusion itself comes at a price of additional myocardial damage, known as ischemia/reperfusion injury (I/R).

The duration of ischemia can only be shortened through coordinated healthcare policies aimed at early detection and transfer of patients to hospitals with angioplasty capabilities. I/R injury, on the other hand, could potentially be reduced by pharmacological approaches; but despite great efforts, no therapy has been shown to consistently limit this phenomenon.

β-blockers are a class of drugs that have been used to treat cardiovascular conditions for several decades. β-blockers reduce mortality when administered after an AMI, and are a class IA indication in this context. What remains unclear is what timing and route of β-blocker administration gives the maximum cardioprotective effect. There is particular debate about whether early β-blocker administration is able to reduce infarct size. Experimental data from our laboratory suggest that the β1-selective blocker metoprolol is able to limit the area of necrosis only when administered before reperfusion.

METOCARD-CNIC is a multicenter randomized clinical trial comparing the effect of early and delayed metoprolol initiation on infarct size and clinical events in more than 200 patients with AMI. More than 60% of the patient population has already been recruited, and more than 300 imaging studies performed in these patients. The studies of patients recruited in Madrid are being performed in the CNIC’s human imaging facility, where the advanced imaging protocol is performed using an innovative, cutting-edge MRI system.

METOCARD-CNIC is the result of a multidisciplinary effort in which investigators from the CNIC, hospitals across Spain, and, importantly, emergency medical services work in close collaboration. The hospitals participating in METOCARD-CNIC are the Hospital Clínico San Carlos, Hospital Puerta de Hierro, Hospital de la Princesa, Hospital 12 de Octubre and Hospital Quirón in Madrid, Hospital Meixoeiro in Vigo, Hospital Marqués de Valdecilla in Santander, and Hospital de León. Emergency medical services actively participating as co-investigators are SUMMA, 061 Galicia, and SAMUR. This initiative is a pilot endeavor that will be followed by larger clinical trials in which more centers will participate in close collaboration with the CNIC.
Translational Platform
Translational Platform

The Translational Platform develops initiatives that foster translational research at the CNIC through collaboration with international partners and Spanish hospitals. The Platform also identifies, promotes, and co-develops CNIC research with potential for industrial application, by facilitating the acquisition of patents and their subsequent development or licensing. The Platform’s own Clinical Research Program provides logistics and methodological support to CNIC researchers and to collaborating institutions and healthcare companies requesting assistance in this area. The Translational Platform is also developing a biobank service to support state-of-the-art specialized cardiovascular research.

The principal objective of the CNIC Project and Technology Transfer Unit is to promote the research carried out at the Center and to stimulate the exploitation of the results generated. The main activities of the Project and Technology Transfer Unit are:

**To stimulate and support the CNIC’s research activities:**
- Supplying CNIC personnel with information about public and private sources of funding for research - grants, contracts, research projects, scientific infrastructure, etc.
- Helping with the preparation and processing of funding proposals written by CNIC staff.
- Administering grants and other funding awarded to CNIC personnel.
- Preparing and processing proposals for central funding for the CNIC.

**To encourage the exploitation of research results generated at the CNIC:**
- Promoting and publicising the CNIC’s R&D assets.
- Providing researchers with advice about the potential for patenting their research results.
- Helping in the preparation of patents.

**To promote and coordinate relations between the CNIC and other sectors in the fields of research and technological innovation:**
- Stimulating collaboration between CNIC researchers and companies interested in their work through formal collaboration agreements or research contracts.
- Promoting participation by CNIC research groups in collaborative research and technology-development programs at regional, national, and European levels.

Departmental Staff

**GROUP LEADER:** Antonio Bernad Miana

**ADMINISTRATIVE SUPPORT:** Ana Gutiérrez Llaneza
Technical Units
The Unit provides state-of-the-art optical and fluorescence microscopy technologies to CNIC scientists. Several brightfield, wide field, confocal and multiphoton microscopes are maintained, and are fully equipped for multicolor immunofluorescence and for a variety of live-cell and in-tissue studies. The Unit has developed customized applications for CNIC scientists, including very large image tiling, cell tracking, shape recognition, 3D-multicolor rendering co-localization, and membrane fluidity (GP) imaging.

The Unit’s capabilities expanded in 2011 to incorporate a two-NDD-channel multiphoton TCSPC-FLIM plus multiline confocal microscope. The new instrument allows second harmonic generation (SHG) and deep multiphoton imaging for 3D rendering in vivo and ex vivo, at subcellular sensitivity. The Unit is also strongly committed to technological innovation and development of new applications of interest to scientists at the CNIC and beyond. Ongoing research collaborations with internal and international groups are developing procedures for fluorescence correlation spectroscopy, SHG, and in vivo phasorFLIM and number and brightness imaging. Multiphoton phasorFLIM imaging is regularly applied in projects assessing protein-protein interaction at high sensitivity in live cells. Last year we further developed this unique imaging technology to enable metabolic fingerprinting in live cells and model organisms. This new field is expected to find innovative applications in cardiovascular development, stem cell differentiation and metabolic research.

The Unit provided four training courses for CNIC scientists in 2011 and participated actively in the ongoing CNIC-JOVEN training plan (ACERCATE, CICERONE and the Master Program) with ‘Introduction to Microscopy’ sessions and hands-on practicals.
Multiphoton microscopy: Unstained section of a mouse aorta imaged for (a) SHG collagen fluorescence and (b) elastin auto-fluorescence. (c) Merged image.

Multiphoton microscopy: Analysis of protein-protein interaction at the immune synapse by phasorFLIM-FRET

**Selected Publications**


The Transgenesis Unit provides a range of services for the production of genetically-modified mice—known as transgenic mice—to serve the needs of the CNIC research groups. The interest is two fold: to understand how genomic activity translates into the complexity of a whole organism, and to generate mouse models of human cardiovascular disease.

Transgenic mice are produced in the Unit by the established methodologies of microinjection of DNA in solution into zygote pronuclei (pronuclear microinjection) or of recombinant lentiviruses beneath the zygote zona pellucida (subzonal or perivitelline microinjection). Chimeric mice for the generation of knockout and knockin mice are produced by a variety of techniques, but mainly by microinjection of genetically-modified mouse embryonic stem cells into eight-cell embryos or blastocysts. Other key services and techniques include rederivation of mouse and rat strains by embryo transfer, cryopreservation of mouse strains (frozen embryos or sperm), in vitro fertilization (IVF), and intracytoplasmic sperm injection (ICSI).

In addition to its routine work, the Unit collaborates with several CNIC groups on specific aspects of their research programs.

As in preceding years, the main activity of the Unit in 2011 was the rederivation of mouse strains, and a total of 69 new mouse strains were rederived to the specific pathogen free area of the Comparative Medicine Unit, bringing the total number of rederived mouse strains at the Center to 231.
Two-cell embryo from a WISTAR rat

Intracytoplasmic sperm injection (ICSI) into a C57BL/6JCrI mouse oocyte

The Genomics Unit provides the latest genomic technologies to the scientific community at the CNIC and beyond, together with expert assistance with experimental design.

With the advent of high-throughput sequencing (next generation sequencing; NGS) as an important technology in modern biomedicine, the Unit now provides massively parallel NGS on the Illumina Genome Analyzer IIx. The Genomics Unit’s NGS services include gene expression and alternative splicing (RNA-Seq), protein-nucleic acid association profiling (ChIP-Seq), and small RNA discovery (small RNA-Seq). The Unit’s tasks in each sequencing project include project consultation, sample quality check, sample library preparation and data generation.

The Unit continues to offer microarray analysis services using Agilent and Affymetrix microarray platforms, the world’s leading DNA chip technologies. Microarray applications include whole-genome differential gene expression analysis (including at the exon level using Exon arrays), microRNA expression analysis and CGH arrays. Other services include the maintenance and management of real-time PCR instruments (one AB 7000 and two ABI 7900HT machines) and a TaqMan array processing service.
TECAN ROBOT FOR AUTOMATED RNA Seq SAMPLE LIBRARY PREPARATION

Culture of human mesenchymal stem cells at low oxygen tension improves growth and genetic stability by activating glycolysis. Cell Death and Differentiation (accepted)


MAJOR GRANTS

Ministerio de Ciencia e Innovación. FIS (PI10/01124)
Reliable culture of pluripotent stem cells requires specialist expertise. At the CNIC, the Pluripotent Cell Technology Service (PCTS) provides centralized support in the culture and manipulation of mouse and human pluripotent stem cells. The PCTS staff supervise two culture rooms, each devoted entirely either to human or to mouse stem cells. The broad range of support services offered includes expert advice and training in the maintenance and differentiation of stem cells and the provision of validated reagents.

One of the unit’s core tasks is to facilitate the generation of genetically-modified mice through homologous recombination in mouse embryonic stem cells (mESCs). Our staff takes charge of all the key steps of the gene targeting protocol: electroporation of the targeting vector, selection, karyotyping, culture, and the preparation of cells for appropriate targeting and screening strategies. The systems developed in the unit achieve efficient transmission of targeted mESCs to the germline, using mESC lines in both the 129 and the 129/B16 genetic backgrounds.

Collaborations with CNIC research groups involve us in the derivation of mutant homozygotic mESC lines and the differentiation of mESCs and mouse induced pluripotent stem (miPS) cells to cardiomyocytes. Last year we also focused on the design and fine-tuning of protocols for generating iPS cells using transposons. This pioneering technology will underpin the use and application of cutting-edge pluripotent cell technologies by CNIC researchers.
Mouse induced pluripotent cells generated using transposons. (a) A colony ready for picking and disaggregation. (b) miPS expansion on feeder cells.

Immunostaining of cardiomyocytes (α-actinin) differentiated in vitro from mouse iPSCs.

MAJOR GRANTS
- Ministerio de Ciencia e Innovación. FIS (CTA0801)

SELECTED PUBLICATIONS
The Proteomics Unit has broad experience in proteomics approaches for the separation, quantification, identification and characterization of proteins in biological systems, and maintains a program of continuous development for the improvement of technologies and protocols to meet the challenging requirements of the research community. During 2011 substantial progress was made in spectrometric analysis and procedures for sample fractionation and enrichment.

For the separation and quantitative analysis of protein expression, we are refining technologies based on nanoHPLC coupled to mass spectrometry. Proteins, peptides and their post-translational modifications are identified and characterized with a MALDI-TOF/TOF, a hybrid triple quadrupole (QqQ), and a linear ion trap coupled to an Orbitrap high resolution mass analyzer. Particular progress has been made in relation to the chromatographic conditions for peptide separation, optimization of fragmentation parameters, and post-acquisition analysis and data visualization employing several validation programs. These approaches make use of shotgun and targeted proteomic analyses. By using high-throughput tandem mass spectrometry methods for global proteome profiling, we are increasing the analysis sensitivity to enable us to reliably quantify and detect low-abundance proteins in complex biological specimens, such as biopsies or cell extracts. For validation purposes and targeted analysis, we use directed approaches in which specific precursor/product ion transitions are selectively monitored (selected reaction monitoring; SRM) to improve overall detection sensitivity, reliability, and quantification. The combination of SRM approaches with mass spectrometry-based techniques (both label-free and using multiplexed isotopic labeling; iTRAQ) allows us to quantify hundreds of proteins in a single experiment.

This robust analytical platform, together with our recognized experience in the field, enables us to take on large and technically demanding research projects that require both qualitative and quantitative proteomic approaches for measuring differential protein expression, studying chemical and posttranslational modifications, and mapping protein-protein interactions in diverse biological systems.

Differential in-gel electrophoresis analysis. Polymorphonuclear neutrophil protein extracts from abdominal aortic aneurysm (AAA) patients and controls were labeled with the corresponding CyDye reagents, mixed, resolved on independent 2D gels, imaged (red, Cy3; green, Cy5; blue, Cy2) and analyzed with DeCyder software. (A) A representative gel image. Proteins were resolved in the 3 to 11 (nonlinear) pH range on the first dimension and by 12% SDS-PAGE on the second dimension. (B) Spots showing statistically significant regulation between the two conditions were excised from silver-stained gels and analyzed by MALDI-MS for protein identification. From Ramos-Mozo et al. 2011.
MS analysis of the binding of dactylolide and zampanolide to tubulin. Total ion chromatogram (TIC) of a precursor ion scanning experiment at selected m/z values for control microtubules (MTB) (blue) or MTB treated with dactylolide (left panel; red in Part A) or zampanolide (right panel; green in Part A) in the Applied 4000 Qtrap mass spectrometer. Subtle differences are detected in the hydrophobic regions of the chromatograms (green boxes), which are highlighted in the corresponding zoomed area (Part B). Further MS/MS analysis allowed us to show that both dactylolide and zampanolide bind covalently to residue Tyr224 of β-tubulin. From Calvo et al., Shotgun and targeted MS analyses pinpoint the zampanolide-tubulin interacting site.


SELECTED PUBLICATIONS


The CNIC Bioinformatics Unit was established in the last quarter of 2010. The main goal of the Unit is to establish a collaborative environment within which to contribute to CNIC research projects, thereby providing CNIC researchers with ad-hoc, state-of-the-art bioinformatics and computational biology solutions to enhance their research.

The Unit focuses on the analysis and interpretation of high-throughput biological data from CNIC research projects, with special emphasis on data generated by the Genomics, Proteomics and Cellomics Units. One of the Unit’s main aims is to develop and implement analysis pipelines using state-of-the-art algorithms specific for each type of data.

Other aims of the Unit are to locally implement genome-related software (for example, genomics browsers such as GBrowse) and the most widely-used bioinformatics tools (Galaxy, Alexa-SEQ, etc.) and to generate local mirrors of public genomic databases (Ensembl, UCSC, NCBI, BioMart) for selected genomes (human, mouse and zebrafish).

The Unit also provides customized advice and training to CNIC researchers in the analysis and interpretation of their experimental data.

**Services**

- Help in experimental design for high-throughput experiments
- Quality control, preprocessing, data management and statistical analysis for microarray/next-generation sequencing (NGS) and other high-throughput technologies.
- Ad-hoc mathematical models for high-throughput data to enable systems biology
- Functional analysis using Ingenuity Pathway Analysis and Open Source Software
- Sequence analysis
- Genome data-mining using genome browsers (ENSEMBL, UCSC)
- Sequence analysis using traditional sequencing methods (Sanger)
- Support in writing bioinformatics and biostatistics texts.

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**RESEARCH INTEREST**

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IGV Browser displaying information from different NGS experiments (Chip-Seq, FAIRE-Seq, RNA-Seq and miRNA-Seq) analyzed at the CNIC.
Gene expression data from infarcted and uninjured heart samples. The figure compares data from RNA-Seq (y-axes) and Exon arrays (x-axes). Genes showing differential expression under the two conditions are highlighted red.


SELECTED PUBLICATIONS


The Cellomics Unit provides services in the two principal cell analytical techniques, flow cytometry and high content screening (HCS), and supports quantitative image-based research.

The Unit assists researchers in experimental design and data interpretation for flow cytometry experiments, providing the necessary technical expertise in the manipulation of equipment and software, which include

- Three latest generation digital analytical flow cytometers: two Becton Dickinson FACSCanto II machines and one Cyan (Beckman Coulter).
- Two high speed flow sorters: A MoFlo (Beckman Coulter) and a custom made FACSAria II (Becton Dickinson).
- Cytometry software (Modfit and FlowJo).

HCS services include design, development (miniaturization, automation, analysis) and performance of siRNA library screening.

- HCS resources include
- A liquid handling workstation connected to a cell culture incubator with 110 plate throughput (Freedom EVO, Tecan).
- An automated confocal microscope for microplate reading (Opera, Perkin Elmer).
- Whole genome human and mouse siRNA libraries (4 individual siRNA-oligos per gene; Thermo Scientific).

The Image Analysis Unit (IAU) was established in 2011 with the aim of providing solutions for image-based scientific applications by developing computational techniques that extract information from biological images. The IAU is equipped with dedicated image analysis software packages (Acapella, Definiens, MatLab).

The Unit conducts research using HCS and quantitative image analysis tools into the regulation of membrane trafficking during cell migration. We are interested in the interplay of Rho GTPases and Rab8, a GTPase that regulates membrane trafficking to the plasma membrane during cell migration.

**RESEARCH INTEREST**

**Head of Unit:** María Montoya

**Support Scientists:** José Manuel Ligos, Hind Azegrouz

**Predoctoral Researchers:** Begoña Díez, Carmen Muñoz

**Technicians:** Raquel Nieto, Mariano Vitón, Mª Montserrat Arroyo, Ignacio Cotillo

**FRET analysis in live cells by flow cytometry.** Carcinoma MDA-MB-231 cells co-transfected with mRFP and FRET control fusion constructs with different lengths (tandem and spectrin), with Raichu Rac construct 1011 (Raichu Rac) for assessing Rac-GTPase activity, or with independent CFP and YFP constructs used as negative control. (A) Dot plot representation of CFP and FRET signals. (B) Histogram representing FRET ratio (CFP/FRET signal). (C) Quantification of median FRET ratio for the different constructs.
Development of a siRNA screen for genes that modulate caveolin expression and localization. Images acquired using the Opera HCS system. (A) Hoechst (red) and tubulin (green) staining, and overlayed Caveolin-GFP signal (blue). (B) Segmentation of cell, nucleus, perinuclear area and cell cortex. (C) 3D reconstruction of cells including nucleus (red) perinuclear (green) and cortical (yellow) segmented areas. (D) Segmentation of high-intensity tubulin areas.

Image analysis development for quantitative cardio-imaging. (A) 3D rendering of nucleus segmentation and classification; nuclear color coding indicates BrdU and cardiomyocyte staining classification. (B) Telomere segmentation based on local maxima seed detection and 3D constrained dilation. (C) 2D representation of nuclei segmentation and classification. Confocal microscopy images acquired and project conducted by Ignacio Flores’s Group.

MAJOR GRANTS

- Ministerio de Ciencia e Innovación. FIS (PS09/01028)

SELECTED PUBLICATIONS


The Viral Vector facility is dedicated to providing high-quality recombinant viruses (lentivirus, adenovirus and adeno-associated virus) for preclinical studies at the CNIC and beyond. The facility’s capabilities expanded in 2011 to complete a collection of more than 80 HIV-derived lentiviral backbones containing promoter, polycistronic and selectable/fluorescent markers. Adeno-associated virus (AAV) derived vectors are currently produced and titrated to widely accepted standards. Of particular interest is the availability of backbones containing polycistronic expression cassettes driven by a cardiac-specific promoter (minimal TnT) that can be serotyped with preferentially tropic capsids. This allows specific and efficient cardiac transcriptional and transductional targeting both in vivo and in vitro. Procedures are currently being developed for large-scale production and purification of AAVs for delivery into large animal models.

Our own research program is aimed at developing novel strategies for gene transfer to specific loci by means of integration-deficient lentivirus and recombinase-mediated cassette exchange (RMCE).

**RESEARCH INTEREST**

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Our own research program is aimed at developing novel strategies for gene transfer to specific loci by means of integration-deficient lentivirus and recombinase-mediated cassette exchange (RMCE).
The promoter-outside trap principle is used to switch markers in order to signal authentic cassette exchange.

Genotyping of the RMCE product demonstrates the fidelity of the exchange reaction.

**SELECTED PUBLICATIONS**


Technical Units

Comparative Medicine

The Comparative Medicine Unit supports in vivo work at the CNIC, and is organized into five core work areas:

- **Animal Husbandry.** This area is staffed by dedicated animal technicians, managers and veterinarians who take charge of the daily husbandry and welfare of animals. Housing and husbandry conditions conform to European and national regulations for the use of animals for experimental and other scientific purposes, including the provision of mandatory training to researchers involved in animal experiments.

- **The Pathology Core (PC),** run by an on-site laboratory animal pathologist. The PC has established collaborations with the Comparative Pathology Laboratory of the Weill Cornell Medical College and the Memorial Sloan-Kettering Center in New York, and with the Phenotyping Core at the Department of Molecular and Comparative Pathobiology, Johns Hopkins Hospital in Baltimore.

- **The Phenotyping Core (PhC),** which provides a comprehensive cardiovascular phenotype evaluation service, includes a Clinical Pathology service, which provides expertise in hematology and clinical biochemistry in a variety of species.

- **The Veterinary Medicine and Experimental Surgery Core (VMESC) provides** specialized expertise in animal medical problems, disease follow-up, surgical procedures, minimally invasive intervention, and life support. The VMESC is run by the Head of the Comparative Medicine Unit, and provides training for resident veterinarians through a program in Laboratory Animal Medicine.

- **The Quality Control Core (QCC) is run by a senior microbiologist and monitors** the health and the genetic status of the animals on site.

The PC and PhC services combine in vivo evaluation, imaging strategies, and clinical and anatomic pathology to characterize complex phenotypes—including multisystemic phenotypes or syndromes—for the development and validation of genetically engineered mouse models.

The Unit has gained ISO 9001 accreditation for all five core work areas.
Appendix

Publications 2011
Training Programs and Courses
Seminars, Events and Awards
Strategic Alliances
Funding
Staff Figures
Appendix

Publications 2011

Publications by CNIC staff are listed by Department, followed by the Technical Units. In each section publications are listed alphabetically by first author. The table at the end summarizes the cumulative and average impact factors in each area, calculated according to de ISI Journal Citation Reports (JCR), 2010. Publications with no IF, for example book chapters or articles published in journals not currently listed by the JCR, are not included in the table.

Cardiovascular Development and Repair


Appendix

Publications 2011


Appendix

Publications 2011

Differential Lipid Partitioning Between Adipocytes and Tissue Macrophages Modulates Macrophage Lipotoxicity and M2/M1 Polarization in Obese Mice.
Diabetes (2011) 60: 797-809
IF: 8.889

Rosello-Diez A, Ros M and Torres M.
Diffusible Signals, Not Autonomous Mechanisms, Determine the Main Proximodistal Limb Subdivision.
Science (2011) 332: 1086-8
IF: 31.364

San Martin N, Cervera AM, Cordova C, Covarello D, McCreath KJ and Galvez BG.
Mitochondria Determine the Differentiation Potential of Cardiac Mesangioblasts.
Stem Cells (2011) 29: 1064-74
IF: 7.871

PGC-1alpha regulates translocated in liposarcoma activity: role in oxidative stress gene expression.
Antioxid Redox Signal (2011) 15: 325-37
IF: 8.209

Shimano M, Ouchi N, Nakamura K, Oshima Y, Higuchi A, Pimentel DR, Lara-Pezzi E, Lee SJ, Sam F and Walsh K.
Cardiac myocyte-specific ablation of follistatin-like 3 attenuates stress-induced myocardial hypertrophy.
IF: 5.328

Induction of the Mitochondrial NDUFA4L2 Protein by HIF-1alpha Decreases Oxygen Consumption by Inhibiting Complex I Activity.
Cell Metab (2011) 14: 768-79
IF: 18.207

mir-335 orchestrates cell proliferation, migration and differentiation in human mesenchymal stem cells.
Cell Death Differ (2011) 18: 985-95
IF: 9.050

Traves PG, Lopez-Fontal R, Luque A and Hortaleno S.
The tumor suppressor ARF regulates innate immune responses in mice.
IF: 5.745
Appendix

Publications 2011

**Epidemiology, Atherothrombosis and Imaging**


Fayad ZA, Mani V, Woodward M, Kallend D, Bansilal S, Pozza J, Burgess T, Fuster V, Rudd JH, Farkouh ME and Tawakol A. Rationale and design of dal-PLAQUE: a study assessing efficacy and safety of dalcetrapib on progression or regression of atherosclerosis using magnetic resonance imaging and 18F-fluorodeoxyglucose positron emission tomography/computed tomography. Am Heart J (2011) 162: 214-221 e2 IF: 5.052


Appendix

Publications 2011


Appendix

Publications 2011


Appendix

Publications 2011

Ruiz-Hornillos PJ, Martinez-Camara F, Elizondo M, Jimenez-Fraile JA, Del Mar Alonso-Sanchez M, Galan D, Garcia-Rubira JC, Macaya C and Ibanez B.
IF: 4.142

Serum uric acid levels predict incident nonalcoholic fatty liver disease in healthy Korean men.
Metabolism (2011) 60: 860-6
IF: 2.538

Sanchez-Moreno C, Ordovas JM, Smith CE, Baraza JC, Lee YC and Garaulet M.
APOA5 Gene Variation Interacts with Dietary Fat Intake to Modulate Obesity and Circulating Triglycerides in a Mediterranean Population.
IF: 4.295

The Fixed-dose Combination Drug for Secondary Cardiovascular Prevention project: Improving equitable access and adherence to secondary cardiovascular prevention with a fixed-dose combination drug. Study design and objectives.
Am Heart J (2011) 162: 811-817 e1
IF: 5.052

Shea MK, O’Donnell CJ, Vermeer C, Magdeleyns EJ, Crosier MD, Gundberg CM, Ordovas JM, Kritchevsky SB and Booth SL.
Circulating Uncarboxylated Matrix Gla Protein Is Associated with Vitamin K Nutritional Status, but Not Coronary Artery Calcium, in Older Adults.
J Nutr (2011) 141: 1529-34
IF: 4.295

Monocytes control natural killer cell differentiation to effector phenotypes.
Blood (2011) 117: 4511-8
IF: 10.564

Spector JT, Navas-Acien A, Fadrowski J, Guallar E, Jaar B and Weaver VM.
Associations of blood lead with estimated glomerular filtration rate using MDRD, CKD-EPI and serum cystatin C-based equations.
IF: 3.564

Spinola-Amilibia M, Rivera J, Ortiz-Lombardia M, Romero A, Neira JL and Bravo J.
The Structure of BRMS1 Nuclear Export Signal and SNX6 Interacting Region Reveals a Hexamer Formed by Antiparallel Coiled Coils.
IF: 4.008

Stranges S, Tabak AG, Guallar E, Rayman MP, Akbaraly TN, Laclaustra M, Alffhan G, Mussalo-Rauhamaa H, Viikari JS, Raitakari OT and And MK.
Selenium Status and Blood Lipids: The Cardiovascular Risk in Young Finns Study.
IF: 5.935

Suarez-Barrientos A and Ibanez B.
The authors’ reply.
Heart (2011) 97: 1359
IF: 4.706

Suarez-Barrientos A and Ibanez B.
The authors’ reply.
Heart (2011) 97: 1895
IF: 4.706

Circadian variations of infarct size in acute myocardial infarction.
Heart (2011) 97: 970-6
IF: 4.706

Tarin C, Lavin B, Gomez M, Saura M, Diez-Juan A and Zaragoza C.
The extracellular matrix metalloproteinase inducer EMMPRIN is a target of nitric oxide in myocardial ischemia/reperfusion.
Free Radic Biol Med (2011) 51: 387-95
IF: 5.707

Tsai AK, Steffen BT, Ordovas JM, Straka R, Zhou X, Hanson NG, Arnett D and Tsai MY.
Short-term fenofibrate treatment reduces elevated plasma Lp-PLA2 mass and SCAM-1 levels in a subcohort of hypertriglyceridemic GOLDN participants.
IF: 2.903
Appendix

Publications 2011


Appendix

Publications 2011


Technical Units


Appendix

Publications 2011

Protein phosphorylation analysis in archival clinical cancer samples by shotgun and targeted proteomics approaches.
Mol Biosyst (2011) 7: 2368-74 IF: 3.859

Garcia-Del Portillo F, Calvo E, D’Orazio V and Pucciarelli MG.
Association of ActA to the peptidoglycan revealed by cell wall proteomics of intracellular Listeria monocytogenes.

Modeling Human Endometrial Decidualization from the Interaction between Proteome and Secretome.
J Clin Endocrinol Metab (2011) 3: 706-16 IF: 6.495

SPARC Promotes Cathepsin B-Mediated Melanoma Invasiveness through a Collagen I/alpha2beta1 Integrin Axis.

Biomechanical remodeling of the microenvironment by stromal caveolin-1 favors tumor invasion and metastasis.
Cell (2011) 146: 148-63 IF: 32.401

Hellriegel C, Caioffa VR, Corti V, Sidenius N and Zamai M.
Number and brightness image analysis reveals ATF-induced dimerization kinetics of uPAR in the cell membrane.

Lomsadze K, Salgado A, Calvo E, Lopez JA and Chankvetadze B.
Comparative NMR and MS studies on the mechanism of enantioseparation of propranolol with heptakis(2,3-diacetyl-6-sulfo)-beta-cyclodextrin in capillary electrophoresis with aqueous and non-aqueous electrolytes.
Electrophoresis (2011) 32: 1156-63 IF: 3.569

Nodal/Activin signaling drives self-renewal and tumorigenicity of pancreatic cancer stem cells and provides a target for combined drug therapy.

Identification of Peroxiredoxin-1 as a Novel Biomarker of Abdominal Aortic Aneurysm.

Muriel O, Echarri A, Hellriegel C, Pavon DM, Beccari L and Del Pozo MA.
Phosphorylated filamin A regulates actin-linked caveolae dynamics.

Ramella NA, Rimoldi OJ, Prieto ED, Schinella GR, Sanchez SA, Jaureguiberry MS, Vela ME, Ferreira ST and Tricerri MA.

Proteomic Analysis of Polymorphonuclear Neutrophils Identifies Catalase as a Novel Biomarker of Abdominal Aortic Aneurysm.
Appendix

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(*) The sum of publications for all Departments and Units in these columns exceeds the total given in the first row because some publications are signed by members from more than one Department or Unit, and these duplicates have been eliminated from the total.
Appendix

Training Programs and Courses

Training is one of the CNIC’s core activities, and the Center has devised a comprehensive training plan, called **CNIC-JOVEN**, which includes programs for people at all levels, from senior high school students to postdoctoral researchers and other professionals.

The **CNIC-JOVEN Training Plan** is designed to bring young people into biomedical research and create a strong base of talented researchers in the cardiovascular area.

**Pre-university & Undergraduate Students**

**ACÉRCATE Program**

The ACÉRCATE Program offers senior high school students studying natural and health sciences the chance to experience life as a biomedical researcher, with the aim of awakening interest in a career in research.

Participants spend two weeks at the CNIC, learning modern techniques used in biomedical research, conducting supervised experiments, operating sophisticated scientific equipment and presenting the results of their work, all under the supervision of our researchers.

**Fellowships in 2011**

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**CICERONE Program**

The CICERONE Program is open to advanced undergraduate students studying toward a biomedicine-related university degree. Participants extend their scientific training through hands-on experience of laboratory-based biomedical research during the summer recess. In addition to carrying out a supervised research project, the students also attend CNIC seminars and workshops.

The aim of the program is to give university students first-hand knowledge of biomedical research so that they can make more informed choices about the possibility of pursuing a scientific career in the future.

**Fellowships in 2011**

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## Fellowship Programs and Courses

### Fellowships in 2011

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**Appendix**

**Training Programs and Courses**

**CICERONE Workshop: “What you need to know about cardiovascular research”**

This workshop, offered in collaboration with the Sociedad Española de Cardiología (SEC), consists of a group of lectures that provide a general introduction to cardiovascular research in Spain, and also give participants the chance to question key researchers and opinion leaders in the field. The 2011 edition of the CICERONE workshop took place in “La Casa del Corazón”, Madrid.

**Date:** 23 and 24 September 2011  
**Attendees:** 92

**VASCULAR BIOLOGY Course**

Dr Valentin Fuster delivers this lecture series, sponsored by the pharmaceutical company Esteve, on “Vascular biology: basic and clinical research” as part of the summer program of the Universidad Internacional Menéndez Pelayo (UIMP) in Santander.

**Dates:** 18-19 July 2011  
**Attendees:** 80

**Recent Graduates**

**CARDIOVASCULAR POSGRADUATE Program**

The CNIC is developing a Cardiovascular Postgraduate Program, run through collaboration with Spanish universities. The first strand in this Program has been established through a formal agreement with the Universidad Autónoma de Madrid (UAM).

In the academic year 2010/2011, the CNIC collaborated in the Masters in Molecular Biomedicine, offering a module in Cardiovascular Disease. This optional module provides a broad overview of cardiovascular biology, including perspectives from basic, clinical and translational research.

**Dates:** 17 January-22 February 2011  
**Venue:** CNIC  
**UAM MSc Students:** 11  
**CNIC PhD students:** 4
Appendix

Training Programs and Courses

MASTER Program

This grants program provides individual funding for study towards a Masters degree at a Spanish university. The program is directed at students who are going to study for a PhD in one of the CNIC's laboratories: completion of an official Masters (Máster Oficial) has been introduced as an obligatory stage towards a PhD in Spain, in accordance with the Bologna process to standardize academic qualifications across Europe.

Fellowships in 2011

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PREDOCTORAL (PhD) Program

The PREDOCTORAL Program provides a common framework for all researchers at the CNIC who are working toward a doctoral degree. All predoctoral researchers are signed up to this program, independently of their funding source.

The aims of the program are as follows:

• To ensure uniform quality of predoctoral training at the CNIC
• To ensure fair and equal access of predoctoral researchers to training opportunities
• To work in accordance with the rights and obligations laid out in Real Decreto 63/2006, which relates to the training of research personnel

Graduate students at the CNIC who obtained their PhD degrees in 2011

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<td>Del Monte Nieto, Gonzalo</td>
<td>Expression and functional analysis of notch signalling during cardiac development with special focus on the epicardium and coronary vasculature</td>
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## Training Programs and Courses

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### Graduate students studying for their PhD theses at the CNIC during 2011

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## Training Programs and Courses

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## Appendix

### Training Programs and Courses

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Appendix

Training Programs and Courses

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<td>Cardiovascular Development and Repair</td>
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CARDIO-IMAGE Program

The CARDIO-IMAGE Program (CNIC-MSSM) has been launched against the backdrop of the Collaboration Agreement signed between the CNIC and the Mount Sinai School of Medicine (MSSM), the aim of which is to create a Joint Training and Research Unit in Cardiovascular Imaging. The goal of this Program is to offer blue-ribbon training in state-of-the-art cardiovascular imaging. This will be achieved through laboratory-based training at the CNIC-MSSM Joint Unit, located on the MSSM campus in New York.

Fellowships in 2011

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<th>Institution</th>
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<tr>
<td>Arias, Teresa</td>
<td>Centro de Investigación Aplicada, Navarra</td>
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<tr>
<td>García Lunar, Inés</td>
<td>Hospital Puerta de Hierro, Madrid</td>
</tr>
<tr>
<td>Mateo de Castro, Jesús</td>
<td>Centro Nacional de Investigaciones Cardiovasculares - Madrid</td>
</tr>
<tr>
<td>Pazos, Pablo</td>
<td>Hospital de Vigo</td>
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</table>
Training Programs and Courses

Postgraduate Students & Medical Professionals

INVESMIR Program

The INVESMIR Program offers medical professionals, during their specialization period as resident interns, the opportunity to further their training through a research project in one of the CNIC’s laboratories, under the supervision of a CNIC scientist.

An important aim of the program is that participants establish contacts and collaborations in the CNIC that will support them, after completion of their MIR specialization training, in pursuing their own research projects at their centers within the Spanish National Health System.

Fellowships in 2011

<table>
<thead>
<tr>
<th>Name</th>
<th>Hospital</th>
<th>CNIC Department</th>
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<td>Fernández, Rodrigo</td>
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<td>Epidemiology, Atherothrombosis and Imaging</td>
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</table>

CARDIOVASCULAR PATHOPHYSIOLOGY Course: “From symptoms to genes”

The course in CARDIOVASCULAR PATHOPHYSIOLOGY is offered in collaboration with the Sociedad Española de Cardiología. This course offers a translational vision of cardiology to medical specialists by introducing them to the study of pathophysiology and basic research. Participants are given an overview of the molecular and genetic factors that underlie cardiac diseases and gain a modern vision of cardiac physiology.

Dates: 25 and 26 November 2011
Venue: CNIC Lecture Hall
Attendees: 80
Appendix

Seminars, Events and Awards

**January**
25
Jan Ruijter  
Academic Medical Center  
Amsterdam, The Netherlands

31  
Toby Lawrence  
Centre d’Immunologie de Marseille  
Luminy CNRS-INSERM  
Université de la Méditerranée  
Marseille, France

**February**
07  
Sussan Nourshargh  
William Harvey Research Institute  
London, UK

28  
Benjamin Cravatt  
The Skaggs Institute for Chemical Biology  
La Jolla, USA

**March**
07  
Douglas Losordo  
Northwestern University  
Feinberg School of Medicine  
Chicago, USA

15  
Daniel Lieber  
Systems Biology  
Harvard University  
Cambridge, USA

23  
Kenneth Walsh  
Whitaker Cardiovascular Institute  
Boston University School of Medicine, USA

28  
Denis Duboule  
University of Geneva, Switzerland

**April**
04  
Douglas C. Wallace  
Center of Mitochondrial and Epigenomic Medicine (CMEM)  
Children’s Hospital of Philadelphia  
University of Pennsylvania, USA

07  
Alexandra Joyner  
Courtney Steel Chair in Pediatric Cancer Research  
Memorial Sloan-Kettering Cancer Center  
New York, USA

11  
Irina Kaverina  
Vanderbilt University Medical Center  
Nashville, USA

14  
Jesús Ruberte París  
Barcelona University, Spain

18  
Frans Van de Werf  
Department of Cardiovascular Medicine  
University Hospitals Leuven, Belgium

**May**
09  
Marino Zerial  
Max Planck Institute of Molecular Cell Biology and Genetics  
Dresden, Germany

11  
Pilar Ruiz-Lozano  
Stanford University School of Medicine, USA

12  
Álvaro Rada-Iglesias  
Stanford University School of Medicine, USA

13  
Alahari Suresh  
Department of Biochemistry and Molecular Biology, School of Medicine, LSUHSC  
New Orleans, USA

18  
Ana Diez-Roux  
School of Public Health  
University of Michigan, USA

23  
Celeste Simon  
University of Pennsylvania School of Medicine Philadelphia, USA

30  
Stephen Miller  
Judy Gugenheim Research Professor  
Director-Interdepartmental Immunobiology Center  
Department of Microbiology-Immunology  
Northwestern University Medical School  
Chicago, USA

**June**
06  
Mike Levine  
University of California at Berkeley, USA

09  
Juan Luis Gutiérrez-Chico  
Biomedical Research Institute  
Vigo & Interventional Cardiology Department  
Erasmus MC,  
Rotterdam, The Netherlands
Appendix

Seminars, Events and Awards

> July

05 José Antonio Cancelas
Children’s Hospital
Cincinnati, USA

11 Bradford Berk
University of Rochester
New York, USA

> August

02 Jorge Moscat
Sanford-Burnham Medical Research Institute
La Jolla, USA

> September

01 Matt Sleeman and Mat Robinson
MedImmune
Cambridge, UK

19 Nils-Göran Larsson
Max Planck Institute for Biology of Ageing
Köln, Germany

> October

03 Ian Chambers
Institute for Stem Cell Research
University of Edinburgh, Scotland

06-07 CNIC Conference
“At the heart of the genome: frontiers in cardiovascular genomics research”

10 Bart N. Lambrecht
Ghent University, Belgium

17 Christian Weber
Direktor, Institut für Prophylaxe und Epidemiologie der Kreislaufkrankheiten
Lehrstuhl für Präventive Vaskuläre Medizin
August-Lenz-Stiftung
Poliklinik
Klinikum der Universität München (KUM), Germany

24 Tom Gridley
The Jackson Laboratory
Bar Harbor, Maine, USA

26 CNIC-IIF - International Incoming Fellowships for Young Group Leaders

> November

14 David Scadden
LMGH Center for Regenerative Medicine
Boston, USA

13 Carlos Iribarren
Kaiser Permanente Division of Research
Broadway, Oakland, USA

15 Kenneth Chien
Harvard Stem Cell Institute
Massachusetts General Hospital
Boston, USA

16 Tobias Schäffter
Philip Harris Chair of Imaging Sciences
Division of Imaging Sciences
King’s College
London, UK

16 Ken Poss
Department of Cell Biology
Duke University Medical Center
Durham, USA

20 CNIC High Content Screening Workshop

22 Jesús Ruiz-Cabello
Departamento de Química-Física II
Facultad de Farmacia
Universidad Complutense de Madrid, Spain

22 Guido Serini
Laboratory of Cell Adhesion Dynamics
IRCC
Torino, Italia

23 IX Reunión Científica del Grupo de Trabajo de Cardiología Experimental

26 Alan Daugherty
Division of Cardiovascular Medicine
University of Kentucky
Lexington, USA

01 Matt Sleeman and Mat Robinson
MedImmune
Cambridge, UK

19 Nils-Göran Larsson
Max Planck Institute for Biology of Ageing
Köln, Germany

03 Michael Schneider
National Heart and Lung Institute
Imperial College London, UK

05 José Antonio Cancelas
Children’s Hospital
Cincinnati, USA

11 Bradford Berk
University of Rochester
New York, USA

17 Christian Weber
Direktor, Institut für Prophylaxe und Epidemiologie der Kreislaufkrankheiten
Lehrstuhl für Präventive Vaskuläre Medizin
August-Lenz-Stiftung
Poliklinik
Klinikum der Universität München (KUM), Germany

26 CNIC-IIF - International Incoming Fellowships for Young Group Leaders

14 David Scadden
LMGH Center for Regenerative Medicine
Boston, USA
Seminars, Events and Awards

15  Semana de la Ciencia
Ven a CNIC: Visita interactiva a sus departamentos para conocer la investigación cardiovascular

18  Semana de la Ciencia
CNIC y la investigación cardiovascular

21  Derek Yellon
The Hatter Cardiovascular Institute
University College London, UK

24  Jean-Jacques Schott
Institut du Thorax – INSERM, Nantes, France
CRG, Barcelona, Spain

25-26  Curso de Fisiopatología Cardiovascular

28  Elaine Dzierzak
Erasmus MC - Medical Faculty
Erasmus Stem Cell Institute
Rotterdam, The Netherlands

December

12  Ben Nichols
Medical Research Council Laboratory of Molecular Biology
Cambridge, UK
Appendix

Seminars, Events and Awards

Awards

**Cardiovascular Development and Repair**

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<th>Award</th>
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<tr>
<td>Awarded to</td>
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Appendix

Seminars, Events and Awards

**Vascular Biology and Inflammation**

Award: First Prize in the X Premio Certamen Universitario "Arquímedes", de Introducción a la Investigación Científica, from the Ministerio de Educación, Cultura y Deporte
Awarded to: Alba Mota

**Epidemiology, Atherotrombosis and Imaging**

Award: 2011 Grand Prix Scientifique from the Lefoulon-Delalande Foundation of the Institut de France
Awarded to: Valentín Fuster

Award: Universal Spaniard (Considered the most influential in Spain, previous awardees including two Nobel Laureates – Rafael Nadal in 2010)
Awarded to: Valentín Fuster

Award: Honorary citizen of Buenos Aires, Argentina
Awarded to: Valentín Fuster

Award: Presidential Award of the German Society of Cardiology
Awarded to: Valentín Fuster

Award: Presidential Award of the French Society of Cardiology
Awarded to: Valentín Fuster

Award: Presidential Award of the Mexican Society of Cardiology
Awarded to: Valentín Fuster

Award: Presidential Award of the Argentine Society of Cardiology
Awarded to: Valentín Fuster

Award: Presidential Award of the Brazilian Society of Cardiology
Awarded to: Valentín Fuster

Award: Presidential Award of the Chilean Society of Cardiology
Awarded to: Valentín Fuster

Award: Premio a la Mejor Comunicación Oral, Annual Meeting of The Spanish Society of Cardiology, Murcia

Award: Prize for the Best Translational Research study from the Fundación Hospital Madrid, for the study “Diagnostic value of coronary artery calcium scoring in low-intermediate risk patients evaluated in the emergency department for acute coronary syndrome”
Awarded to: Leticia Fernandez Friera

Award: Member of the Spanish Royal Academy of Pharmacy
Awarded to: José Mª Ordovás

Award: Panamerican Nutrition and Food Research Award from Grupo Bimbo
Awarded to: José Mª Ordovás

Award: Gregorio Marañón Nutrition Award from The Spanish Royal Gastronomic Society
Awarded to: José Mª Ordovás

Award: Jose Mataix Nutrition Award from The Spanish Academy of Nutrition
Awarded to: José Mª Ordovás
Strategic Alliances

The CNIC forms alliances to investigate, train, innovate and transfer

The central aim of biomedical research is to translate knowledge generated in basic research laboratories into improved and innovative clinical practice, and reciprocally to stimulate research into questions raised in healthcare centers. Excellence in this area therefore requires close contact with clinical institutions. In the period 2008-2011, the CNIC has established a strategic network with institutions within the Spanish National Health System and collaborations with the Spanish Society of Cardiology (Sociedad Española de Cardiología) to develop translational research projects and to identify and train the best investigators for these types of projects.

Innovation, the development of new therapies and drugs, and the application of advanced technologies in the field of biomedical research require close collaboration with the industrial sector. The CNIC has established partnerships with companies from different sectors (including pharmaceutical, biotechnology, medical technology and imaging) to take on cutting-edge research projects in these fields. Two of the CNIC’s main translational projects are based on this type of collaboration: the PESA study (Progression of early subclinical atherosclerosis), run with Banco Santander and the Marcelino Botín Foundation; and the Polypill project, run through a private-public partnership with Ferrer International.

Since research is one of the most globalized sectors, where competition is international, the CNIC is also very active in establishing collaborations with other countries, particularly in Europe and North America. Currently, the CNIC is collaborating with six institutes in the USA and 33 in Europe.

At the level of training, the CNIC-JOVEN Training Plan is advancing thanks to collaborations that the Center has established with prestigious Spanish universities and scientific societies such as the Universidad Autónoma de Madrid and the Spanish Society of Cardiology, as well as foreign biomedical research institutions such as Mount Sinai Medical School of Medicine (New York, USA) and Johns Hopkins University (Baltimore, USA).
Appendix

Funding

Public-Private Partnership

In spite of the enormous advances in diagnosis and treatment witnessed over the last 20 years, cardiovascular diseases continue to be the main cause of death in the developed world. The costs generated in economic, social and human terms are immense. In response to this reality, the Spanish Government, through the Instituto de Salud Carlos III (Carlos III Health Institute, created the CNIC to bring together the best of Spanish cardiovascular research and provide it with a modern infrastructure and ample funding to carry out world-leading biomedical research.

To achieve the funding necessary for its ambitious plan, The Spanish government appealed to the sense of social obligation of some of the major players in Spanish civil society, by inviting the largest businesses in the country to make an active and long-term commitment to this project. The outcome was an agreement, signed in December 2005, between the Spanish Government and a group of some of the most important Spanish businesses. Under the terms of this agreement these companies pledged their commitment to funding the CNIC up until 2012. This commitment was recently extended until 2020.

Shortly after the agreement was signed, on January 24, 2006, this group of companies was formally constituted as the Pro CNIC Foundation. Through its creation, the participating companies have made a long-term commitment to biomedical research that represents the most significant act of business sponsorship in recent years in terms of the amount of funding it provides, its social significance, the group of companies involved, and the anticipated outcomes.

Since the signing of this agreement, the CNIC’s funding has been based on a public-private partnership of a broad, socially-committed nature. In this innovative PPP, state funding is complemented by financing through the Pro CNIC Foundation (http://www.fundacionprocnic.org).

New companies have since joined the Pro CNIC Foundation, and there are now 13 members: Acciona, BBVA, Fundación Botín, Endesa, Fundación Abertis, Fundación Ramón Areces, Gas Natural Fenosa, Grupo Prisa, Inditex, La Caixa, Fundación Repsol, Fundación Mutua Madrileña, and Telefónica. This funding scheme allows the CNIC to fund special programs for the discovery and training of young investigators, to award extramural grants aimed at integrating basic and clinical research to answer specific questions, to acquire specialized research equipment that would otherwise be difficult to fund, and to run programs to incentivize and retain valuable investigators.

But the Pro CNIC Foundation not only provides the CNIC with money; it also contributes its accumulated managerial and business expertise. Representatives of the Pro CNIC Foundation sit on the CNIC’s Board of Trustees, and actively participate in the management, planning and decision taking related to the Center. In this way, some of the most important organizations in the private sector in Spain have committed themselves to a direct involvement in biomedical research and the fight against cardiovascular diseases.

A major strength of this socially-committed PPP model is that it provides a more solid base than traditional forms of charitable financing, giving the CNIC a more stable financial support than it would have if it depended on sporadic donations from benefactors. This stability gives the CNIC greater freedom to commit itself to long-term, high-return research strategies in collaboration with public and private institutions, and allows for a more effective use of its own resources generated through competitive projects and the exploitation of intellectual property rights.
Appendix

Funding

Public Funding

Private Funding

Fundación proCnic
The CNIC attracts international resources

The presence of the CNIC in international projects of excellence, achieved through competitive bidding processes, has increased markedly in recent years. This positive trend will continue in the coming years.

Evidence for this is provided by the fact that CNIC groups submitted six proposals in the latest call for European Research Council Starting Grants (ERC StG), the most prestigious and competitive call for basic research in Europe.

The CNIC is already host to three ERC StGs, as well as another project in the same program but in the category of senior researchers (ERC Advanced Grant).

The CNIC also coordinates two cooperative research projects of international scope within the 7th Framework Programme of the European Union.

Furthermore, the CNIC has positioned itself as a leader in cooperative networks for training young researchers (Initial Training Networks, ITN) and projects to attract talent (COFUND).

Another international award garnered by the CNIC is that from the US-based Howard Hughes Medical Institute to Dr. Mendez-Ferrer this year, which will be officially presented in January 2012.

**ERCs:**

- Immune functions of myeloid Syk-coupled C-type lectin receptors sensing necrosis – CLR Sensing Necrosis – Dr. David Sancho Madrid.
- Role of obesity in the development of hepatocellular carcinoma (OBECAN) – Dr. Guadalupe Sabio Buzo.
- Molecular mechanisms of mature B cell lymphomagenesis (BCLYM) – Dr. Almudena Ramiro.

**HHMI:**

- Howard Hughes Medical Institute International Early Career Scientist – Dr. Simón Méndez Ferrer.

**Seventh Framework Programme (highlights):**

- Fixed dose combination drug for secondary cardiovascular prevention – Dr. Ginés Sanz / Dr. Valentín Fuster.
- Cardio Repair European Multidisciplinary Initiative (CARE-MI) - Dr. Antonio Bernad.
- Translational Training network on the Cellular and Molecular Bases of Heart Homeostasis and Repair “CardioNeT” – Dr. Enrique Lara / Dr. Miguel Torres / Dr. José Luis de la Pompa.

**CNIC International Incoming Fellowships.**

**National and Regional Funds (public and private):**

- From 2008 to 2011 the CNIC won about 150 grants (projects, grants and other subsidies) on a competitive basis.
Appendix

Funding

Each year the CNIC improves its competitive funding figures.

In total, from 2008 to 2011 the CNIC raised around €40 million in competitive bidding projects, including funding from national and international sources.

The CNIC promotes the transfer of knowledge to the industrial sector

The protection of research findings: a tool for creating value

- So far, 23 inventions have been protected by the CNIC.
- There are currently over 11 patent families being actively marketed. In total more than 25 documents are being processed.
- Since 2008, we have analyzed 24 ideas that have given rise to 16 priority patent applications.
- 18 of these inventions have been developed in cooperation with other entities.

AWARD COMPETITIVE (summary 2008 - 2011)

<table>
<thead>
<tr>
<th>Year</th>
<th>National</th>
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<tbody>
<tr>
<td>2008</td>
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<tr>
<td>2011</td>
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</tbody>
</table>

Funds awarded annually and disbursed over several years (average 3.5 years). 2011 includes the Severo Ochoa prize and estimates pending the definitive granting and final distribution of the funds.

The protection of research findings: a tool for creating value

- So far, 23 inventions have been protected by the CNIC.
- There are currently over 11 patent families being actively marketed. In total more than 25 documents are being processed.
- Since 2008, we have analyzed 24 ideas that have given rise to 16 priority patent applications.
- 18 of these inventions have been developed in cooperation with other entities.
Encouraging a culture of innovation

• Our researchers are increasingly involved in generating inventions, protected by the Center. In recent years interest has grown in cooperation with business and technological development.

• To increase the number of researchers participating in these initiatives, a strategy is being designed for communication and internal training in the transfer of technology and innovation.

The transfer of knowledge ensures its translation into clinical practice and a return on R&D investment

• The CNIC has signed two licensing agreements since 2008, with returns already expected for 2012. One is for the development of the Polypill and the other, renewed in 2011, provides for the transfer of knowhow and material to the company Proalt.

• These agreements ensure that the investment in research will be reflected in products and services that improve patient care, generate an economic return for the CNIC, and support knowledge-based economic development.

Proactive management: essential for working with third parties

• The CNIC offers its researchers active and continued monitoring in their relations with all players in the science and technology sectors.

• The protection of results with patents is managed in collaboration with industrial property agencies specializing in biomedicine.

• The CNIC uses standard models for material transfer and confidentiality agreements approved by the State Legal Service.

• Around 240 MTAs (Material Transfer Agreements) have been signed, including 200 since 2008.

• 21 CDAs (confidentiality agreements) have been signed since 2008 with national and international institutions.
Appendix

Funding

Innovation: a priority for the CNIC

- In June 2011 the CNIC Translational Platform was launched to serve as a link between the Center and the other players in the science and technology system.
- The Center’s activity is built on three pillars: technological development, technology transfer, and clinical.
Appendix

Staff Figures

CNIC staff 2011 (361)

- Scientific Departments: 269
- Technical Units: 18
- Administration: 27
- Scientific Services: 47

CNIC research staff 2011 (316)

- Head of Laboratory/Unit: 169
- Research Scientists: 41
- Postdoctoral Researchers: 68
- Predoctoral Researchers: 7
- Technicians: 31
Appendix

Staff Figures

Staff by department 2011 (269)

- Epidemiology, Atherothrombosis and Imaging: 79
- Vascular Biology and Inflammation: 70
- Cardiovascular Development and Repair: 113
- Translational Platform: 7

Gradual growth and current status

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</tbody>
</table>
Appendix

Staff Figures

Gender distribution 2011

- Male: 61
- Female: 39

Age distribution 2011

- <30: 20
- 30-39: 25
- 40-50: 6
- >50: 49