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Fundación
Centro Nacional de
Investigaciones
Cardiovasculares
Carlos III

EXCELENCIA
SEVERO
OCHOA

SCIENTIFIC REPORT 2011

SCIENTIFIC REPORT 2011

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Valentín Fuster. *General Director*

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 (Valentín 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.

Miguel Torres. *Associate Director*

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 in 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.

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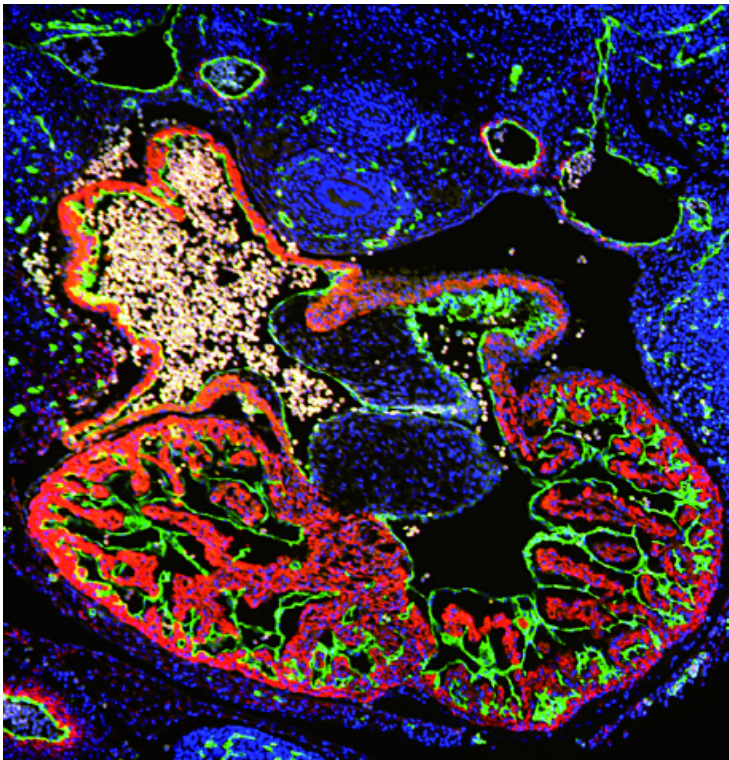
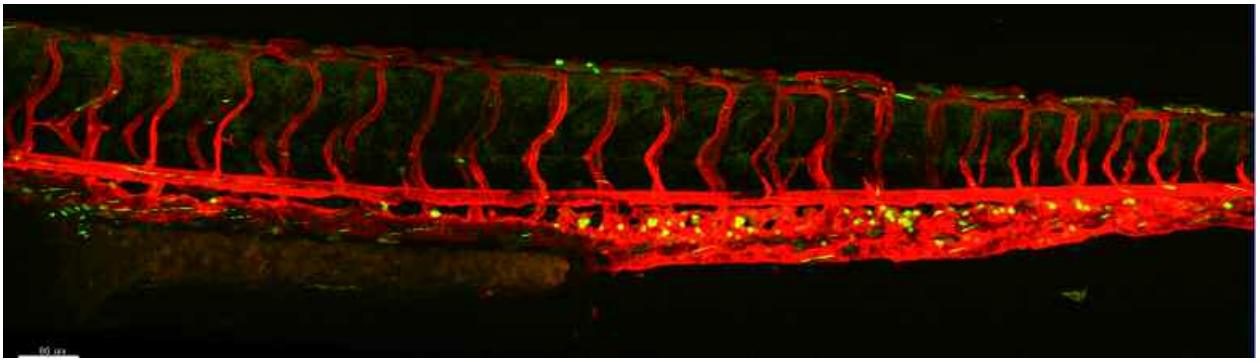
Research Departments

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 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 Enviromental Health, Munchen, Germany (20 Jul – 5 Aug)

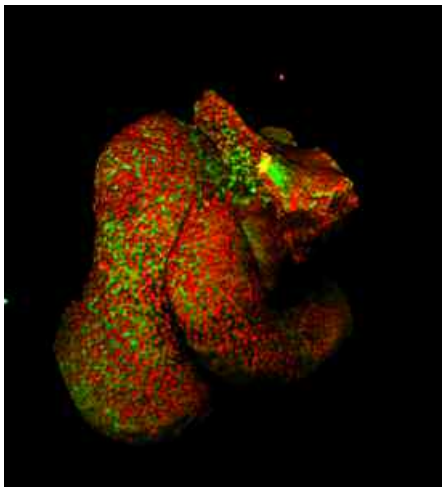
RESEARCH INTEREST

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.

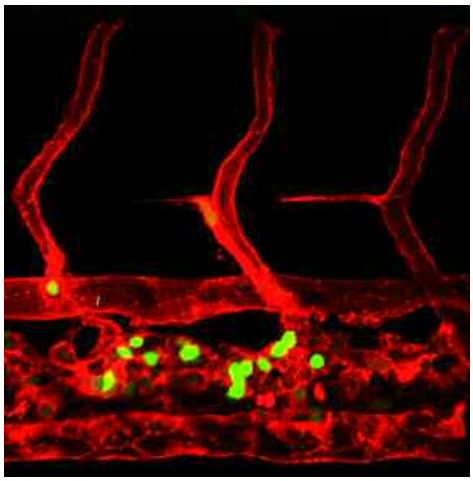


Cell-resolution 3D reconstruction from confocal microscopy data of a mosaic E10.5 mouse heart. *The image shows a lateral view of the heart tube, comprising the atrioventricular canal, outflow tract and the left and right ventricles. Nuclei are depicted in red.*

Research Departments

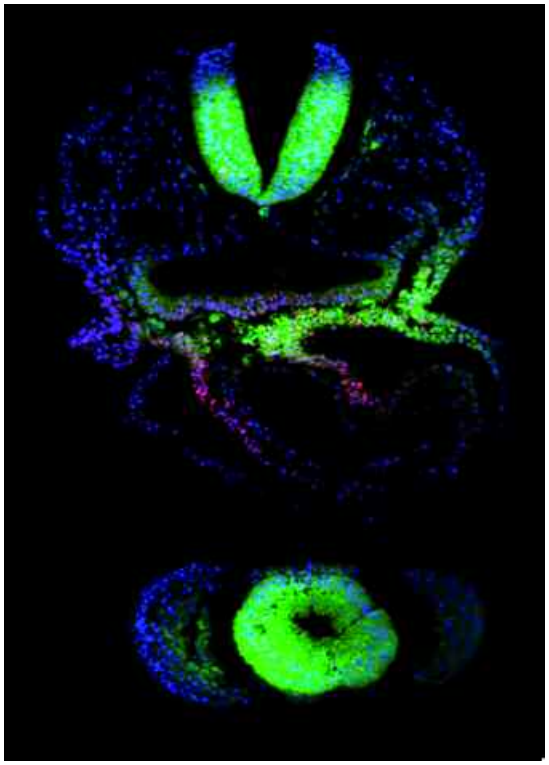
1 Cardiovascular Development and Repair

A. Cardiovascular Developmental Biology



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

Roselló-Díez A, Ros MA, Torres M. **Diffusible signals, not autonomous mechanisms, determine the main proximodistal limb subdivision.** *Science* (2011) 332: 1086-1088

JM González-Rosa, Martín V, Peralta M, Torres M, Mercader N. **Extensive scar formation and regression during heart regeneration after cryoinjury in zebrafish.** *Development* (2011) 138: 1663-74

A Roselló-Díez, 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

Carramolino L, Fuentes J, Garcia-Andres C, Azcoitia V, Riethmacher D, Torres M. **Platelets play an essential role in separating the blood and lymphatic vasculatures during embryonic angiogenesis.** *Circ Res* (2010) 106: 1197-201

Research Departments

1 Cardiovascular Development and Repair

A. Cardiovascular Developmental Biology

Intercellular signaling in cardiac development, disease and tissue homeostasis

Head of Laboratory: *José Luis de la Pompa*

Postdoctoral Researchers: *Jesús Fernández Casanova*
Luis Luna Zurita
Donal MacGrogan
Beatriz Martínez Poveda
Meritxell Nus
Belén Prados

Predoctoral Researchers: *Gonzalo del Monte*
Gaetano D'Amato
Álvaro González Rajal
Guillermo Luxán
Juliane Münch
Stanislao I. Travisano

Technicians: *Vanesa Bou*
Ana Cabrero
Patricia Martínez

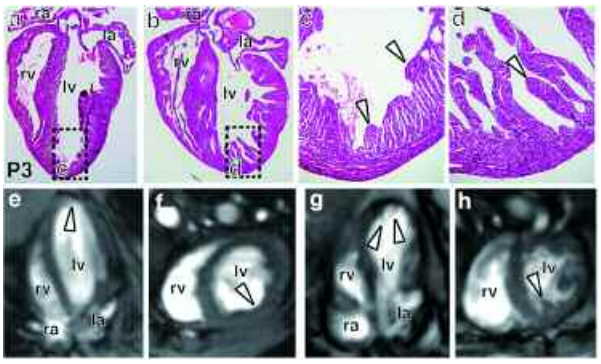
Visiting Scientist: *José María Pérez Pomares*

RESEARCH INTEREST

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.

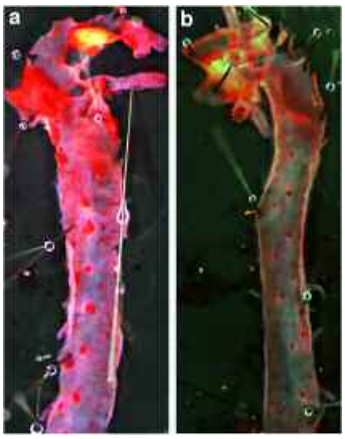


Defective chamber development in Notch mutants. *(a-d) Hemotoxilin-eosin (H&E) staining showing large trabeculae in newborn* Mib1 *mutants (b, d) compared with wild types (a, c). (e-h) Cardiac MRI images of 6 month-old mice showing normal chambers in wild-type (e, g) and large trabeculae in* Mib1 *mice (f, h).*

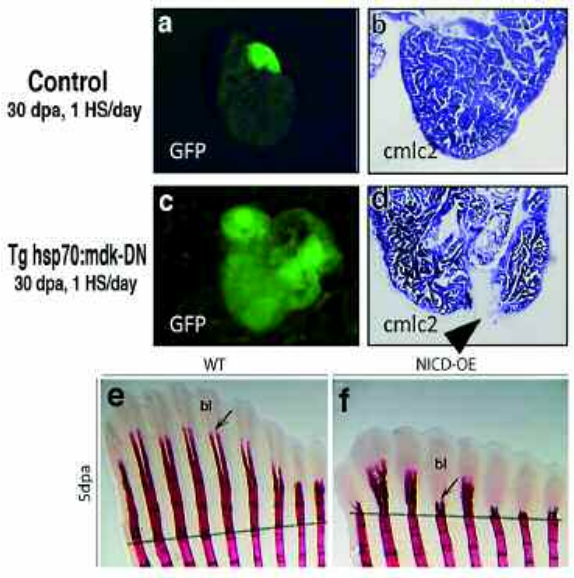
Research Departments

1 Cardiovascular Development and Repair

A. Cardiovascular Developmental Biology



Endothelial-specific deletion of* RBPJk *reduces atherosclerotic plaque formation. *Oil red staining of atherosclerotic plaques in aortas from (a)* ApoE$^{-/-}$;RBP$^{flox/flox}$ *and (b)* ApoE$^{-/-}$; VEcadCreERT/+;RBP$^{flox/flox}$ *mice.*



Inhibition of regeneration in zebrafish. *(a-d) Syndecan-2 inhibition impairs heart regeneration. (a, b) Normal regeneration in a control GFP line, heat shocked (HS) daily for one hour over a 30-day regeneration period. (c,d) Regeneration is disrupted in a transgenic line expressing DN-syndecan-2 under a heat shock promoter. The arrowhead marks the non-regenerated area that does not express the mature cardiomyocyte marker cmlc2. (e,f) Notch activity inhibits blastema differentiation during fin regeneration. (e) Regenerating caudal fin of a wild-type zebrafish, 5 days post amputation (dpa), stained with alizarin red. (f) NICD overexpression leads to an expansion of the blastema (bl) and a blockade of bone formation (arrow).*

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

Pérez-Pomares JM, de la Pompa JL. **Signaling during epicardium and coronary vessel development.** *Circ Res* (2011) 109: 1429-42

Rodríguez P, Higueras MA, González-Rajal A, Alfranca A, Fierro-Fernández M, García-Fernández RA, Ruiz-Hidalgo MJ, Monsalve M, Rodríguez-Pascual F, Redondo JM, de la Pompa JL, Laborda J, Lamas S. **The non-canonical NOTCH ligand DLK1 exhibits a novel vascular role as a strong inhibitor of angiogenesis.** *Cardiovasc Res.* (accepted)

MacGrogan D, Luna-Zurita L, de la Pompa JL. **Notch signaling in cardiac valve development and disease.** *Birth Defects Res A Clin Mol Teratol.* (2011) 91: 449-59

Nus M, MacGrogan D, Martínez-Poveda B, Benito Y, Casanova JC, Fernández-Avilés F, Bermejo J, de la Pompa JL. **Diet-induced aortic valve disease in mice haploinsufficient for the Notch pathway effector RBPJK/CSL.** *Arterioscler Thromb Vasc Biol* (2011) 31: 1580-8

del Monte G, Casanova JC, Guadix JA, MacGrogan D, Burch JB, Pérez-Pomares JM, de la Pompa JL. **Differential Notch signaling in the epicardium is required for cardiac inflow development and coronary vessel morphogenesis.** *Circ Res.* (2011) 108: 824-36

Research Departments

1 Cardiovascular Development and Repair

A. Cardiovascular Developmental Biology

Stem cells in organ generation, regeneration and aging

Head of Laboratory: *Ignacio Flores*

Postdoctoral Researchers: *Tania Aguado*
Cristina González Estévez

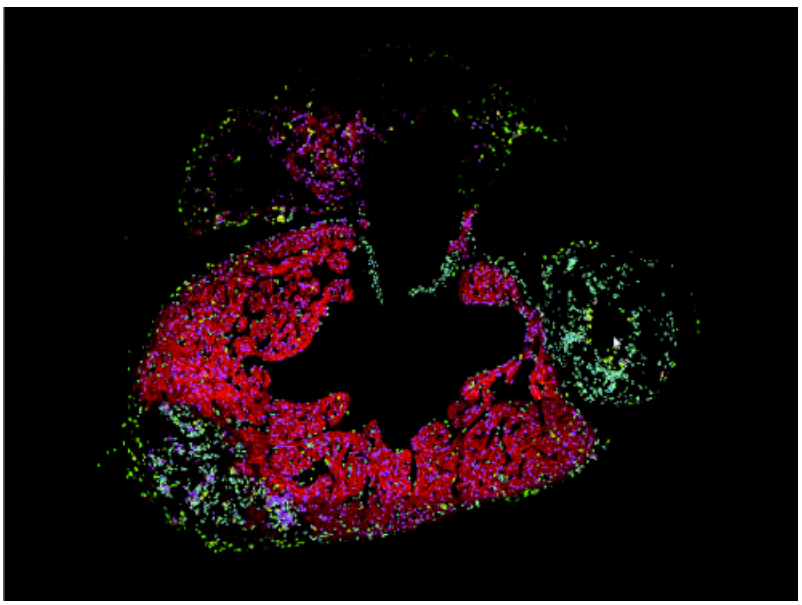
Predoctoral Researchers: *Esther Aix*
Dorotha Bednarek
Maria del Mar de Miguel

Technician: *Irene de Diego*

RESEARCH INTEREST

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.

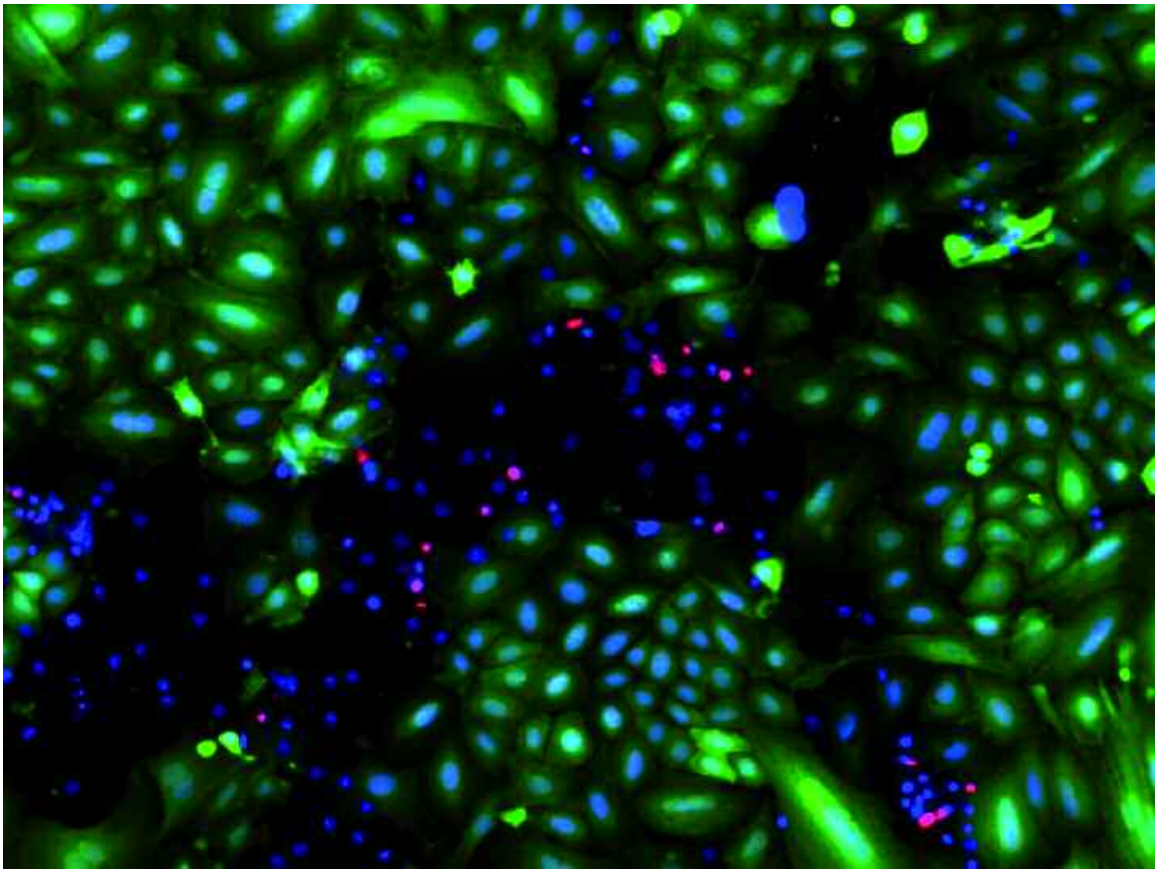


Quantitative analysis of cardiac proliferation in zebrafish heart after infarction.
A specifically tailored image analysis program was used to segment, classify and quantify proliferation of different subtypes of cardiac cells after infarction. This work was done in collaboration with Hind Azegrouz of the Cellomics Unit.

Research Departments

1 Cardiovascular Development and Repair

A. Cardiovascular Developmental Biology



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

Flores I, Blasco MA. **The role of telomeres and telomerase in stem cell aging.** *FEBS Lett* (2010) 584: 3826-30

Blázquez C, Chiarlone A, Sagredo O, Aguado T, Pazos MR, Resel E, Palazuelos J, Julien B, Salazar M, Börner C, Benito C, Carrasco C, Diez-Zaera M, Paoletti P, Díaz-Hernández M, Ruiz C, Sendtner M, Lucas JJ, de Yébenes JG, Marsicano G, Monory K, Lutz B, Romero J, Alberch J, Ginés S, Kraus J, Fernández-Ruiz J, Galve-Roperh I, Guzmán M. **Loss of striatal type 1 cannabinoid receptors is a key pathogenic factor in Huntington's disease.** *Brain* (2011) 134: 119-36

González-Estévez C, Saló E. **Autophagy and apoptosis in planarians.** *Apoptosis* (2010) 15: 279-92

Research Departments

1 Cardiovascular Development and Repair

A. Cardiovascular Developmental Biology

Development of the epicardium and its role during regeneration

Head of Laboratory: *Nadia Mercader*

Predoctoral Researchers: *Juan Manuel González-Rosa*
Marina Peralta

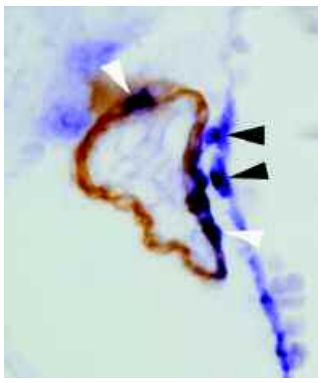
Visiting Scientists: *Francisco J. Enguita, University of Lisbon, Portugal (Jun 18-22)*
Caroline Pellet-Many, University College London, UK (Dec 12-19)
Sophie Rodius, CRP-Santé, Luxemburg (Nov 21-26)
Jana Koth and Ana F da Costa, King's College London, UK (Nov 16-18)

RESEARCH INTEREST

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.

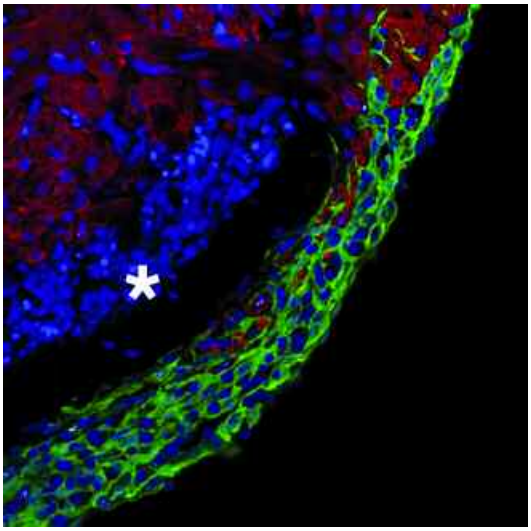


Development of the epicardium in the zebrafish. *In situ hybridization of* tbx18 *mRNA on a sagittal section of a 2 day old zebrafish heart, marking proepicardial (PE) cells in the pericardial wall (black arrowheads) and PE cells attaching to the myocardium (white arrowheads). The myocardium is revealed by anti-myosin heavy chain immunohistochemistry (brown staining).*

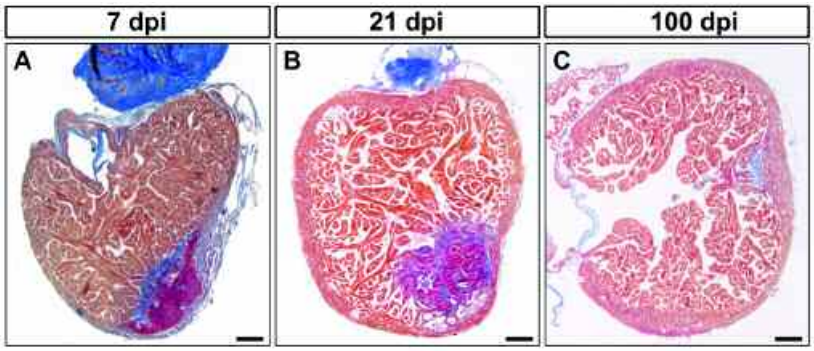
Research Departments

1 Cardiovascular Development and Repair

A. Cardiovascular Developmental Biology



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)

Kovacic JC, Mercader N, Torres M, Boehm M, Fuster V. **Epithelial- and Endothelial- to Mesenchymal Transition: from Cardiovascular Development to Disease.** *Circulation* (accepted)

González-Rosa JM, Martín V, Peralta M, Torres M, Mercader N. **Extensive scar formation and regression during heart regeneration after cryoinjury in zebrafish.** *Development* (2011) 138: 1663-74

González-Rosa JM, Padrón-Barthe L, Torres M, Mercader N. **[Lineage tracing of epicardial cells during development and regeneration].** *Rev Esp Cardiol* (2010) 63: 36-4

Research Departments

1 Cardiovascular Development and Repair

A. Cardiovascular Developmental Biology

Role of new genes in cardiovascular development

Head of Laboratory: *Juan José Sanz Ezquerro*

Predoctoral Researchers: *Verónica Uribe Sokolov*
Laura González Calero

Technician: *Claudio Badía Careaga*

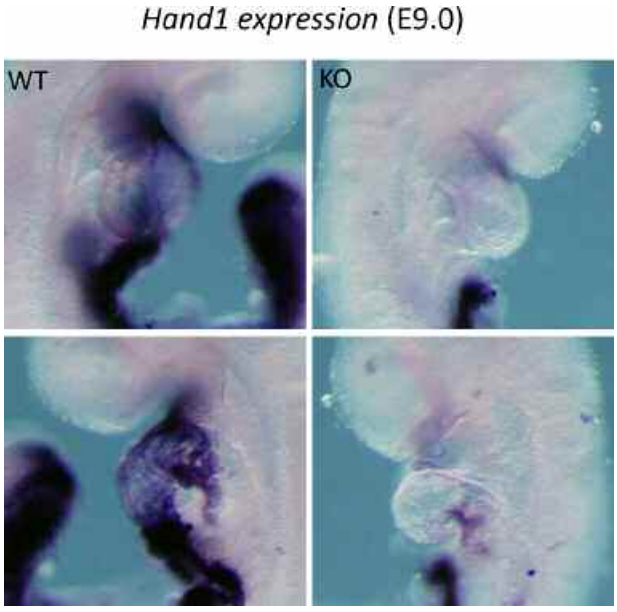
RESEARCH INTEREST

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.

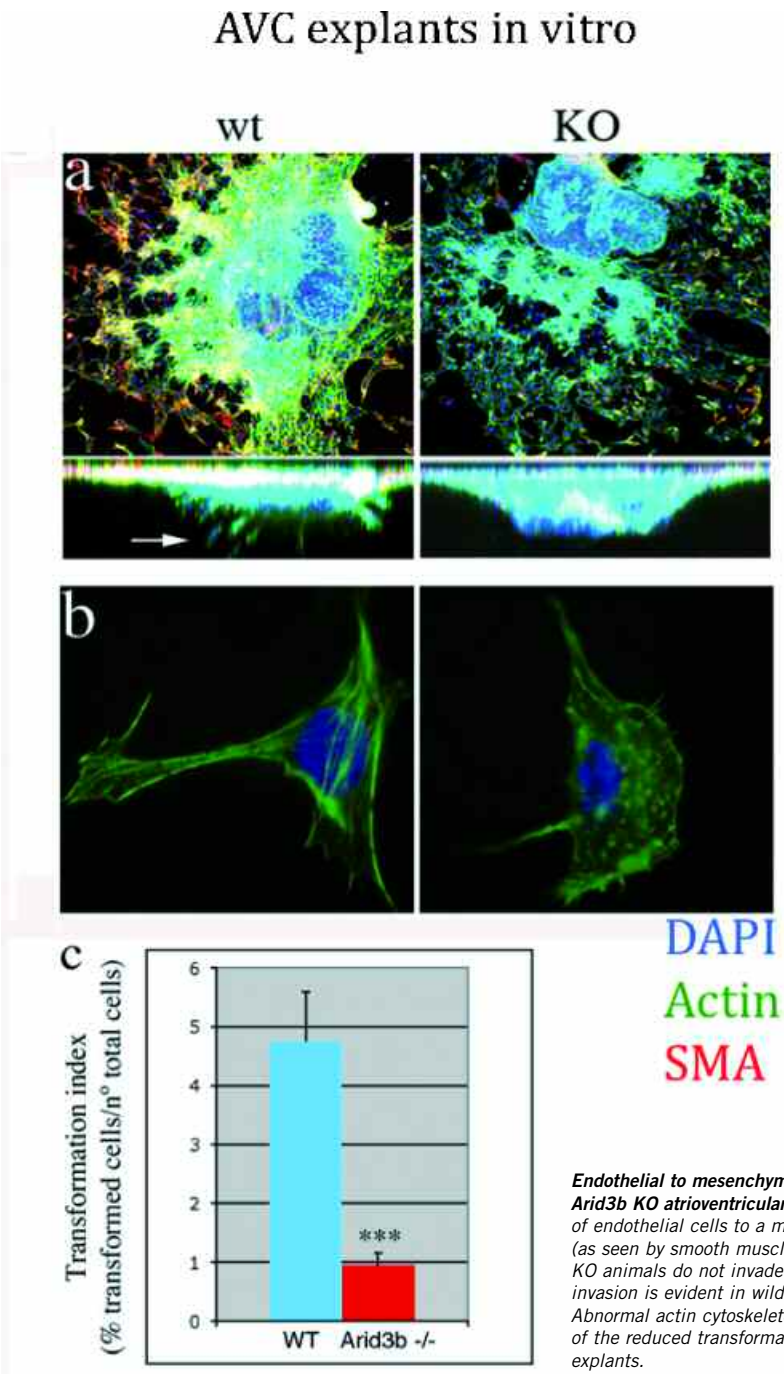


Expression of the transcription factor Hand1 is absent from the ventricular myocardium of Arid3b KO mice at E9.0.

Research Departments

1 Cardiovascular Development and Repair

A. Cardiovascular Developmental Biology



Endothelial to mesenchymal transition is defective in Arid3b KO atrioventricular canal explants. *a) Transformation of endothelial cells to a mesenchymal phenotype is reduced (as seen by smooth muscle actin staining). Also, cells from KO animals do not invade the collagen gel, whereas gel invasion is evident in wild-type embryos (arrows); b) Abnormal actin cytoskeleton in KO cells; c) Quantification of the reduced transformation index in KO versus wild-type explants.*

MAJOR GRANTS

- **Fundació La Marató TV3 (082031)**
- **Ministerio de Ciencia e Innovación. FIS (CP07/00251)**

SELECTED PUBLICATIONS

Casanova JC, Uribe V, Badia-Careaga C, Giovinazzo G, Torres M, Sanz-Ezquerro JJ. **Apical Ectodermal Ridge morphogenesis in limb development is controlled by Arid3b-mediated regulation of cell movements.** *Development* (2011) 138: 1195-205

Research Departments

1 Cardiovascular Development and Repair

B. Stem Cell Biology

Functional genomics of embryonic pluripotency and heart development

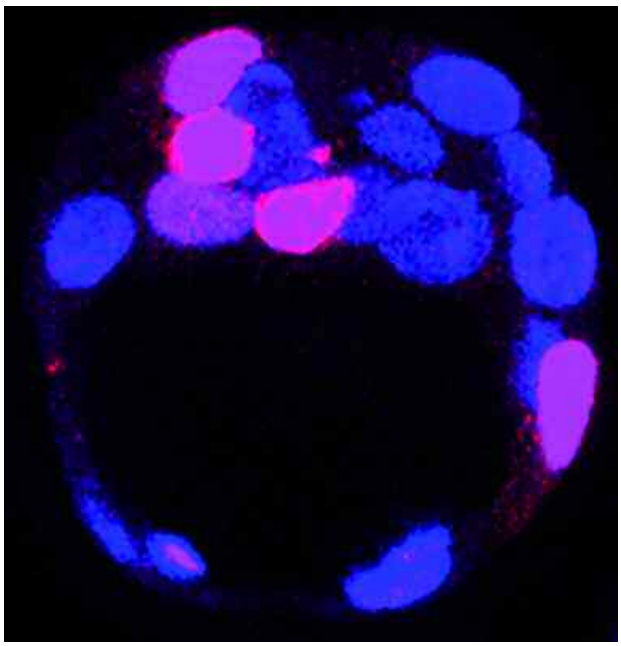
Head of Laboratory:	*Miguel Manzanares*
Postdoctoral Researchers:	*M. Eva Alonso* *Beatriz Fernández-Tresguerres* *Luis Augusto Aguirre Pérez* *Elena López Jiménez* *Cristina Arias Sánchez*
Predoctoral Researchers:	*Teresa Rayón* *Melisa Gómez Velázquez*
Master Student:	*Julio González Sainz de Aja*
Technician:	*Inmaculada Ors*
Visiting Student:	*Sergio Menchero Fernández*

RESEARCH INTEREST

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.

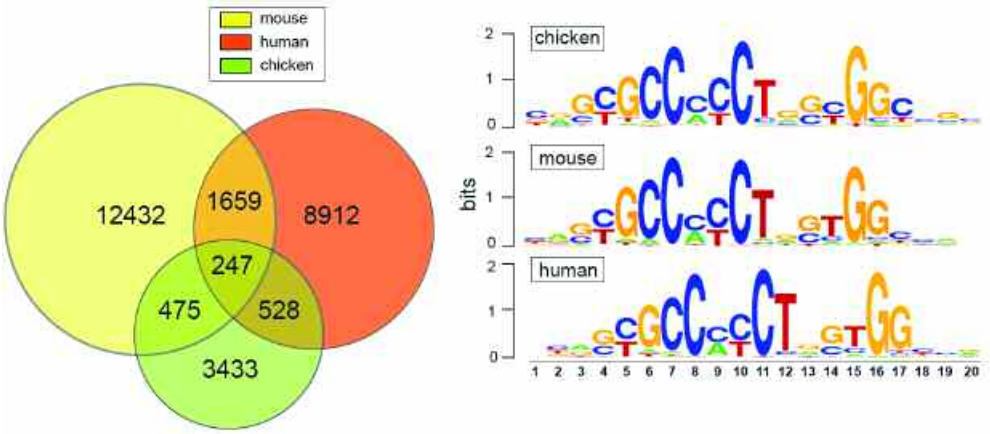


Transgenic mouse blastocyst showing activity of RFP (red fluorescent protein) driven by an Oct4 *regulatory element that predominantly functions in the inner cell mass.*

Research Departments

1 Cardiovascular Development and Repair

B. Stem Cell Biology



CTCF binds at multiple positions in the genome, where it is necessary for the function of boundary, insulator and looping elements. ***A****, Venn diagram of interspecies conservation of CTCF bound sites, showing a core of 247 positions bound in three vertebrate species.* ***B****, Canonical CTCF motifs are obtained by de novo motif discovery from each species-specific bound subset.*

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

Pernaute B, Spruce T, Rodriguez TA, Manzanares M. **miRNA-mediated regulation of cell signaling and homeostasis in the early mouse embryo.** *Cell Cycle* (2011) 10: 584-91

Cañon S, Fernandez-Tresguerres B, Manzanares M. **Pluripotency and lineages in the mammalian blastocyst: an evolutionary view.** *Cell Cycle* (2011) 10: 1731-8

Tena JJ, Alonso ME, de la Calle-Mustienes E, Splinter E, de Laat W, Manzanares M, Gómez-Skarmeta JL. **An evolutionarily conserved three-dimensional structure in the vertebrate Irx clusters facilitates enhancer sharing and coregulation.** *Nat Commun* (2011) 2: 310

Martin D, Pantoja C, Fernández Miñán A, Valdes-Quezada C, Moltó E, Matesanz F, Bogdanoviç O, de la Calle-Mustienes E, Domínguez O, Taher L, Furlan-Magaril M, Alcina A, Cañón S, Fedetz M, Blasco MA, Pereira PS, Ovcharenko I, Recillas-Targa F, Montoliu L, Manzanares M, Guigó R, Serrano M, Casares F, Gómez-Skarmeta JL. **Genome-wide CTCF distribution in vertebrates defines equivalent sites that aid the identification of disease-associated genes.** *Nat Struct Mol Biol* (2011) 18: 708-14

Fernandez-Tresguerres B, Cañon S, Rayon T, Pernaute B, Crespo M, Torroja C, Manzanares M. **Evolution of the mammalian embryonic pluripotency gene regulatory network.** *Proc Natl Acad Sci U S A* (2010) 107: 19955-6

Research Departments

1 Cardiovascular Development and Repair

B. Stem Cell Biology

Gene expression and genetic stability in adult stem cells

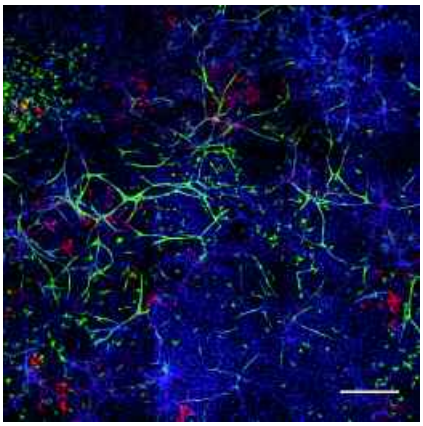
Head of Laboratory:	*Antonio Bernad*
Research Scientists:	*Manuel Ángel González de la Peña* *Enrique Samper*
Postdoctoral Researchers:	*Isabel Moscoso* *Juan A Bernal* *José Luis Torán* *Beatriz Escudero* *Susana Cañón* *Alberto Izarra* *Marta Evangelista* *Kausalia Vijayaragavan* *Xonia Carvajal-Vergara*
Predoctoral Researchers:	*Juan Camilo Estrada* *María Tomé* *Íñigo Valiente* *Francisco Miguel Cruz*
Masters Student:	*Elvira Alonso*
Support Scientists:	*Candelas Carreiro* *Carmen Albo*
Technicians:	*Juan Carlos Sepúlveda* *Yaima Torres* *Rosa María Carmona* *Vanessa Blanca* *Susana Aguilar*
Visiting Scientist:	*Guadalupe Gómez*

RESEARCH INTEREST

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.

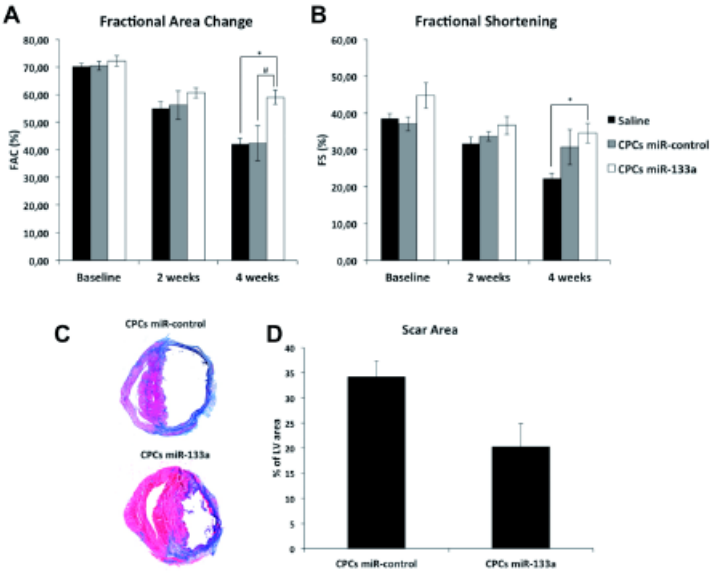


Mouse CPCs, defined by expression of the Polycomb gene Bmi-1, differentiate toward vascular lineages in response to defined stimuli: green, Bmi1; red, smooth-muscle actin; blue, the endothelial marker CD31.

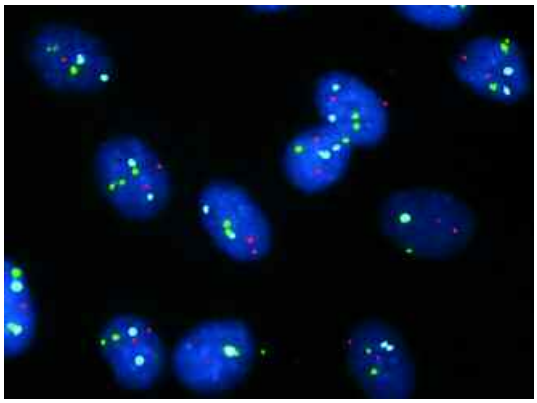
Research Departments

1 Cardiovascular Development and Repair

B. Stem Cell Biology



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

- **European Commission FP7. European Multidisciplinary Initiative (FP7-HEALTH -2009 CAREMI). PI: A. Bernad (coordinator)**
- **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 (PIFNOMEC08). PI: A. Bernad**
- **CNIC Translational Grants (13-2007). Sub-project coordinator: A. Bernad**
- **Comunidad de Madrid (S2010/BMD-2402)**

SELECTED PUBLICATIONS

Estrada JC, Albo C, Benguría A, Dopazo A, López-Romero P, Carrera-Quintanar L, Roche E, Clemente EP, Enríquez JA, Bernad A, Samper E.**Culture of human mesenchymal stem cells at low oxygen tension improves growth and genetic stability by activating glycolysis.** *Cell Death Differ* (accepted)

Fuster JJ, Gonzalez-Navarro H, Vinue A, Molina P, Andres-Manzano MJ, Nakayama KI, Nakayama K, Diez-Juan A, Bernad A, Rodriguez C, Martinez-Gonzalez J, Andres V. **Deficient p27 phosphorylation at serine 10 increases macrophage foam cell formation and aggravates atherosclerosis through a proliferation-independent mechanism.** *Arterioscler Thromb Vasc Biol* (2011) 31: 2455-63

Tome M, Lopez-Romero P, Albo C, Sepulveda JC, Fernandez-Gutierrez B, Dopazo A, Bernad A, and González MA. **miR-335 orchestrates cell proliferation, migration and differentiation in human mesenchymal stem cells.** *Cell Death Differ* (2011) 18: 985-95

Josowitz R, Carvajal-Vergara X, Lemischka IR, Gelb BD. **Induced pluripotent stem cell-derived cardiomyocytes as models for genetic cardiovascular disorders.** *Curr Opin Cardiol* (2011) 26: 223-9

Mittelbrunn M, Gutiérrez-Vázquez C, Villarroya-Beltri C, González S, Sánchez-Cabo F, González MA, Bernad A, Sánchez-Madrid F. **Unidirectional transfer of microRNA-loaded exosomes from T cells to antigen-presenting cells.** *Nat Commun* (2011) 2: 282

Research Departments

1 Cardiovascular Development and Repair

B. Stem Cell Biology

Stem cell niche pathophysiology

Head of Laboratory: *Simón Méndez Ferrer*

Postdoctoral Researchers: *Joan Isern*
Abel Sánchez-Aguilera

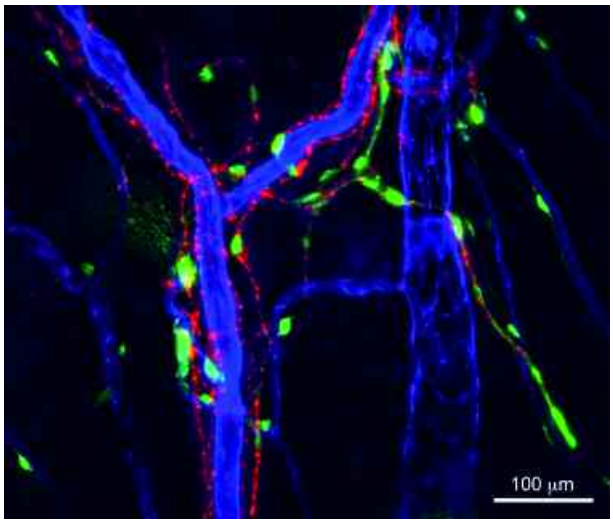
Predoctoral Researcher: *Ana Lázaro*

Technicians: *Ana María Martín de Ana*
Daniel Martín Pérez

RESEARCH INTEREST

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 fulfils 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.



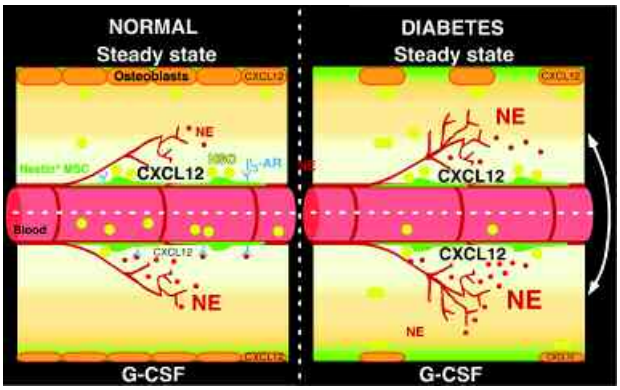
Peri-vascular nestin$^+$ mesenchymal stem cells are innervated by sympathetic fibers in the bone marrow. Projection stack (~100 µm) of fluorescent images showing the distribution of Nestin-*GFP$^+$ cells (green), CD31/PECAM$^+$ vascular endothelial cells (blue) and tyrosine hydroxylase$^+$ sympathetic nerve fibers (red) after whole mount staining of the skull bone marrow (from Isern and Méndez-Ferrer, 2011).*

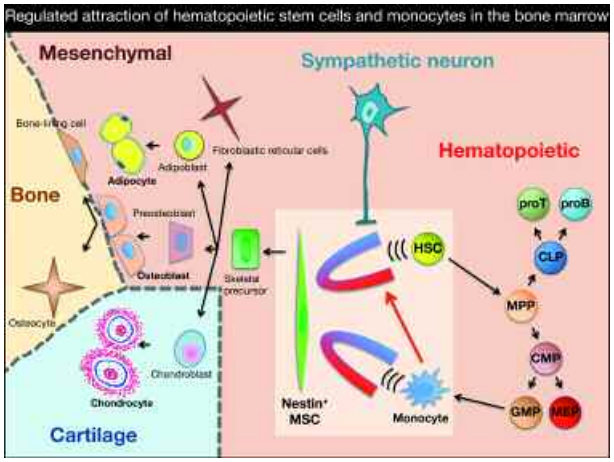
Research Departments

1 Cardiovascular Development and Repair

B. Stem Cell Biology

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

Ferraro F, Lymperi S, Méndez-Ferrer S, Saez B, Spencer JA, Yeap BY, Masselli E, Graiani G, Prezioso L, Rizzini EL, Mangoni M, Rizzoli V, Sykes SM, Lin CP, Frenette PS, Quaini F, Scadden DT. **Diabetes impairs hematopoietic stem cell mobilization by altering niche function.** *Sci Translat Med* (2011) 3: 104ra101

Isern J, Méndez-Ferrer S. **Stem cell interactions in a bone marrow niche.** *Curr Osteopor Rep* (2011) 9: 210-8

Shi C, Jia T, Méndez-Ferrer S, Hohl TM, Serbina NV, Lipuma L, Leiner I, Li MO, Frenette PS, Pamer EG. **Bone marrow mesenchymal stem and progenitor cells induce monocyte emigration in response to circulating TLR-ligands.** *Immunity* (2011) 34: 590-601

Chow A, Lucas D, Hidalgo A, Méndez-Ferrer S, Hashimoto D, Scheiermann C, Battista M, Leboeuf M, Prophete C, van Rooijen N, Tanaka M, Merad M, Frenette PS. **Bone marrow CD169+ macrophages promote the retention of hematopoiteic stem and progenitor cells in the mesenchymal stem cell niche.** *J Exp Med* (2011) 208: 261-71

Méndez-Ferrer S*, Michurina TV, Ferraro F, Mazloom AR, Macarthur BD, Lira SA, Scadden DT, Ma'ayan A, Enikolopov GN, Frenette PS* **Mesenchymal and haematopoietic stem cells form a unique niche in the bone marrow.** *Nature* (2010) 466: 829-34. (Full Article)
**Corresponding authors*

Research Departments

1 Cardiovascular Development and Repair

B. Stem Cell Biology

Cardiovascular related risks of obesity

Head of Laboratory: *Beatriz González Gálvez*

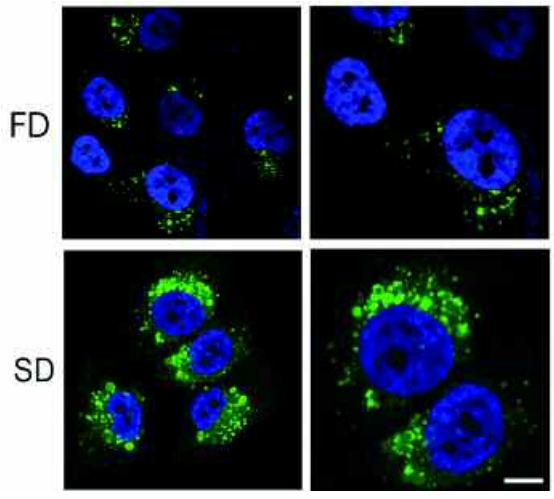
Predoctoral Researchers: *Aurora Bernal*
Laura Martín Pérez

Technician: *Nuria San Martín*

RESEARCH INTEREST

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 mesoangioblasts 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 mesoangioblast differentiation and raise the interesting possibility that treatments that increase cellular mitochondrial content may have applications in cardiac stem cell therapy.

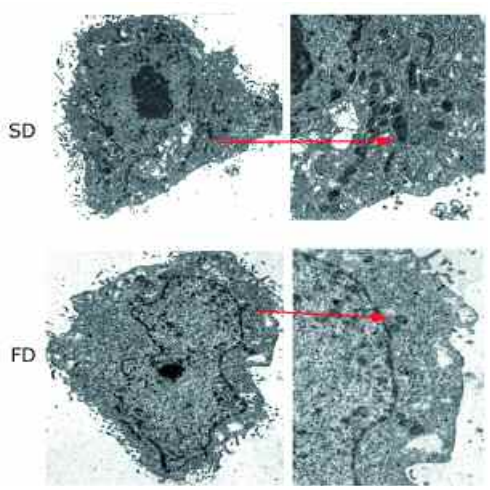


Mitotracker staining for mitochondrial load in fast dividing (FD) and slow (SD) dividing cardiac precursors.

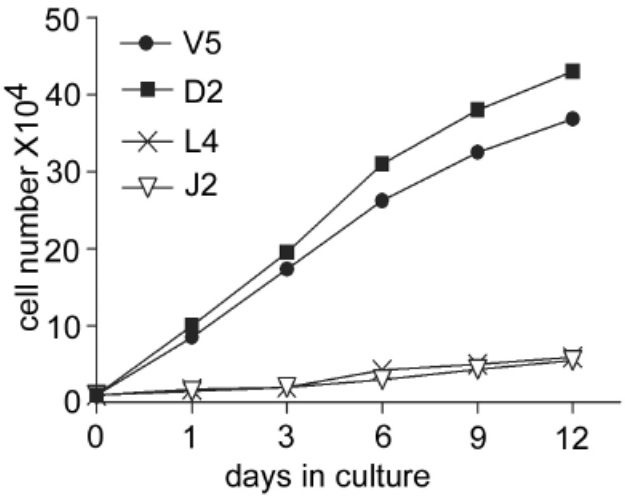
Research Departments

1 Cardiovascular Development and Repair

B. Stem Cell Biology



Electron micrograph showing mitochondrial morphology in slow dividing cardiac precursors.



Growth curves for fast (black) and slow (white) dividing cardiac precursors.

MAJOR GRANTS

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

SELECTED PUBLICATIONS

Bernal A, San Martin N, Fernandez M, Covarello D, Molla F, Soldo A, Latini R, Cossu G, Galvez BG. **L-selectin and SDF-1 enhance the migration of mouse and human cardiac mesoangioblasts.** *Cell Death Differ* (accepted)

Crippa S, Cassano M, Messina G, Galli D, Galvez BG, Curk T, Altomare C, Ronzoni F, Toelen J, Gijsbers R, Debyser Z, Janssens S, Zupan B, Zaza A, Cossu G, Sampaolesi M. **miR669a and miR669q prevent skeletal muscle differentiation in postnatal cardiac progenitors.** *J Cell Biol* (2011) 193: 1197-212

San Martín N, Gálvez BG. **A new paradigm for the understanding of obesity: the role of stem cells.** *Arch Physiol Biochem* (2011) 117: 188-94

San Martin N, Cervera AM, Cordova C, Covarello D, McCreath KJ, Galvez BG. **Mitochondria determine the differentiation potential of cardiac mesoangioblasts.** *Stem Cells* (2011) 29: 1064-74

Research Departments

1 Cardiovascular Development and Repair

B. Stem Cell Biology

Cellular signaling

Head of Laboratory: *Kenneth J. McCreath*

Research Scientist: *Ana M. Cervera*

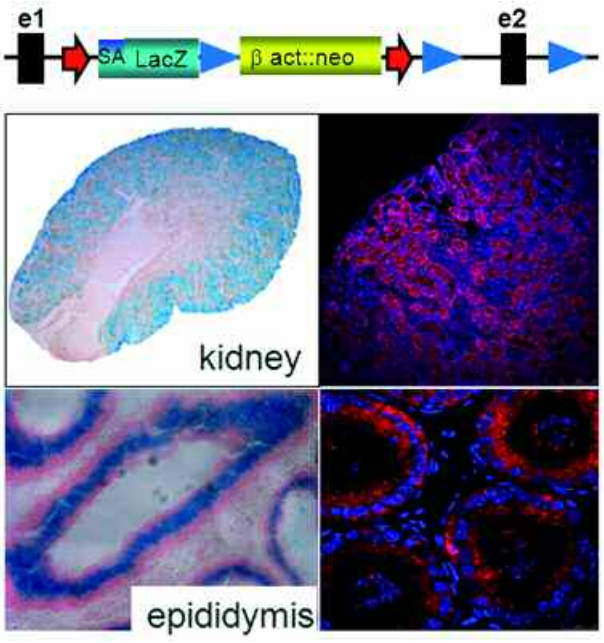
Postdoctoral Researcher: *Sandra Espada Serrano*

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.

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.



Top: Whole animal reporter and conditional deletion of the SUCNR1 *locus is achieved through targeting using the knockout-first approach. Left panels:* SUCNR1 *gene expression pattern in male mice using X-gal staining shows high activity in the kidney cortex and epididymis.* Right panels: *Immunohistochemical staining using an antibody to SUCNR1 confirms reporter activity.*

MAJOR GRANTS

- Ministerio de Ciencia e Innovacion (SAF2009-07965)

SELECTED PUBLICATIONS

San Martin N, Cervera AM, Cordova C, Covarello D, McCreath KJ, Galvez BG. **Mitochondria determine the differentiation potential of cardiac mesoangioblasts.** *Stem Cells* (2011) 29: 1064-74

Crespo FL, Sobrado VR, Gomez L, Cervera AM, McCreath KJ. **Mitochondrial reactive oxygen species mediate cardiomyocyte formation from embryonic stem cells in high glucose.** *Stem Cells* (2010) 28: 1132-42

Research Departments

1 Cardiovascular Development and Repair

C. Tissue Homeostasis and Repair

Functional genetics of the oxidative phosphorylation system

Head of Laboratory: *José Antonio Enríquez*

Research Scientists:
Acisclo Pérez
Patricio Fernández-Silva
Erika Fernández-Vizarra
Nuria Garrido
Pilar Bayona

Postdoctoral Researchers:
Carmen Colas
Rebeca Acín
Raquel Meade
Patricia Meade
Ester Perales

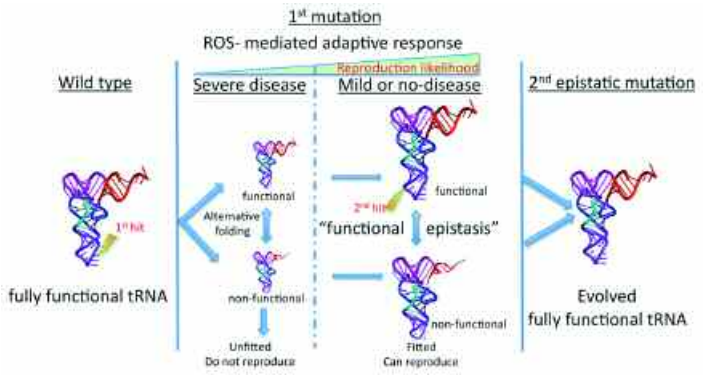
Predoctoral Researchers:
Ricardo Marco
Adela Guaras
Ana Latorre
Esther Lapuente
Elena de Tomás.

Support Scientists:
Mª Concepción Jiménez
Marta Roche
Nieves Movilla

Visiting Scientists:
Eduardo Balsa
Sara Cogliati
Ana Martín

RESEARCH INTEREST

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 neurodenerative 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.

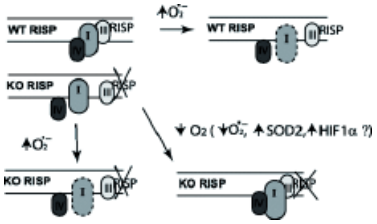


Model of the potential consequences of "functional epistasis" on disease penetrance and sequence evolution of mitochondrial tRNAs.

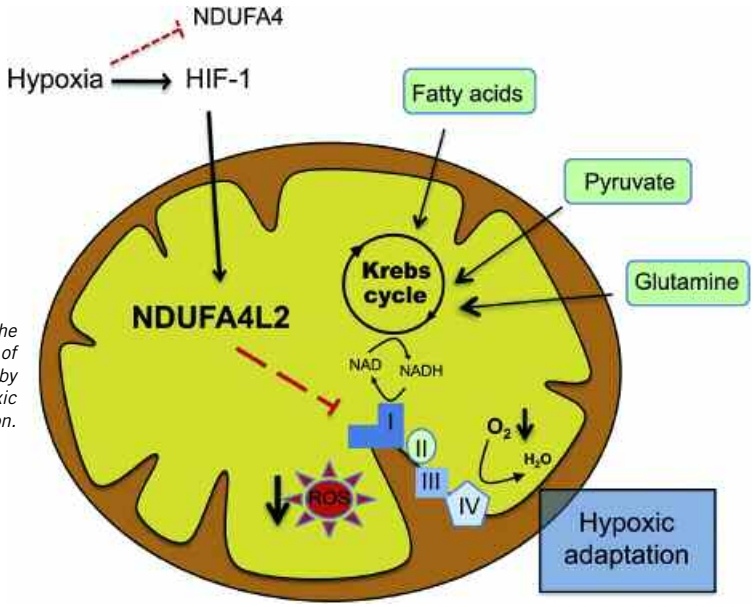
Research Departments

1 Cardiovascular Development and Repair

C. Tissue Homeostasis and Repair



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



Model showing the involvement of NDUFA4L2 induction by HIF-1α in hypoxic adaptation.

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**

SELECTED PUBLICATIONS

Diaz F, Enríquez JA, Moraes CT. **Cells lacking Rieske iron-sulfur protein have a reactive oxygen species-associated decrease in respiratory complexes I and IV.** *Mol Cell Biol.* (2012) 32:415-29

Moreno-Loshuertos R, Ferrin G, Acin-Perez R, Gallardo ME, Viscomi C, Perez-Martos A, Zeviani M, Fernandez-Silva P, Enriquez JA. **Evolution meets disease: penetrance and functional epistasis of mitochondrial tRNA mutations.** *PLoS Genet* (2011) 7: e1001379

Perales-Clemente E, Fernandez-Silva P, Acin-Perez R, Perez-Martos A, Enriquez JA. **Allotopic expression of mitochondrial-encoded genes in mammals: achieved goal, undemonstrated mechanism or impossible task?** *Nucleic Acids Res* (2011) 39: 225-34

Tello D*, Balsa E*, Acosta-Iborra B, Fuertes-Yebra E, Elorza A, Ordóñez A, Corral-Escariz M, Soro I, López-Bernardo E, Perales-Clemente E, Martínez-Ruiz A, Enríquez JA, Aragonés J, Cadenas S, Landázuri MO.
Induction of the mitochondrial NDUFA4L2 protein by HIF-1α decreases oxygen consumption by inhibiting complex I activity. *Cell Metab.* (2011) 14: 768-79
*Joint 1st authors

Bayona-Bafaluy MP, Sanchez-Cabo F, Fernandez-Silva P, Perez-Martos A, Enriquez JA. **A genome-wide shRNA screen for new OxPhos related genes.** *Mitochondrion* (2011) 11: 467-7

Research Departments

1 Cardiovascular Development and Repair

C. Tissue Homeostasis and Repair

Stem cell aging

Head of Laboratory: *Susana González*

Postdoctoral Researcher: *Lorena Arranz*

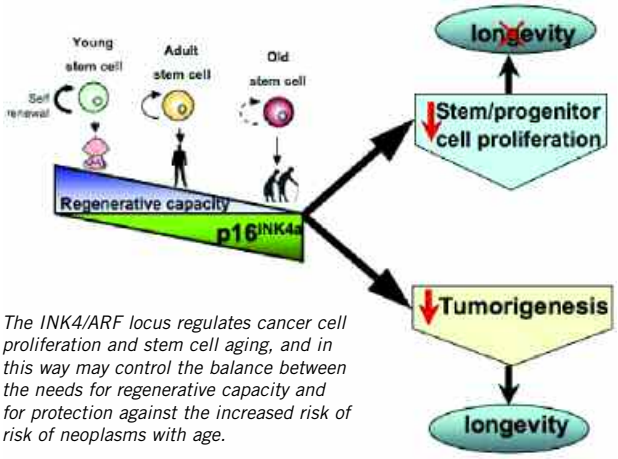
Predoctoral Researchers: *Antonio Herrera*
Isabel Hidalgo

Visiting Scientist: *Ana Branco*

RESEARCH INTEREST

The INK4b-ARF-INK4a locus encodes three tumor suppressors, p15INK4b, ARF, and p16INK4a. Together, these factors constitute one 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.



The INK4/ARF locus regulates cancer cell proliferation and stem cell aging, and in this way may control the balance between the needs for regenerative capacity and for protection against the increased risk of risk of neoplasms with age.

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

Herrera-Merchan A, Arranz L, Ligos JM, Dominguez O, de Molina A, Gonzalez S. **Ectopic expression of the histone methyltransferase Ezh2 in hematopoietic stem cells causes myeloproliferative disease.** *Nature Communications* (accepted)

Arranz L, Herrera-Merchán A, Ligos JM, Dominguez O, Molina A, Gonzalez S. **Bmi1 is critical to prevent Ikaros-mediated lymphoid priming in hematopoietic stem cells.** *Cell Cycle* (accepted)

Arranz L, Herrera-Merchan A, Gonzalez S. **Therapeutic Polycomb targeting in human cancer.** *Recent Patents on Regenerative Medicine* (accepted)

Herrera-Merchan A, Cerrato C, Luengo G, Dominguez O, Piris MA, Serrano M, Gonzalez S. **miR-33-mediated downregulation of p53 controls hematopoietic stem cell self-renewal.** *Cell Cycle* (2010) 16: 3277-85

Research Departments

1 Cardiovascular Development and Repair

C. Tissue Homeostasis and Repair

Nuclear receptor signaling

Head of Laboratory: *Mercedes Ricote*

Postdoctoral Researchers: *Piedad Menéndez*
Tamas Röszer
Lucía Fuentes

Predoctoral Researchers: *Daniel Alameda*
Marta Cedenilla

Technician: *Vanessa Núñez*

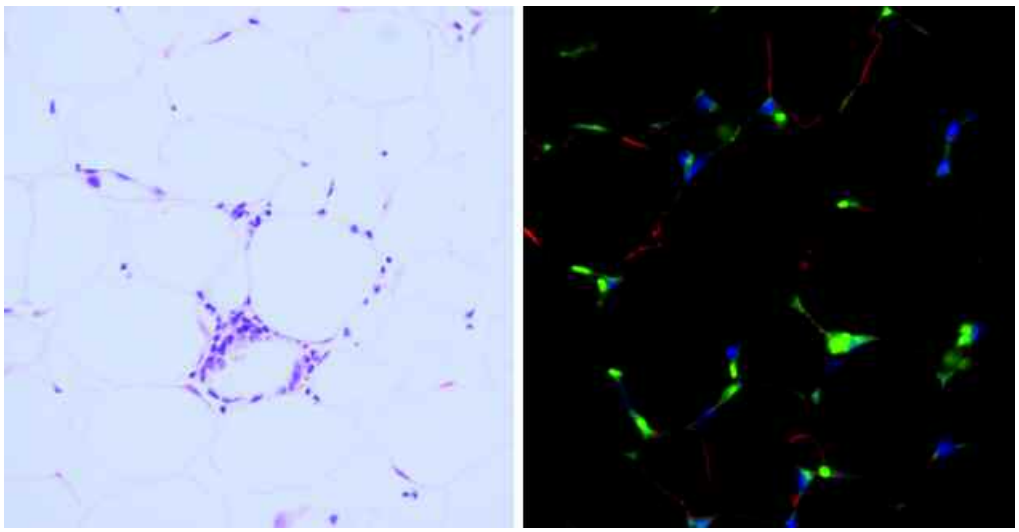
RESEARCH INTEREST

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-infacted 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.

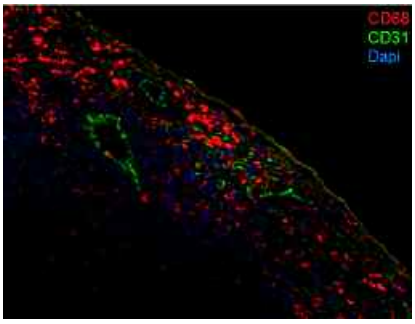


Macrophage infiltration of visceral adipose tissue (VAT) is a hallmark of type 2 diabetes. Left: *Hematoxylin-eosin stained section of VAT from a mouse fed a high-fat diet for 16 weeks. Macrophages form a crown-like structure around a dying adipocyte.* Right: *F4/80-positive (green) macrophages in VAT. Nuclei are counterstained blue, and red staining highlights collagen fibers surrounding adipocytes.*

Research Departments

1 Cardiovascular Development and Repair

C. Tissue Homeostasis and Repair



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 Felloships for Career Development (FP7-PEOPLE-IEF-2008) PI T Röszer**

SELECTED PUBLICATIONS

Prieur X, Mok CY, Velagapudi VR, Nunez V, Fuentes L, Montaner D, Ishikawa K, Camacho A, Barbarroja N, O'Rahilly S, Sethi J, Dopazo J, Oresic M, Ricote M,* Vidal-Puig A*. **Differential lipid partitioning between adipocytes and tissue macrophages modulates macrophage lipotoxicity and M2/M1 polarization in obese mice.** *Diabetes* (2011) 60: 797-809
*Corresponding authors

Roszer T, Menendez-Gutierrez MP, Lefterova MI, Alameda D, Nunez V, Lazar MA, Fischer T, Ricote M. **Autoimmune kidney disease and impaired engulfment of apoptotic cells in mice with macrophage peroxisome proliferator-activated receptor γ or retinoid X receptor α deficiency.** *J Immunol* (2011) 186: 621-31

Núñez V, Alameda D, Rico D, Mota R, Gonzalo P, Cedenilla M, Fischer T, Boscá L, Glass CK, Arroyo AG, Ricote M. **Retinoid X receptor α controls innate inflammatory responses through the up-regulation of chemokine expression.** *Proc Natl Acad Sci U S A* (2010) 107: 10626-31

Röszer T, Ricote M. **PPARs in the renal regulation of systemic blood pressure.** *PPAR Res* (2010) 2010: 698730

Prieur X, Roszer T, Ricote M. **Lipotoxicity in macrophages: evidence from diseases associated with the metabolic syndrome.** *Biochim Biophys Acta* (2010) 1801: 327-37

Research Departments

1 Cardiovascular Development and Repair

C. Tissue Homeostasis and Repair

Molecular regulation of heart development and disease

Head of Laboratory: *Enrique Lara Pezzi*

Predoctoral Researcher: *Jesús Gómez*

Visiting Scientist: *Maria Villalba Orero*

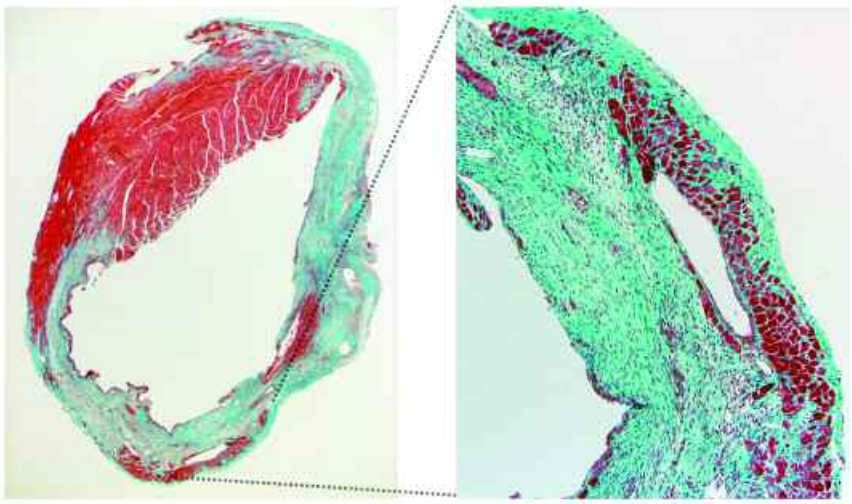
Technician: *Marina López*

RESEARCH INTEREST

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.

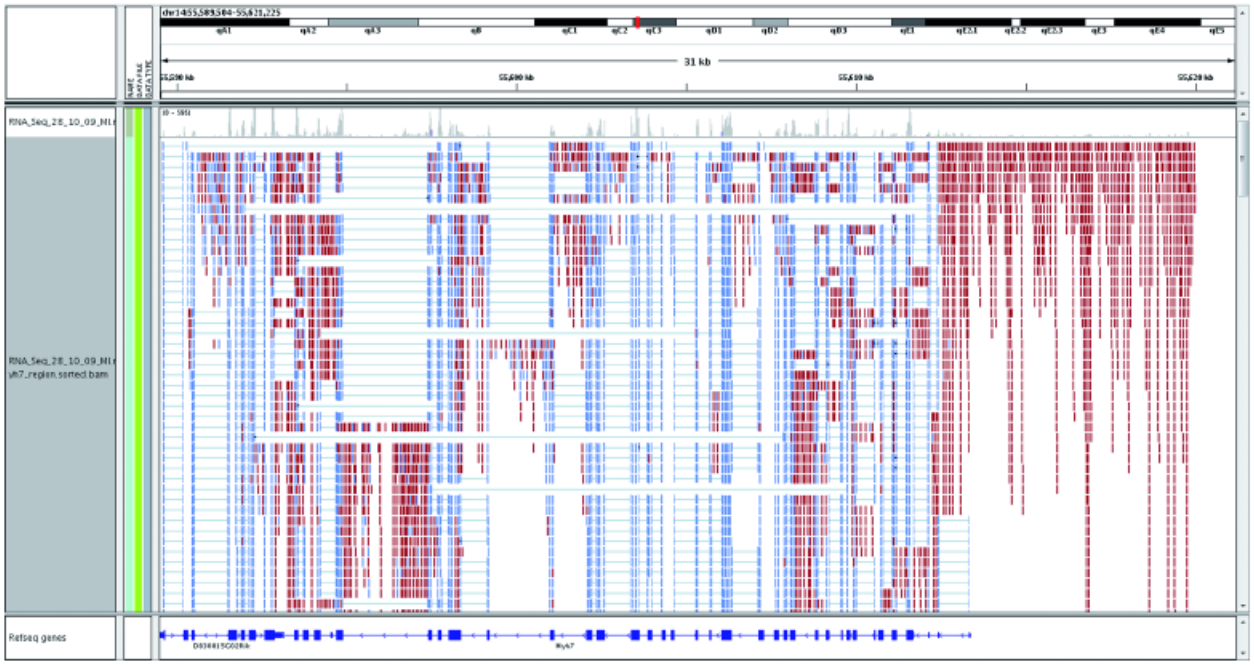


Histological analysis of an infarcted mouse heart. To induce a myocardial infarction, the left coronary artery was occluded for 30 minutes and then reperfused. After 28 days the heart was fixed and analyzed by the Masson's Trichrome method. Cardiomyoctes and collagen fibers are stained in red and blue, respectively.

Research Departments

1 Cardiovascular Development and Repair

C. Tissue Homeostasis and Repair



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 Commision 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

Felkin LE, Narita T, Germack R, Shintani Y, Takahashi K, Sarathchandra P, López-Olañeta MM, Gómez-Salinero JM, Suzuki K, Barton PJ, Rosenthal N and Lara-Pezzi E. **Calcineurin splicing variant calcineurin Aβ1 improves cardiac function after myocardial infarction without inducing hypertrophy.** *Circulation* (2011) 123: 2838-2847

Shimano M, Ouchi N, Nakamura K, Oshima Y, Higuchi A, Pimentel DR, Panse KD, Lara-Pezzi E, Lee SJ, Sam F and Walsh K. **Cardiac myocyte-specific ablation of follistatin-like 3 attenuates stress-induced myocardial hypertrophy.** *J Biol Chem* (2011) 286: 9840-8

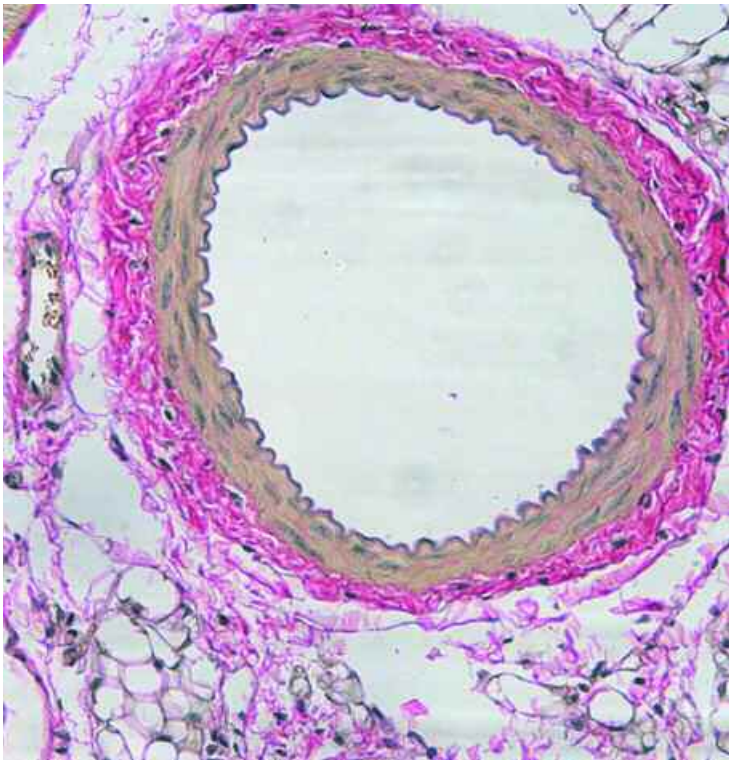
Felkin LE, Lara-Pezzi E, Hall JL, Birks EJ and Barton PJ. **Reverse remodelling and recovery from heart failure are associated with complex patterns of gene expression.** *J Cardiovasc Transl Res* (2011) 4: 321-31

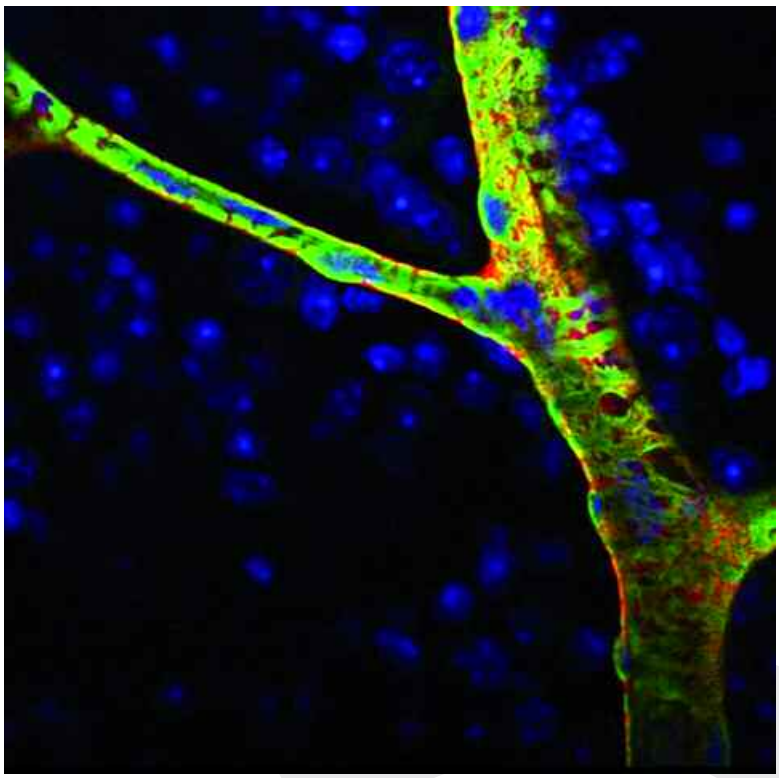
Bochmann L, Sarathchandra P, Mori F, Lara-Pezzi E, Lazzaro D and Rosenthal N. **Revealing new mouse epicardial cell markers through transcriptomics.** *PLoS One* (2010) 5: e11429

Lara-Pezzi E, Rosenthal N. **Genetic enhancement of cardiac regeneration.** In: Rosenthal N and Harvey R (Eds) *Heart Development and Regeneration* (2010) 2nd Ed. New York: Academic Press, 981-97

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

Research Departments

2 Vascular Biology and Inflammation

Gene regulation in cardiovascular and inflammatory diseases

Head of Laboratory: *Juan Miguel Redondo*

Postdoctoral Researchers: *Pablo Gómez-del Arco*
Sara Martínez-Martínez
Aránzazu Alfranca
Miriam Zeini
Vanesa Esteban

Predoctoral Researchers: *Katia Urso*
Amelia Escolano
Nerea Méndez
Noelia Lozano

Masters Students: *Jorge Oller*
María del Mar Torres

Technicians: *Dolores López Maderuelo*
Felipe Were
Raquel Sánchez
Gema Benito
Beatriz Carolina Ornés

RESEARCH INTEREST

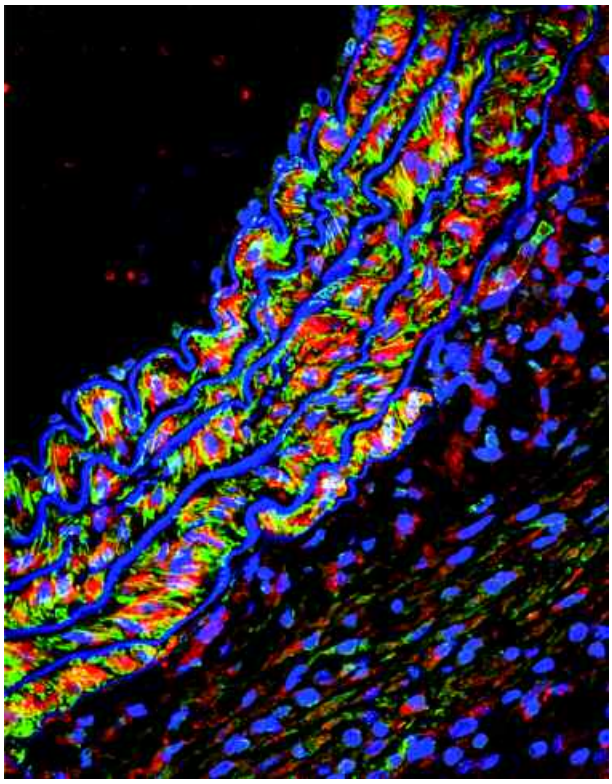
Many important biological processes, including the regulation and development of the immune and cardiovascular systems, are regulated by the calcineurin-NFAT (CN-NFAT) pathway. Much of our previous work relates to molecular interactions of CN with substrates. We are now studying the regulation and function 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.

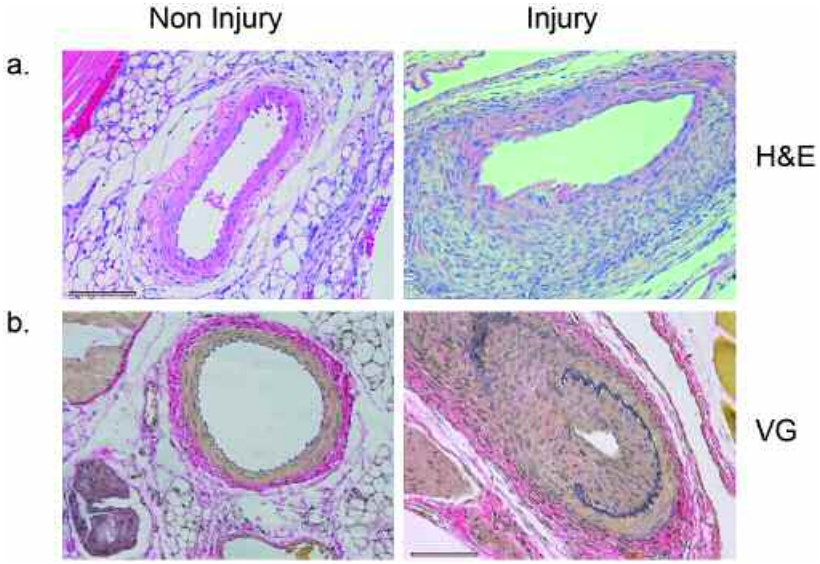
We are also dissecting signaling pathways involved in vascular wall remodeling, a major feature of vascular diseases such as atherosclerosis, aneurysm, and restenosis. We have set up animal models of these pathologies, and have generated mice deficient for AngII-target molecules that are regulated by 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.



Merged confocal microscopy images of Rcan1 (red) and SMA (green) immunostaining and nuclear staining (blue) on an abdominal aortic cross-section from an AngII-treated Apoe-/- mouse. The image shows maximal projections of a complete z-series.

Research Departments

2 Vascular Biology and Inflammation



Cross-sections of uninjured and injured mouse femoral arteries stained with hematoxilin-eosin (H&E) and Van Gieson's stain (VG).

MAJOR GRANTS

- **Ministerio de Ciencia e Innovación (SAF2009-10708)**
- **Ministerio de Ciencia e Innovación. FIS RETICS (RECAVA II: RD06/0014/0005)**
- **Fundación Genoma España**
- **Fundació La Marató TV3 (081731)**

SELECTED PUBLICATIONS

Rodríguez P, Higueras MA, González-Rajal A, Alfranca A, Fierro-Fernández M, García-Fernández RA, Ruiz-Hidalgo MJ, Monsalve M, Rodríguez-Pascual F, Redondo JM, de la Pompa JL, Laborda J, Lamas S. **The non-canonical NOTCH ligand DLK1 exhibits a novel vascular role as a strong inhibitor of angiogenesis.** *Cardiovasc Res* **(accepted)**

Esteban V, Méndez-Barbero N, Jiménez-Borreguero LJ, Roqué M, Novensá L, García-Redondo AB, Salaices M, Vila L, Arbonés ML, Campanero MR, Redondo JM. **Regulator of calcineurin 1 mediates pathological vascular wall remodeling**. *J Exp Med* (2011) 208: 2125-39

Urso K, Alfranca A, Martínez-Martínez S, Escolano A, Ortega I, Rodríguez A, Redondo JM. **NFATc3 regulates the transcription of genes involved in T-cell activation and angiogenesis**. *Blood* (2011) 118: 795-803

Bonzon-Kulichenko E, Pérez-Hernández D, Núñez E, Martínez-Acedo P, Navarro P, Trevisan-Herraz M, Ramos Mdel C, Sierra S, Martínez-Martínez S, Ruiz-Meana M, Miró-Casas E, García-Dorado D, Redondo JM, Burgos JS, Vázquez J. **A robust method for quantitative high-throughput analysis of proteomes by ^{18}O labeling**. *Mol Cell Proteomics* (2011) 10: 10.1074/mcp.M110.003335: 1–14

Gómez-del Arco P, Kashiwagi M, Jackson AF, Naito T, Zhang J, Liu F, Kee B, Vooijs M, Radtke F, Redondo JM, Georgopoulos K. **Alternative promoter usage at the Notch1 locus supports ligand-independent signaling in T cell development and leukemogenesis**. *Immunity* (2010) 33: 685-98

Research Departments

2 Vascular Biology and Inflammation

CNIC-UAM COLLABORATIVE PROGRAM: Intercellular communication in the inflammatory response

Head of Laboratory: *Francisco Sánchez-Madrid*

Research Scientist: *Gloria Martínez del Hoyo*

Postdoctoral Researchers: *Olga Barreiro*
Hortensia de la Fuente
Noa B. Martín
María Mittelbrunn
Vera Rocha

Predoctoral Researchers: *Francesc Baixauli*
Aránzazu Cruz
Cristina Gutierrez
Giulia Morlino
Norman Núñez
Emilio Tejera
Carolina Villarroya

Technicians: *Marta Esther Ramirez*
María José López
Ana Dominguez Maldonado

RESEARCH INTEREST

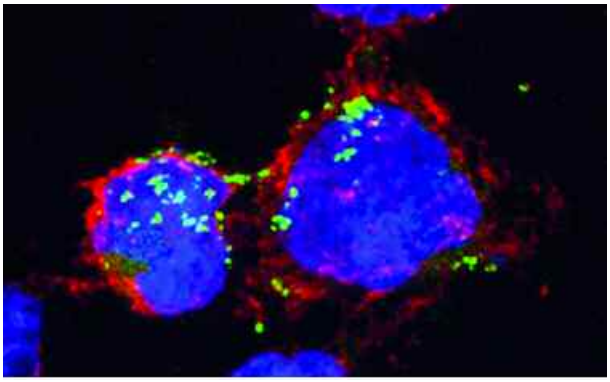
The group's present work focuses on key cell-to-cell communication events during cognate immune interactions. A key goal is to define how the microtubule organizing complex (MTOC), by controlling cytoskeletal rearrangements at the immune synapse (IS), provides a mechanism for macromolecular transport 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.

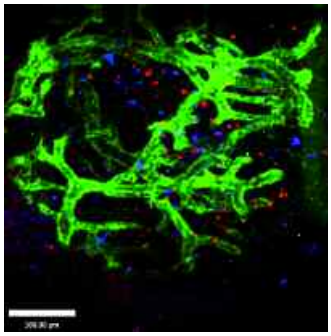
3. To image immune-inflammatory responses in vivo in order to define the role of immunoregulatory molecules in autoimmune inflammatory diseases



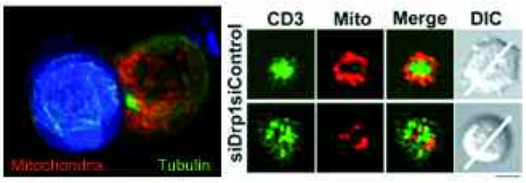
T cells transfer microRNA-loaded exosomes to antigen presenting cells. *The image shows confocal microscopy detection of the exosomal marker CD63-GFP (green) on the surface of recipient APCs (Raji) after incubation with J77-CD63-GFP exosomes. CD45 is stained red and nuclei are blue.*

Research Departments

2 Vascular Biology and Inflammation



Distribution of dendritic cells in peripheral lymph nodes. *Dendritic cells from wild-type (red) and CD69$^{-/-}$ (blue) mice were transferred into C57BL6 recipient mice together with a marker of high endothelial venules (green). Two-photon analysis of draining lymph nodes showed that DCs were mostly located near high endothelial venules and the outer T cell zone inside the lymph node.*



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

Mittelbrunn M, Gutiérrez-Vázquez C, Villarroya-Beltri C, González S, Sánchez-Cabo F, González MÁ, Bernad A, Sánchez-Madrid F. **Unidirectional transfer of microRNA-loaded exosomes from T cells to antigen-presenting cells.** *Nat Commun* (2011) 2: 282

Nuñez-Andrade N, Lamana A, Sancho D, Gisbert JP, Gonzalez-Amaro R, Sanchez-Madrid F, Urzainqui A. **P-selectin glycoprotein ligand-1 modulates immune inflammatory responses in the enteric lamina propria.** J *Pathol* (2011) 224: 212-21

Martín P, Sánchez-Madrid F. **CD69: an unexpected regulator of TH17 cell-driven inflammatory responses.** *Sci Signal* (2011) 4: pe14

Lamana A, Martin P, *de la Fuente H*, Martinez-Muñoz L, Cruz-Adalia A, Ramirez-Huesca M, Escribano C, Gollmer K, Mellado M, Stein JV, Rodriguez-Fernandez JL, Sanchez-Madrid F, del Hoyo GM. **CD69 modulates sphingosine-1-phosphate-induced migration of skin dendritic cells.** *J Invest Dermatol* (2011) 131: 1503-12

Baixauli F, Martín-Cófreces NB, Morlino G, Carrasco YR, Calabia-Linares C, Veiga E, Serrador JM, Sánchez-Madrid F. **The mitochondrial fission factor dynamin-related protein 1 modulates T-cell receptor signalling at the immune synapse.** *EMBO J* (2011) 30: 1238-50

Research Departments

2 Vascular Biology and Inflammation

Integrin signaling

Head of Laboratory:	*Miguel Ángel del Pozo*
Research Scientist:	*Asier Echarri*
Postdoctoral Researchers:	*Susana Minguet* *Ana Cerezo* *Jacky Goetz* *Inmaculada Navarro* *Raffaele Strippoli* *Teijo Pellinen*
Predoctoral Researchers:	*Marta C. Guadamillas* *Olivia Muriel*
Masters Student:	*Roberto Moreno Vicente*
Technicians:	*Sara Sánchez Perales* *Dacil M. Pavón* *Teresa Osteso Ibañez*
Visiting Scientist:	*Aleix Sala*

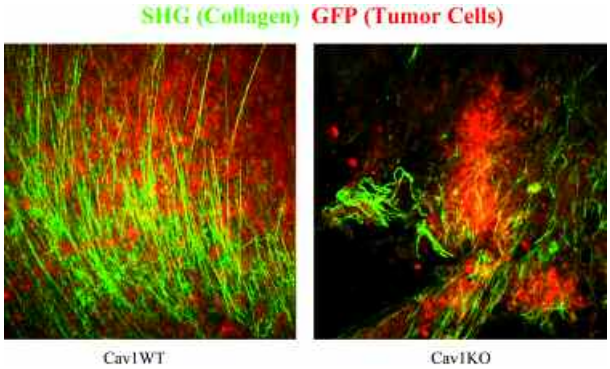
RESEARCH INTEREST

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.

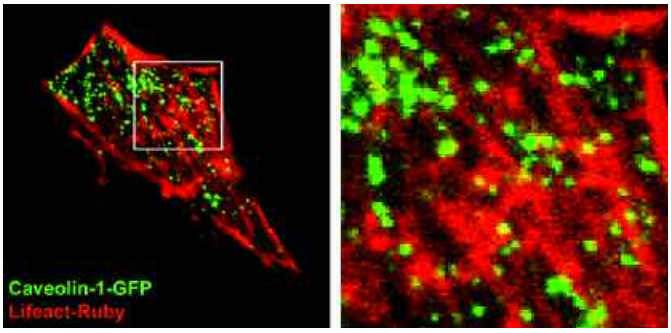
Other recent work has delineated how filamin A regulates actin-linked caveolae dynamics at the PM, and shows that Cav1-membrane inward trafficking depends on the actin polymerization machinery, microtubules (MT), dynamin2, and phosphorylation of filamin A by PKCα. Upon loss of tension caused by loss 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.



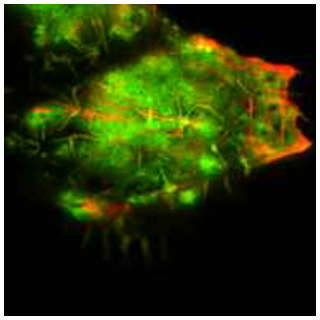
Orthotopic mammary gland allografts injected into wild-type (left) or Cav1-deficient mice (right) were imaged for SHG (second harmonic generation). In the wild-type background the collagen fibers are highly aligned and perpendicular to the tumor-stroma interface, which correlates with a highly invasive behavior.

Research Departments

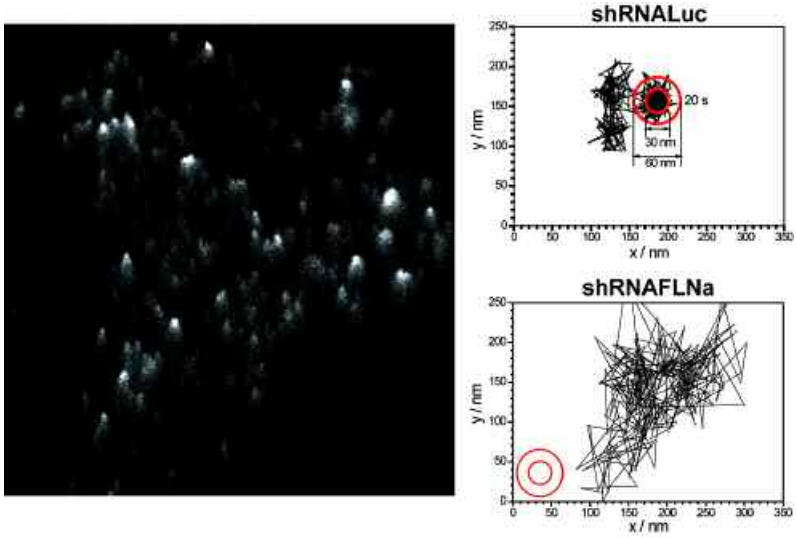
2 Vascular Biology and Inflammation



Total internal reflection fluorescence (TIRF) microscopy image at 90 nm penetration showing caveolin vesicles and actin fibers (stained with RFP-Ruby-Lifeact) in HeLa cells.



TIRF microscopy showing colocalization of GFP-tagged wild-type Rac1 (green) with the actin marker Lifeact (red) on the ventral surface of live COS7 cells.



High spatio-temporal resolution particle tracking of Cav1-GFP vesicles by TIRFm in control HeLa cells (shRNALuc) or filamin A depleted cells (shRNAFLNa). Sequential vesicle positions were recorded at 85 ms intervals and connected by straight lines. Outer circles show the threshold for an anchoring event (60 nm diameter); inner circles show the positioning accuracy (30 nm). Duration of anchoring events is indicated.

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

Navarro-Lérida I, Sánchez-Perales S, Calvo M, Rentero C, Zheng Y, Enrich C, Del Pozo MA. **A palmitoylation switch mechanism regulates Rac1 function and membrane organization.** *EMBO J* (accepted)

Guadamillas MC, Cerezo A, Del Pozo MA. **Overcoming anoikis – pathways to anchorage-independent growth in cancer.** *J Cell Sci* (2011) 124: 3189-97

Castro-Castro A, Ojeda V, Barreira M, Sauzeau V, Navarro-Lérida I, Muriel O, Couceiro JR, Pimentel-Muíños FX, Del Pozo MA, Bustelo XR. **Coronin 1A promotes a cytoskeletal-based feedback loop that facilitates Rac1 translocation and activation.** *EMBO J* (2011) 30: 3913-27

Muriel O, Echarri A, Hellriegel C, Pavón DM, Beccari L, Del Pozo MA. **Phosphorylated filamin A regulates actin-linked caveolae dynamics.** *J Cell Sci* (2011) 124: 2763-76

Goetz JG, Minguet S, Navarro-Lérida I, Lazcano JJ, Samaniego R, Calvo E, Tello M, Osteso-Ibáñez T, Pellinen T, Echarri A, Cerezo A, Klein-Szanto AJ, Garcia R, Keely PJ, Sánchez-Mateos P, Cukierman E, Del Pozo MA. **Biomechanical remodeling of the microenvironment by stromal caveolin-1 favors tumor invasion and metastasis.** *Cell* (2011) 146: 148-63

Research Departments

2 Vascular Biology and Inflammation

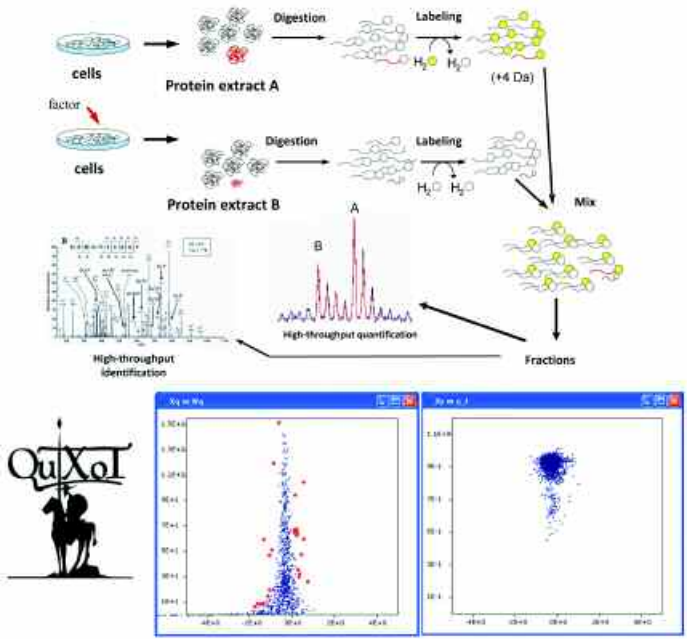
Cardiovascular proteomics

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 Múñoz*

RESEARCH INTEREST

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.

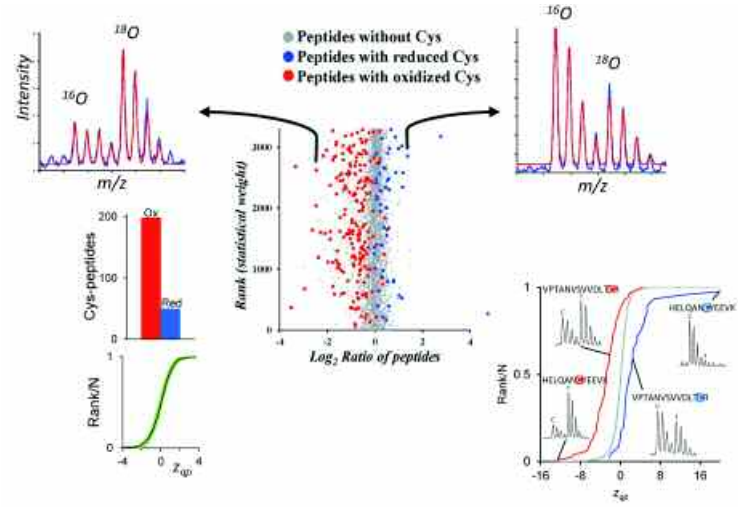
We are applying these developments to the study of key aspects of cardiovascular disease, with the aim of defining molecular mechanisms and identifying specific protein factors for use as pharmacological targets or biomarkers. One area of interest is the dynamic expression changes to the secretome and 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.



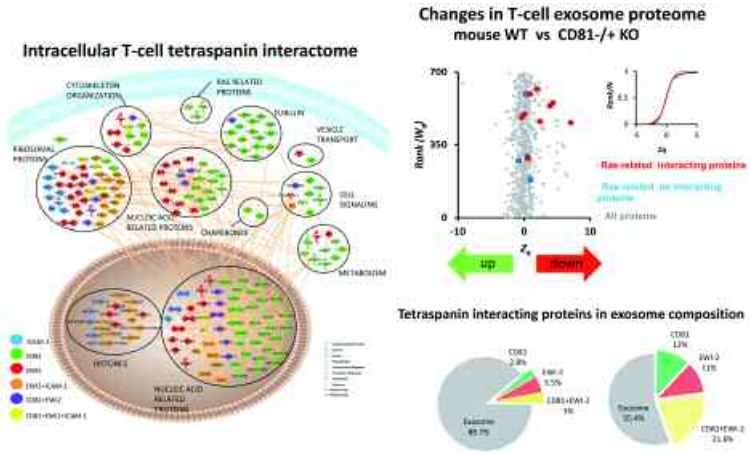
Top: *Workflow scheme for high-throughput quantification of proteomes by stable isotope labeling.* Bottom: *The "Quixot" bioinformatics platform developed in the laboratory for identification, quantification and statistical analysis of mass spectrometry data.*

Research Departments

2 Vascular Biology and Inflammation



Determination of changes in the redox state of cysteine-containing peptides in high-throughput proteomics experiments using GELSILOX technology. The figure shows the effect 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 log2-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)**
- **Insitituto Madrileño para el Desarrollo (IMADE): Programa PIE-IMADE (MAXPRO)**

SELECTED PUBLICATIONS

Bonzon-Kulichenko E, Martínez-Martínez S, Trevisan-Herraz M, Navarro P, Redondo JM, Vázquez J. **Quantitative in-depth analysis of the dynamic secretome of activated Jurkat T-cells.** *J Proteomics* (2011) 75: 561-71

Carrera M, Cañas B, López-Ferrer D, Pineiro C, Vázquez J, Gallardo J M. Fast **Monitoring of Species-Specific Peptide Biomarkers Using High Intensity Focused Ultrasound Assisted Tryptic Digestion and Selected MS/MS Ion Monitoring Mass Spectrometry.** *Anal Chem* (2011) 83: 5688-95

Bonzon-Kulichenko E, Pérez-Hernández D, Núñez E, Martínez-Acedo P, Navarro P, Trevisan-Herraz M, Ramos Mdel C, Sierra S, Martínez-Martínez S, Ruiz-Meana M, Miró-Casas E, García-Dorado D, Redondo JM, Burgos JS, Vázquez J. **A robust method for quantitative high-throughput analysis of proteomes by ^{18}O labeling.** *Mol Cell Proteomics* (2011) 10: 10.1074/mcp.M110.003335: 1–14

Redondo-Horcajo M, Romero N, Martínez-Acedo P, Martínez-Ruiz A, Quijano C, Lourenço CF, Movilla N, Enríquez JA, Rodríguez-Pascual F, Rial E, Radi R, Vázquez J, Lamas S. **Cyclosporine A-induced nitration of tyrosine 34 MnSOD in endothelial cells: role of mitochondrial superoxide.** *Cardiovasc Res* (2010) 87: 356-65

Pérez-Martínez M, Gordón-Alonso M, Román Cabrero J, Barrero-Villar M, Rey M, Mittelbrunn M, Lamana A, Morlino G, Calabia C, Yamazaki H, Shirao T, Vázquez J, González-Amaro R, Veiga E, Sánchez-Madrid F. **F-actin-binding protein drebrin regulates CXCR4 recruitment to the immune synapse.** *J Cell Sci* (2010) 123: 1160-70

Research Departments

2 Vascular Biology and Inflammation

Matrix metalloproteinases in angiogenesis and inflammation

Head of Laboratory: *Alicia G. Arroyo*

Postdoctoral Researchers: *Pilar Gonzalo*
Rubén A. Mota

Predoctoral Researchers: *Cristina Clemente*
María Victoria Hernández de Riquer
Agnieszka Koziol
Mara Martín
Vanessa Moreno

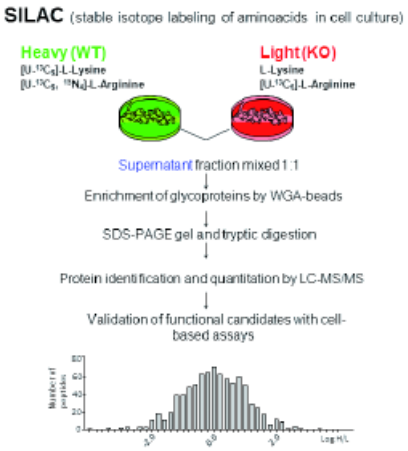
Technicians: *Ángela Pollán*
Laura Balonga

Visiting Scientist: *Olga Alicia Nieto*

RESEARCH INTEREST

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.

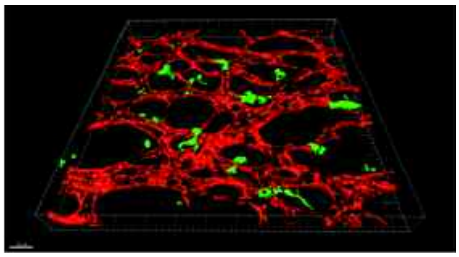
Through these efforts we aim to extend our knowledge of where, when and how MT-MMPs and their substrates modulate endothelial, smooth-muscle cell and leukocyte behavior during the establishment and progression of chronic inflammatory disorders.



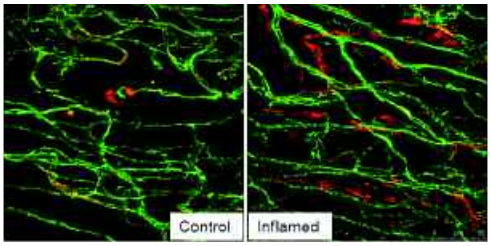
Proteomics for proteases. *SILAC (stable isotype labeling of aminoacids in culture) is a quantitative proteomics approach that we use to identify the collection of substrates (degradome) of specific proteases in cell types involved in inflammation and angiogenesis. The figure shows the general outflow of SILAC applied to primary cells derived from wild type and protease-deficient mice.*

Research Departments

2 Vascular Biology and Inflammation



***Imaging cellular crosstalk in angiogenesis**. 3D-reconstruction with Imaris software of whole-mount staining shows the close association of vessels (red) and macrophages (green) in the developing vasculature of mouse retinas six days postpartum.*



***Analysis of vascular integrity**. Intravascular injection of fluorescent-dextran (red) in mice allows the analysis of vascular integrity in basal and inflamed conditions. Vessels are stained green.*

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

Arroyo AG, Iruela-Arispe ML. **Extracellular matrix, inflammation, and the angiogenic response.** *Cardiovasc Res* (2010) 86: 226-35

Gonzalo P, Arroyo AG. **MT1-MMP: A novel component of the macrophage cell fusion machinery.** *Commun Integr Biol* (2010) 3: 1-4

Gonzalo P, Guadamillas MC, Hernández-Riquer MV, Pollán A, Grande-García A, Bartolomé RA, Vasanji A, Ambrogio C, Chiarle R, Teixidó J, Risteli J, Apte SS, del Pozo MA, Arroyo AG. **MT1-MMP is required for myeloid cell fusion via regulation of Rac1 signaling.** *Dev Cell* (2010) 18: 77-89

Gonzalo P, Moreno V, Galvez BG, Arroyo AG. **MT1-MMP and integrins: Hand-to-hand in cell communication.** *Biofactors* (2010) 36: 248-54

Nunez V, Alameda D, Rico D, Mota R, Gonzalo P, Cedenilla M, Fischer T, Bosca L, Glass CK, Arroyo AG, Ricote M. **Retinoid X receptor alpha controls innate inflammatory responses through the up-regulation of chemokine expression.** *Proc Natl Acad Sci USA* (2010) 107: 10626-31

Research Departments

2 Vascular Biology and Inflammation

B cell biology

Head of Laboratory: *Almudena R Ramiro*

Research Scientists: *Virginia G de Yébenes*
Maria Pilar Delgado

Postdoctoral Researchers: *Laura Belver*
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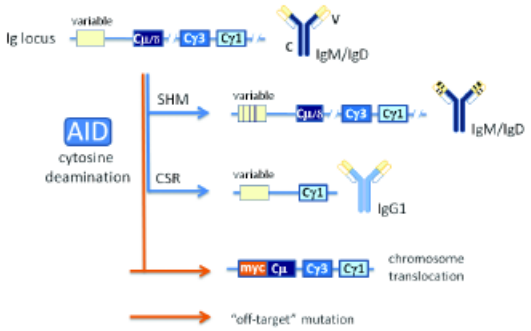
Nahikari Bartolomé
Arantxa Pérez-García
Isora V Sernández

Technician: *Sonia Mur*

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.

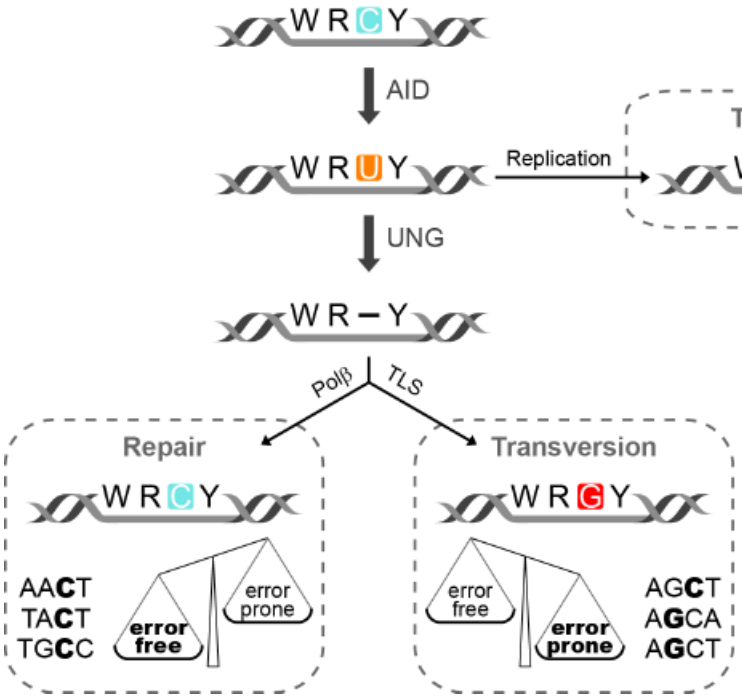
Over the last several years we have focused on understanding AID function and microRNA-regulatory mechanisms in germinal centers. We addressed the overall function of microRNAs in late B cell differentiation in CD19-Cre$^{ki/+}$ Dicer$^{fl/fl}$ mice, finding that in the absence of Dicer, late B cell 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.



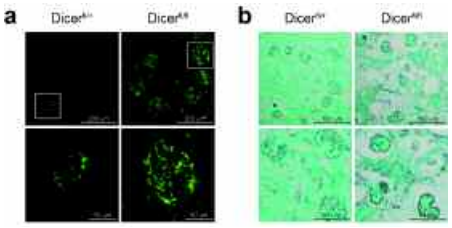
AID activity in germinal center B lymphocytes. By deaminating cytosines in the DNA of the immunoglobulin locus, AID initiates both reactions of antibody diversification that take place in germinal centers: somatic hypermutation (SHM) and class switch recombination (CSR). SHM and CSR allow the generation of specialized antibody isotypes with high affinity for antigen, and are therefore critical for the immune response. However, AID activity can also promote chromosome translocations and mutations outside immunoglobulin genes, potentially leading to oncogenic transformation.

Research Departments

2 Vascular Biology and Inflammation



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.



MicroRNA depletion in CD19-Cre$^{ki/+}$ Dicer$^{fl/fl}$ mice promotes immunocomplex deposition and kidney damage. Kidney sections from 40-60 week old CD19-Cre$^{ki/+}$Dicer$^{fl/+}$ and CD19-Cre$^{ki/+}$Dicer$^{fl/fl}$ animals were stained with anti-IgG antibodies (A) or were subjected to Silver-PAS staining (B).

MAJOR GRANTS

- **Ministerio de Ciencia e Innovación (SAF2010-21394)**
- **European Commission. European Research Council Starting Independent Researcher Grant (ERC-BCLYM 2007)**

SELECTED PUBLICATIONS

Belver L, Papavasiliou FN, Ramiro AR. **MicroRNA control of lymphocyte differentiation and function.** *Curr Opin Immunol* (2011) 23: 368-373

Belver L, de Yébenes VG, Ramiro AR. **MicroRNAs prevent the generation of autorreactive antibodies.** *Immunity* (2010) 33: 713-22

Research Departments

2 Vascular Biology and Inflammation

Immunobiology of inflammation

Head of Laboratory: *David Sancho Madrid*

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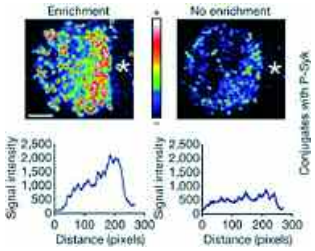
Helena M. Izquierdo Fernández
María Martínez López

Master Student: *Jaime Fernández Barrera*

RESEARCH INTEREST

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.

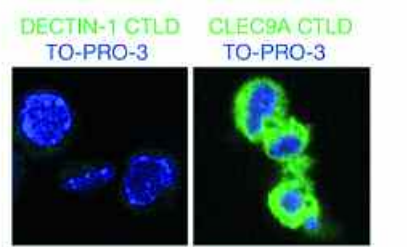
Our hypothesis is that recognition of cell death by Syk-coupled CLRs in myeloid cells might lie at the root of immune pathologies associated with an accumulation of dead cells. We are characterizing signaling and gene induction via CLEC9A as a model of innate sensing of necrotic cells by DCs. We are 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.



CLEC9A-dependent enrichment for phospho-Syk at the contact area between DCs and dead cells.
DC–dead-cell conjugates were formed and stained for P-Syk. P-Syk concentrates in the contact area of conjugated cells only in the presence of CLEC9A.

Research Departments

2 Vascular Biology and Inflammation



CLEC9A ligand is preformed and intracellular. *Fixed and permeabilized mouse embryonic fibroblasts were labeled with the ligand binding domain monomers DECTIN-1 (negative control) or CLEC9A, in green, and counterstained with TO-PRO-3 (nuclear dye) before confocal microscopy. Original magnification, x630.*

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

Mourão-Sá D, Robinson MJ, Zelenay S, Sancho D, Chakravarty P, Larsen R, Plantinga M, Van Rooijen N, Soares MP, Lambrecht B, Reis e Sousa C. **CLEC-2 signaling via Syk in myeloid cells can regulate inflammatory responses.** *Eur J Immunol* (2011) 41: 3040-53

Nuñez-Andrade N, Lamana A, Sancho D, Gisbert JP, Gonzalez-Amaro R, Sanchez-Madrid F, Urzainqui A. **P-selectin glycoprotein ligand-1 modulates immune inflammatory responses in the enteric lamina propria.** *J Pathol* (2011) 224: 212-2

Poulin LF, Salio M, Griessinger E, Anjos-Afonso F, Craciun L, Chen JL, Keller AM, Joffre O, Zelenay S, Nye E, Le Moine A, Faure F, Donckier V, Sancho D, Cerundolo V, Bonnet D, Reis e Sousa C. **Characterization of human DNGR-1+ BDCA3+ leukocytes as putative equivalents of mouse CD8alpha+ dendritic cells.** *J Exp Med* (2010) 207: 1261-71

Joffre OP, Sancho D, Zelenay S, Keller AM, Reis e Sousa C. **Efficient and versatile manipulation of the peripheral CD4+ T-cell compartment by antigen targeting to DNGR-1/CLEC9A.** *Eur J Immunol* (2010) 40: 1255-65

Research Departments

2 Vascular Biology and Inflammation

Stress kinases in diabetes, cancer and cardiovascular disease

Head of Laboratory: *Guadalupe Sabio*

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Antonia Tomás Loba

Predoctoral Researchers: *María Ángeles Verdugo*
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Bárbara González
Edgar Bernardo

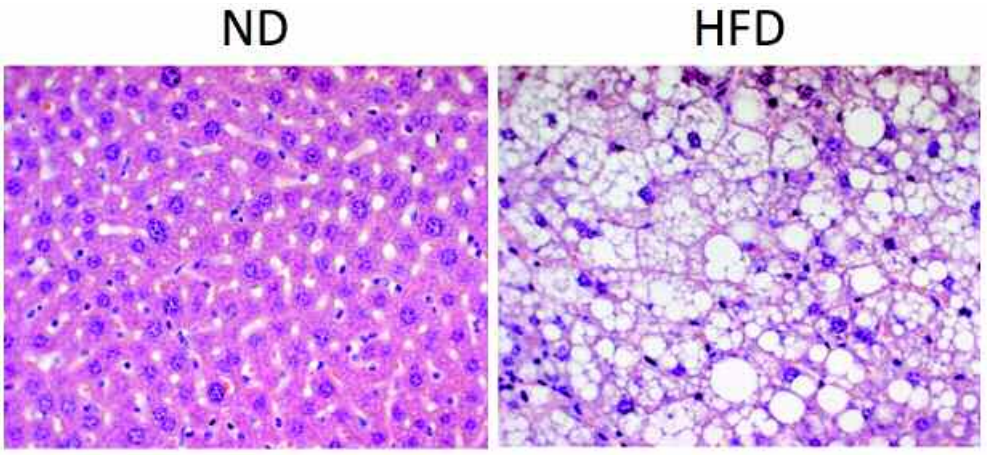
Technicians: *Luis Leiva*
Elena González

RESEARCH INTEREST

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 us 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 metabolim, obesity, dyslipidemia and glucose intolerance through its actions in various tissues.

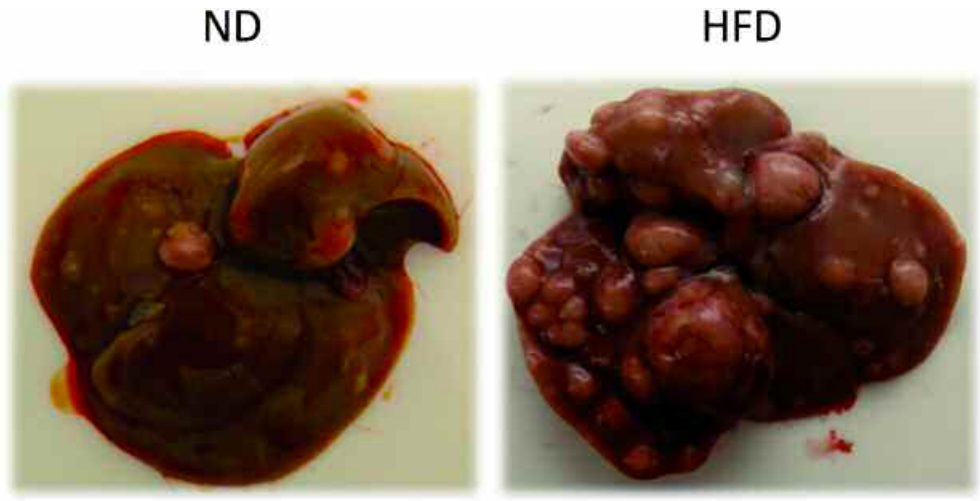


Hematoxylin and eosin (H&E)-stained section of liver from C57Bl6/J mice fed a normal diet (ND) or a high-fat diet (HFD) for 16 weeks.

Research Departments

2 Vascular Biology and Inflammation

C57Bl6/J mice fed a high-fat diet (left) or normal chow diet (right).



Hepatocellular carcinoma in liver from C57Bl6/J mice fed a normal diet (ND) or a high-fat diet (HFD).

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)**

SELECTED PUBLICATIONS

Noubade R, Krementsov DN, Del Rio R, Thornton T, Nagaleekar V, Saligrama N, Spitzack A, Spach K, Sabio G, Davis RJ, Rincon M, Teuscher C. **Activation of p38 MAPK in CD4 T cells controls IL-17 production and autoimmune encephalomyelitis.** *Blood* (2011) 118: 3290-300

Cellurale C, Sabio G, Kennedy NJ, Das M, Barlow M, Sandy P, Jacks T, Davis RJ
Requirement of c-Jun NH(2)-terminal kinase for Ras-initiated tumor formation. *Mol Cell Biol* (2011) 31: 1565-76

Sabio G, Cerezo-Guisado MI, Del Reino P, Inesta-Vaquera FA, Rousseau S, Arthur JS, Campbell DG, Centeno F, Cuenda A. **p38gamma regulates interaction of nuclear PSF and RNA with the tumour-suppressor hDlg in response to osmotic shock.** *J Cell Sci* (2010) 123: 2596-604

Sabio G, Davis RJ. **cJun NH2-terminal kinase 1 (JNK1): roles in metabolic regulation of insulin resistance.** *Trends Biochem Sci* (2010) 35: 490-6

Sabio G, Cavanagh-Kyros J, Barrett T, Jung DY, Ko HJ, Ong H, Morel C, Mora A, Reilly J, Kim JK, Davis RJ. **Role of the hypothalamic-pituitary-thyroid axis in metabolic regulation by JNK1.** *Genes Dev* (2010) 24: 256-64

Research Departments

2 Vascular Biology and Inflammation

Regulatory molecules of inflammatory processes

Head of Laboratory: *Pilar Martín*

Postdoctoral Researcher: *José Rodríguez Cortés*

Predoctoral Researchers: *Adela Matesanz Marín*
Elena Giulia Rodríguez Bovolenta

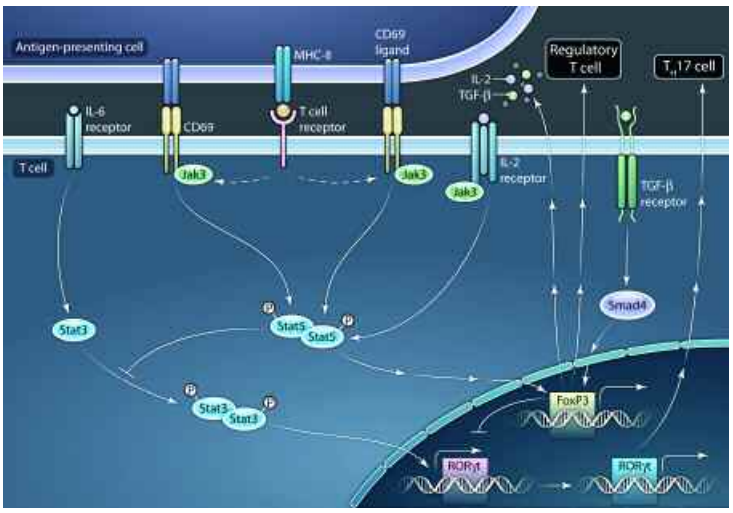
Visiting Scientist: *César Augusto Henríquez Camacho*

Undergraduate Student: *Sara Gutiérrez Ángel*

RESEARCH INTEREST

Understanding peripheral mechanisms operating in autoinmmune 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.

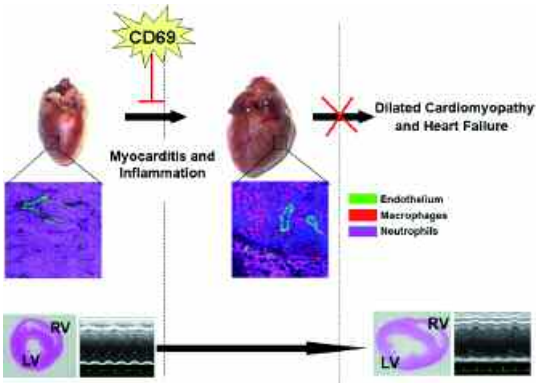
The early leukocyte activation antigen CD69 is a membrane receptor of the family of type II C-type lectins. CD69 is rapidly induced after cell activation in all bone marrow derived cells except erythrocytes. Expression in vivo is restricted to positively selected thymocytes and leukocytes undergoing activation, particularly at inflammation sites. Engagement of CD69 with monoclonal antibodies in the presence of phorbol esters induces a Ca^{2+} influx that activates ERK, induces IL-2 and IFN-γ 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 RORγt 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.



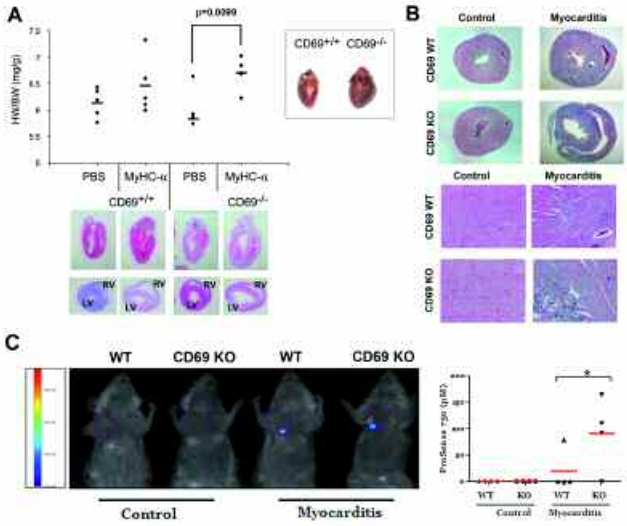
CD69 receptors are expressed on the membrane of T cells following activation. The cytoplasmic tail of CD69 associates with Jak3 and Stat 5 proteins, triggering phosphorylation of Stat5 and its translocation to the nucleus where it can activate the transcription factor FoxP3, stimulating the differentiation of regulatory T cells. CD69 engagement can also induce expression of IL-2 and TGFβ. 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 RORγt activation.

Research Departments

2 Vascular Biology and Inflammation



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

Martín P, Sanchez-Madrid F. **CD69: an unexpected regulator of Th17-driven inflammatory responses.** *Sci Signal* (2011) 4: pe14

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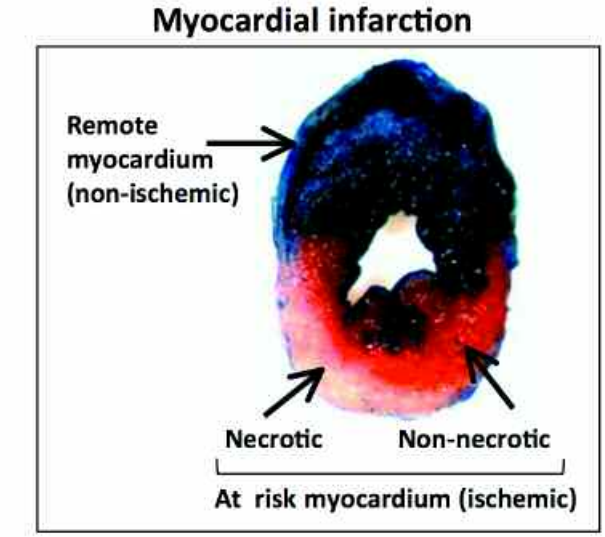
Martín P, Gómez M, Lamana A, Ramírez-Huesca M, Cruz-Adalia A, Ursa MA, Yáñez-Mo M, Sánchez-Madrid F. **CD69 association with Jak3/Stat5 proteins regulates Th17 cell differentiation.** *Mol Cell Biol* (2010) 30: 4877-89

Martín P, Gómez M, Lamana A, Marín AM, Cortés JR, Ramírez-Huesca M, Barreiro O, Lopez-Romero P, Gutierrez-Vazquez C, de la Fuente H, Cruz-Adalia A, Sánchez-Madrid F. **The leukocyte activation antigen CD69 limits allergic asthma and skin contact hypersensitivity.** *J Allergy Clin Immunol* (2010) 126: 355-65

Barreiro O, Martín P, González-Amaro R and Sánchez-Madrid F. **Molecular cues guiding the inflammatory responses.** *Cardiovasc Res* (2010) 86: 174-82

Research Departments

3 *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 Macías* *Ana Vanesa Alonso*
STUDY NURSE:	*Maite Dubraska Rodríguez Cabrera*
ADMINISTRATIVE SUPPORT:	*Eeva Inari Soininen* *Ana Gutiérrez*

Research Departments

3 Epidemiology, Atherothrombosis and Imaging

Cardiovascular imaging

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

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Jesús Mateo
Oliver Michel Weber (CNIC, Philips Healthcare)
Leticia Fernández Friera (CNIC, Hospital de Valdecilla. Santander)
Marta Tomás (CNIC, Fundación Jiménez Díaz. Madrid)

Project Managers: *Laura García Leal*
Luz Álvarez Vilela

CardioImage Fellow: *Gabriela Guzmán (CNIC, Hospital de La Paz, Madrid)*

Technicians: *Carolina Rojas Murcia*
Natalia Serrano Juzgado
Isabel Pérez García
Aurora Del Barrio Mantecas
Alberto Ávila Morales
Ricardo Ponce Sánchez
Sergio Cárdenas Melero
Rosa Villa Povo
Rosario Pérez Rubiño

RESEARCH INTEREST

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.

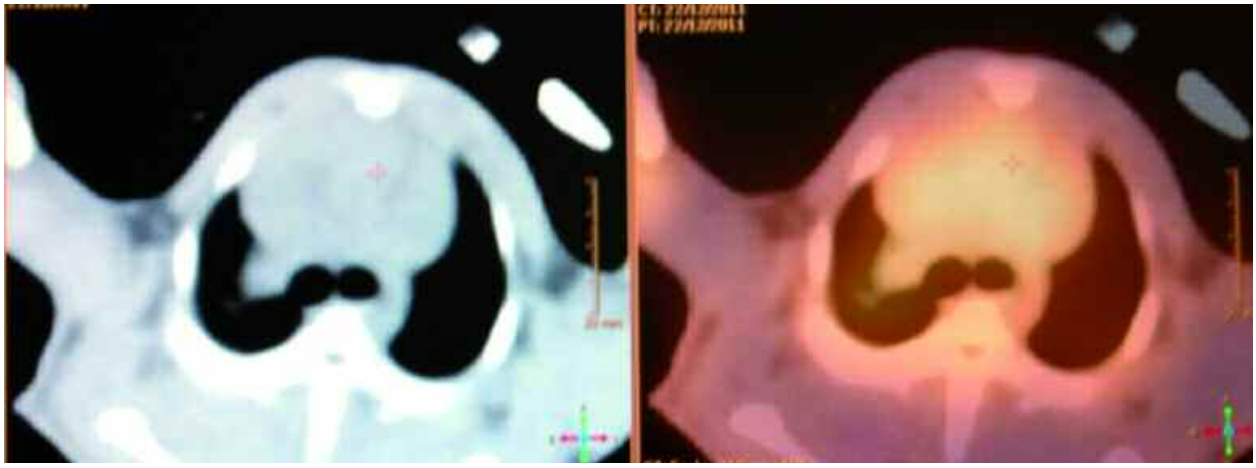
We are directly involved in the development of two large cohort studies (PESA and AWHS, see multidepartamental projects), where we are evaluating the application of non-invasive imaging for tracking atherosclerosis development and stratifying risk in asymptomatic populations. Last year we established 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 nanoprobes 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.

Research Departments

3 Epidemiology, Atherothrombosis and Imaging



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

Fuster V, Kelly BB, Vedanthan R. **Promoting global cardiovascular health: moving forward.** *Circulation* (2011) 123: 1671-8

Kovacic JC, Moreno P, Hachinski V, Nabel EG, Fuster V. **Cellular senescence, vascular disease, and aging: part 1 of a 2-part review.** *Circulation* (2011) 123: 1650-60

Kovacic JC, Moreno P, Nabel EG, Hachinski V, Fuster V. **Cellular senescence, vascular disease, and aging: part 2 of a 2-part review: clinical vascular disease in the elderly.** *Circulation* (2011) 123: 1900-10

Fayad ZA, Mani V, Woodward M, Kallend D, Abt M, Burgess T, Fuster V, Ballantyne CM, Stein EA, Tardif JC, Rudd JH, Farkouh ME, Tawakol A. **Safety and efficacy of dalcetrapib on atherosclerotic disease using novel non-invasive multimodality imaging (dal-PLAQUE): a randomised clinical trial.** *Lancet* (2011) 378: 1547-59

Lobatto ME, Fuster V, Fayad ZA, Mulder WJ. **Perspectives and opportunities for nanomedicine in the management of atherosclerosis.** *Nat Rev Drug Discov* (2011) 10: 835-52

Research Departments

3 Epidemiology, Atherothrombosis and Imaging

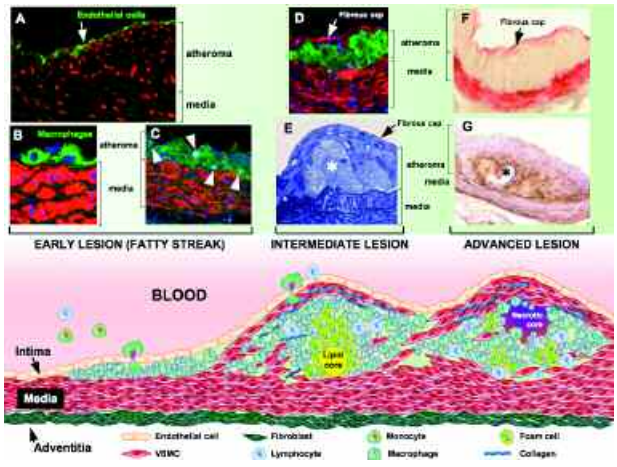
Molecular and genetic cardiovascular pathophysiology

Head of Laboratory:	*Vicente Andrés García*
Postdoctoral Researchers:	*Raphaël Chèvre* *José Javier Fuster Ortuño* *José María González Granado* *Oscar Muñiz Pello* *José Rivera Torres* *Laia Trigueros Motos* *Ricardo Villa Bellosta*
Predoctoral 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 INTEREST

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.

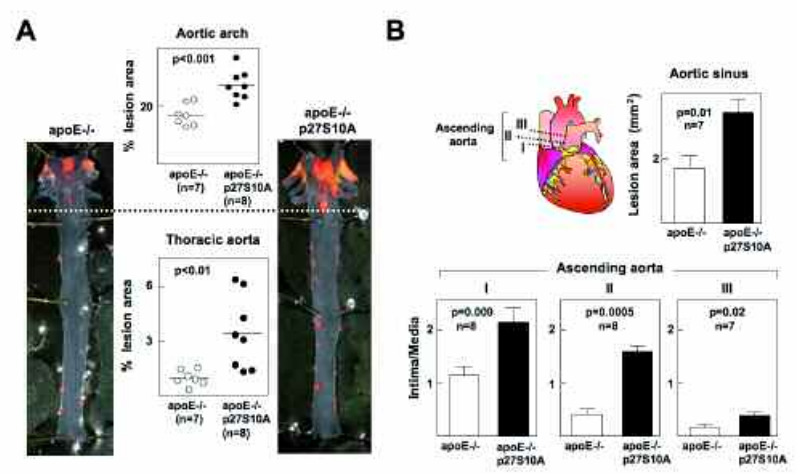
We investigate cellular, molecular and genetic mechanisms that underlie the development of atherosclerosis and restenosis. Our main interest is in regulatory circuits that control gene transcription and cell proliferation, and our long-term goal is to identify novel therapeutic targets and provide the 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.



Atherosclerotic plaque development. The scheme shows the main events that occur in the vessel wall during atheroma progression, starting with a healthy artery wall (left) and progressing to an advanced plaque (right).

Research Departments

3 Epidemiology, Atherothrombosis and Imaging



Deficiency of p27 phosphorylation at serine 10 increases atherosclerosis burden in apoE-null mice.

MAJOR GRANTS

- **Ministerio de Ciencia e Innovación. FIS RETICS (RECAVA, RD06/0014/0021)**
- **Ministerio de Ciencia e Innovación (SAF2010-16044)**

SELECTED PUBLICATIONS

Fuster JJ*, Molina P*, Jovaní D, Vinué A, Serrano M, Andrés V. **Increased gene dosage of the Ink4/Arf locus does not attenuate atherosclerosis development in hypercholesterolaemic mice.** *Atherosclerosis* (accepted)

*Joint 1st authors

Pello OM, Silvestre C, De Pizzol M, Andrés V. **A glimpse on the phenomenon of macrophage polarization during atherosclerosis.** *Immunobiology* (2011) 216: 1172-6

Fuster JJ, González-Navarro H, Vinué A, Molina P, Andrés-Manzano MJ, Nakayama KI, Nakayama K, Díez-Juan A, Bernad A, Rodríguez C, Martínez-González J, Andrés V. **Deficient p27 phosphorylation at serine 10 increases macrophage foam cell formation and aggravates atherosclerosis through a proliferation-independent mechanism.** *Arterioscl Thromb Vasc Biol* (2011) 31: 2455-63

González JM, Andrés V. **Synthesis, transport and incorporation into the nuclear envelope of A-type lamins and inner nuclear membrane proteins.** *Biochem Soc Trans* (2011) 39: 1758-63

Osorio FG, Navarro CL, Cadiñanos J, López-Mejía IC, Quirós PM, Bartoli C, Rivera J, Tazi J, Guzmán G, Varela I, Depetris D, de Carlos F, Cobo J, Andrés V, De Sandre-Giovannoli A, Freije JMP, Lévy N, López-Otín C. **Splicing-directed therapy in a new mouse model of human accelerated aging.** *Sci Trans Med* (2011) 3: 106ra107

Editors' choice: Splicing therapy comes of age. ***Science*** (2011) 334: 739

Research Departments

3 Epidemiology, Atherothrombosis and Imaging

Imaging in experimental cardiology

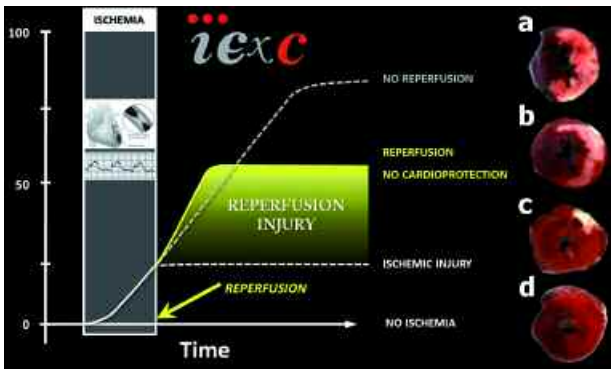
Head of Laboratory:	*Borja Ibáñez Cabeza (CNIC, Hospital Clínico San Carlos)*
Postdoctoral Researchers:	*David Sanz-Rosa* *Leticia Fernández Friera (CNIC, Hospital de Valdecilla)* *Gonzalo Pizarro Sánchez (CNIC, Hospital Quirón Madrid)*
Predoctoral Researcher:	*Jaime García-Prieto*
Invesmir Fellows:	*Carmen Olmos Blanco* *Eduardo Franco Díez*
CardioJoven Fellows:	*José Manuel García Ruiz* *Ana García Álvarez*
CardioImage Fellows:	*Jesús González Mirelis*
Research Coordinator:	*Noemi Escalera*
Technicians:	*Jose Luis Martín Revillo* *Mario Nuño Ayala* *Parvin Rupa Khaton*
Visiting Scientists:	*Alonso Antonio Mateos Rodríguez* *Daniel Pereda Arnau*

RESEARCH INTEREST

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.

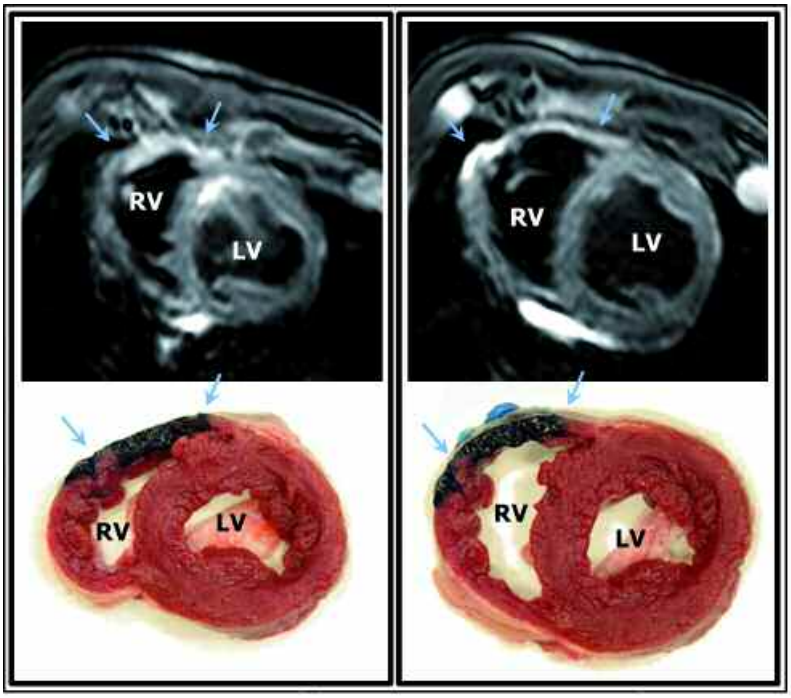
In the clinical setting, our team is a key participant in the METOCARD-CNIC trial, which uses magnetic resonance imaging to evaluate the effectiveness of a cardioprotective strategy based on beta adrenergic modulation in patients with a previous myocardial infarction. We have already launched the metocard-CLOCK 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 (b); 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.

Research Departments

3 Epidemiology, Atherothrombosis and Imaging



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 edemic areas by MRI.

MAJOR GRANTS

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

SELECTED PUBLICATIONS

Ruiz-Hornillos PJ, Martinez-Camara F, Elizondo M, Jimenez-Fraile JA, Del Mar Alonso-Sanchez M, Galan D, Garcia-Rubira JC, Macaya C, Ibanez B. **Systemic fibrinolysis through intraosseous vascular access in ST-segment elevation myocardial infarction.** *Ann Emerg Med* (2011) 57: 572-4

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Cimmino G, Ibanez B, Giannarelli C, Prat-Gonzalez S, Hutter R, Garcia M, Sanz J, Fuster V, Badimon JJ. **Carvedilol administration in acute myocardial infarction results in stronger inhibition of early markers of left ventricular remodeling than metoprolol.** *Int J Cardiol* (2011) 153: 256-61

Ibanez B, Cimmino G, Prat-Gonzalez S, Vilahur G, Hutter R, Garcia MJ, Fuster V, Sanz J, Badimon L, Badimon JJ. **The cardioprotection granted by metoprolol is restricted to its administration prior to coronary reperfusion.** *Int J Cardiol* (2011) 147: 428-32

Ibanez B, Fuster V, Jimenez-Borreguero J, Badimon JJ. **Lethal myocardial reperfusion injury: A necessary evil?** *Int J Cardiol* (2011) 151: 3-11

Research Departments

3 Epidemiology, Atherothrombosis and Imaging

Imaging cardiovascular inflammation and the immune response

Head of Laboratory: *Andrés Hidalgo Alonso*

Postdoctoral Researchers: *Maria Nácher Espuig*
Vinatha Sreeramkumar

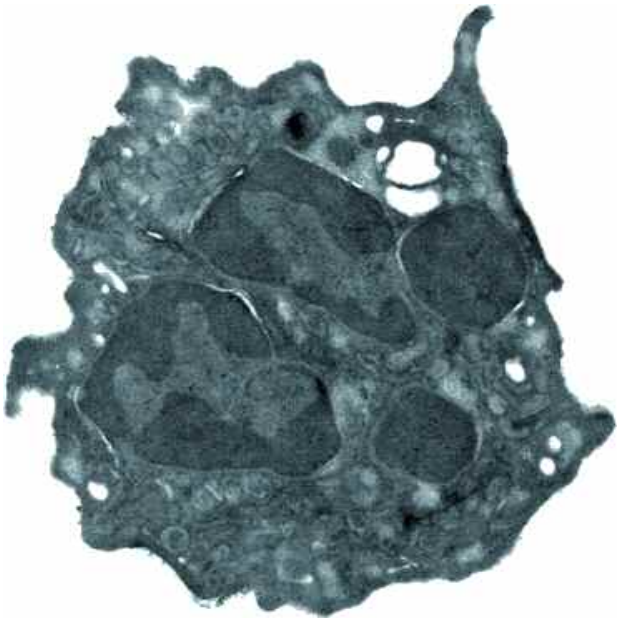
Predoctoral Researcher: *María Casanova Acebes*

Technician: *Christophe Pitaval*

Visiting Scientist: *Linnea Weiss*

RESEARCH INTEREST

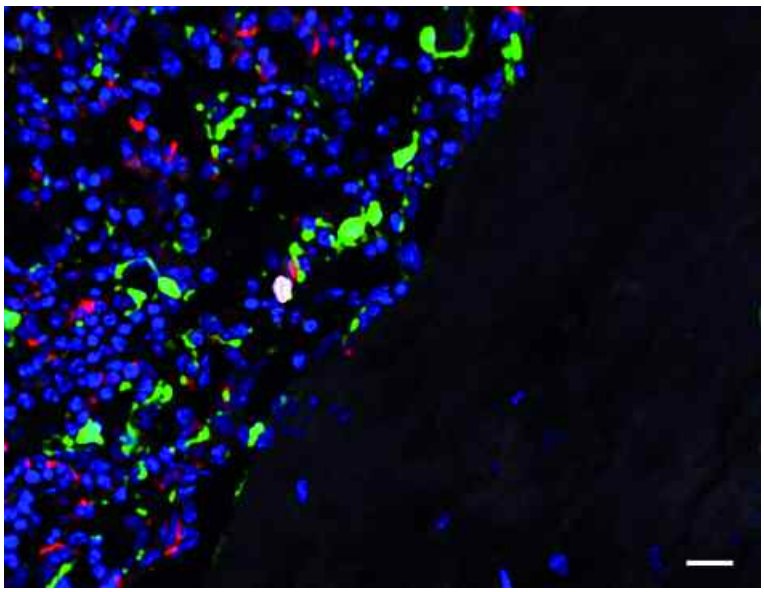
Our group is interested in the roles that neutrophils and other leukocytes play in the body. Part of their functions relate to their well-known functions during inflammation, as the first and critical cellular response to injury. Our current program includes studies of the ability of neutrophils to interact 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.



Electron microscopy image of a naturally-occurring aged neutrophil purified from the blood of a selectin knockout mouse.

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 μm.

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

Zarbock A, Ley K, McEver RP, Hidalgo A. **Leukocyte ligands for endothelial selectins: specialized glycoconjugates that mediate rolling and signaling under flow.** *Blood* (2011) 118: 6743-51

Nacher M, Hidalgo A. **Fire within the vessels: Interactions between blood cells and inflammatory vascular injury.** *Front Bioscience* (Schol Ed) (2011) S3: 1089-100

Chow A, Lucas D, Hidalgo A, Mendez-Ferrer S, Hashimoto D, Scheiermann C, Battista M, Leboeuf M, Prophete C, van Rooijen N, Merad M, Frenette PS. **Bone Marrow CD169+ macrophages promote the retention of hematopoietic stem and progenitor cells in the mesenchymal stem cell niche.** *J Exp Med* (2011) 208: 261-71

Nácher M, Blázquez AB, Shao B, Matesanz A, Prophete C, Berin MC, Frenette PS and Hidalgo A. **Physiological contribution of CD44 as a ligand for E-selectin during inflammatory T cell recruitment.** *Am J Pathol* (2011) 178: 2437-46

Soderquest K, Powell N, Luci C, van Rooijen N, Hidalgo A, Geissmann F, Walzer T, Lord GM, Martin-Fontecha A. **Monocytes control natural killer cell differentiation to effector phenotypes.** *Blood* (2011) 117: 4511-18

Research Departments

3 Epidemiology, Atherothrombosis and Imaging

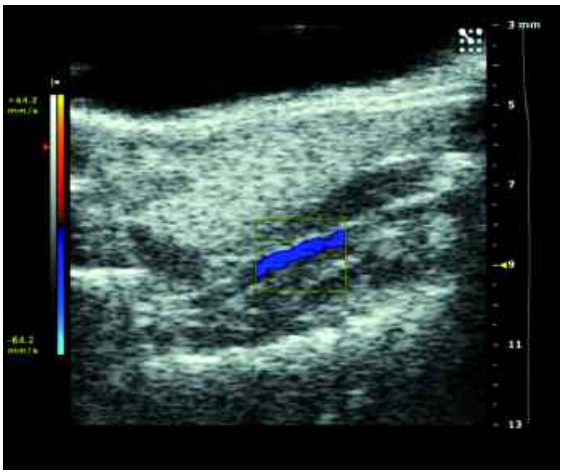
Vascular wall remodeling and cardiovascular disease

Head of Laboratory:	*Carlos Zaragoza Sánchez*
Postdoctoral Researcher:	*Beatriz Herranz Sánchez*
Predoctoral Researcher:	*Begoña Lavin Plaza*
Technician:	*Mónica Gómez Parrizas*
Visiting Scientist:	*Borja Castejón*

RESEARCH INTEREST

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 thearpeutical implications.

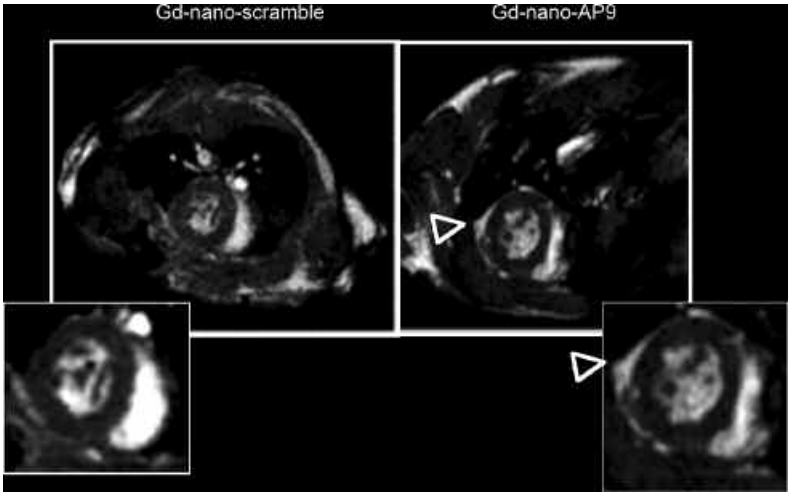
Our group participates in the European Commission funded HYPERImage project for the development of new imaging technologies.



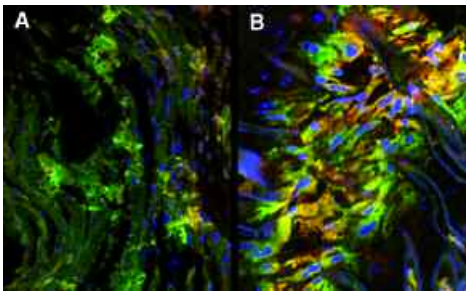
Color doppler ultrasound of a mouse right common carotid artery.

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

Herranz B, Marquez S, Guijarro B, Aracil E, Aicart-Ramos C, Rodriguez-Crespo I, Rodríguez-Puyol M, Zaragoza C*, Saura M.* **Integrin-linked kinase regulates vasomotor function by preventing endothelial nitric oxide synthase uncoupling: role in atherosclerosis.** *Circ Res* (accepted)

*Corresponding authors

Klink A, Hyafil F, Rudd J, Faries P, Fuster V, Mallat Z, Meilhac O, Mulder WJ, Michel JB, Ramirez F, Storm G, Thompson R, Turnbull IC, Egido J, Martín-Ventura JL, Zaragoza C, Letourneur D, Fayad ZA. **Diagnostic and therapeutic strategies for small abdominal aortic aneurysms.** *Nat Rev Cardiol* (2011) 8:338-47

Tarin C, Lavin B, Gomez M, Saura M, Diez-Juan A, 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

Klink A, Heynens J, Herranz B, Lobatto ME, Arias T, Sanders HM, Strijkers GJ, Merkx M, Nicolay K, Fuster V, Tedgui A, Mallat Z, Mulder WJ, Fayad ZA. **In vivo characterization of a new abdominal aortic aneurysm mouse model with conventional and molecular magnetic resonance imaging.** *J Am Coll Cardiol* (2011) 58: 2522-30

Zaragoza C, Gomez-Guerrero C, Martin-Ventura JL, Blanco-Colio L, Lavin B, Mallavia B, Tarin C, Mas S, Ortiz A, Egido J. **Animal models of cardiovascular diseases.** *J Biomed Biotechnol* (2011) 2011: 497841

Research Departments

3 Epidemiology, Atherothrombosis and Imaging

Cardiovascular epidemiology and population genetics

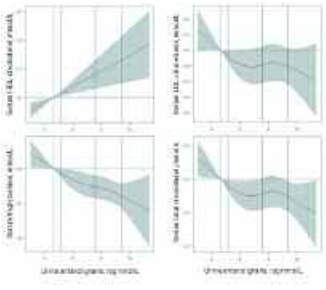
Head of Laboratory:	*Valentín Fuster (CNIC, Mt. Sinai Medical Center, New York)*
Research Scientists:	*Manuel Franco* *Martín Laclaustra* *José Luis Peñalvo*
Visiting Scientists:	*Eliseo Guallar (CNIC, Johns Hopkins Bloomberg School of Public Health, Baltimore)* *José Mª Ordovás (CNIC, Tufts University, 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*

RESEARCH INTEREST

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.

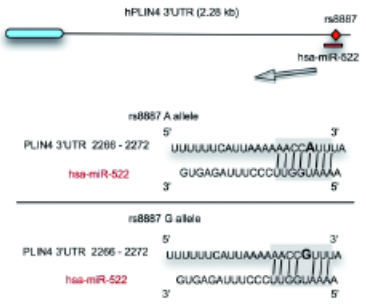
The members of the group also continue to make significant contributions to leading international studies such as the Framingham Heart Study, the Atherosclerosis Risk in Communities (ARIC) Study, the Multiethnic Study of Atherosclerosis (MESA), the Strong Heart Study, the US National Health and Nutrition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



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).

Research Departments

3 Epidemiology, Atherothrombosis and Imaging



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**
- **Comunidad de Madrid (P2009/AGR-1469). PI: JL Peñalvo**
- **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

Tellez-Plaza M, Navas-Acien A, Caldwell KL, Menke A, Muntner P, Guallar E. **Reduction in cadmium exposure in the United States population, 1988-2008: The contribution of declining smoking rates.** *Environ Health Perspect* (accepted)

Laclaustra M, Ordoñez B, Leon M, Andres EM, Cordero A, Pascual-Calleja I, Grima A, Luengo E, Alegria E, Pocovi M, Civeira F, Casasnovas-Lenguas JA. **Metabolic syndrome and coronary heart disease among Spanish male workers: A case-control study of MESYAS.** *Nutr Metab Cardiovasc Dis* (accepted)

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)

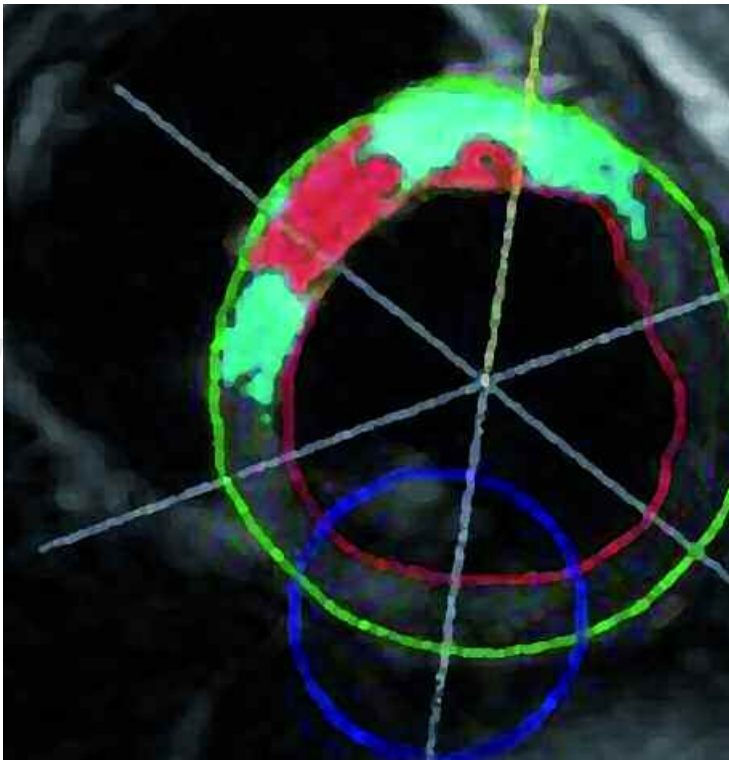
Rayman MP, Stranges S, Griffin B, Wong M, Guallar E. **Effect of supplementation with high-selenium yeast on plasma lipids: a randomized, controlled trial.** *Ann Intern Med* (2011) 154: 656-65

Franco M, Cooper R, Bilal U, Fuster V. **Challenges and Opportunities for Cardiovascular Disease Prevention.** *Am J Med* (2011) 124: 95-102

Richardson K, Louie-Gao Q, Arnett DK, Parnell LD, Lai CQ, Davalos A, Fox CS, Demissie S, Cupples LA, Fernandez-Hernando C, Ordovas JM. **The PLIN4 variant rs8887 modulates obesity related phenotypes in humans through creation of a novel miR-522 seed site.** *PLoS One* (2011) 6:e17944

Multi-departmental Clinical Projects

4



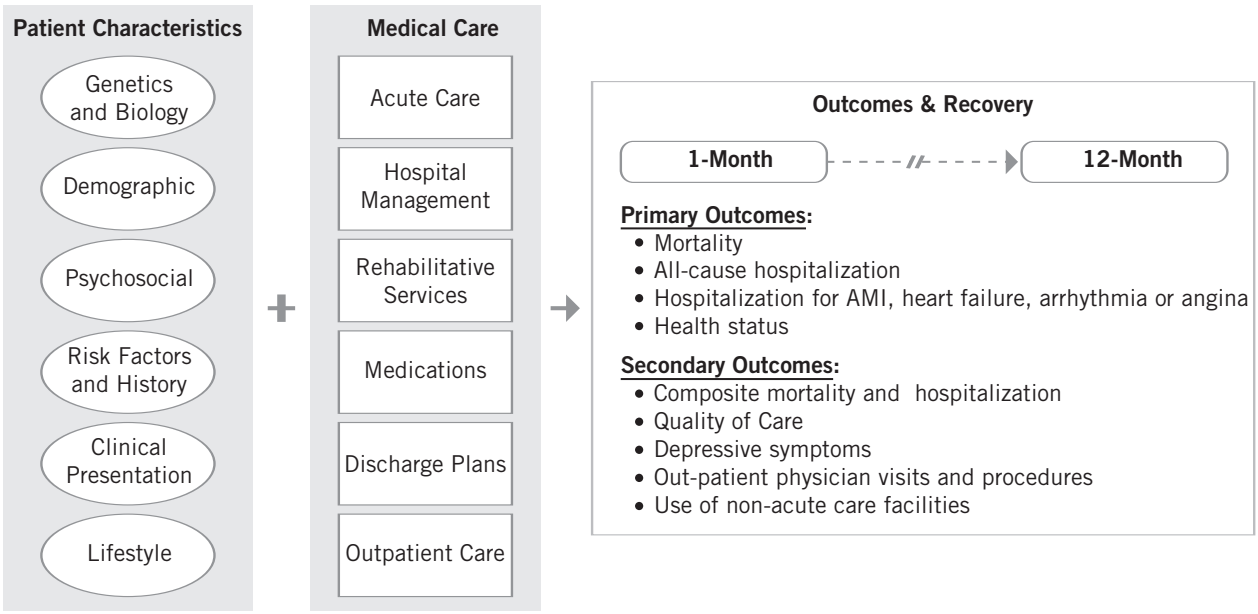
Multi-departmental Clinical Projects

IMJOVEN Study

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.



Multi-departmental Clinical Projects

AWHS

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.

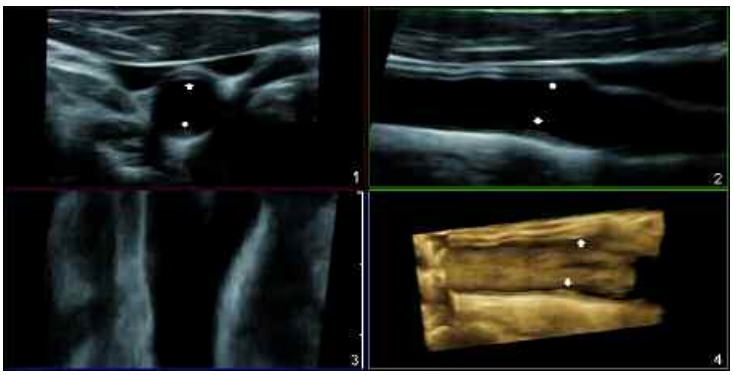
Strategies to identify individuals with subclinical alterations indicating increased risk of cardiovascular disease have been boosted by the development of basic imaging techniques (3D ultrasound) and advanced non-invasive imaging techniques (magnetic resonance imaging, positron emission tomography, 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.



Multi-departmental Clinical Projects

POLYPILL CNIC-FERRER

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).

Multi-departmental Clinical Projects

METOCARD-CNIC

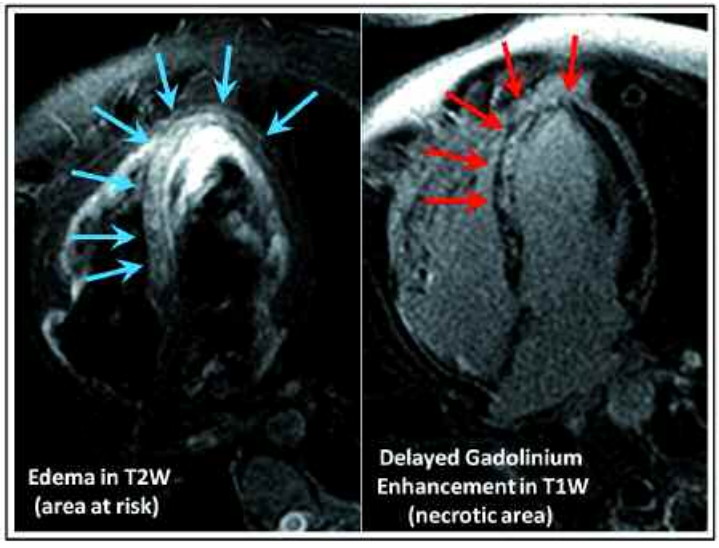
Acute myocardial infarction (AMI) is the main cause of death in western countries. The best strategy to limit myocardial damage is to perform an early coronary reperfusion. However, reperfusion itself comes at a price of additional myocardial damage, known as ischemia/reperfusion injury (I/R).

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.



Imaging of human heart after an acute myocardial infarction.
Apical four chamber view of a human heart 6 days after an acute myocardial infarction. Left: Edema (without contrast infusion). Arrows delineate the area of the left ventricle at risk. Right: Necrotic area (revealed by gadolinium contrast injection). Arrows mark the necrotic area in the left ventricle. Note that the area at risk is larger than the necrotic area, evidencing areas of cardioprotection (areas at risk with no necrosis).

Top: *Members of the METOCARD-CNIC initiative at the recently launched CNIC human cardiovascular imaging laboratory at the Hospital Carlos III, in Madrid.*

Bottom: *Members of the METOCARD-CNIC steering committee and partners from the emergency medical service SUMMA 112. The contribution of the emergency medical services, in close collaboration with Hospitals, is critical to the effective conduct of this revolutionary study.*

Translational Platform

5

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.

Departamental Staff

GROUP LEADER: *Antonio Bernad Miana*

ADMINISTRATIVE SUPPORT: *Ana Gutiérrez Llaneza*

Technical Units

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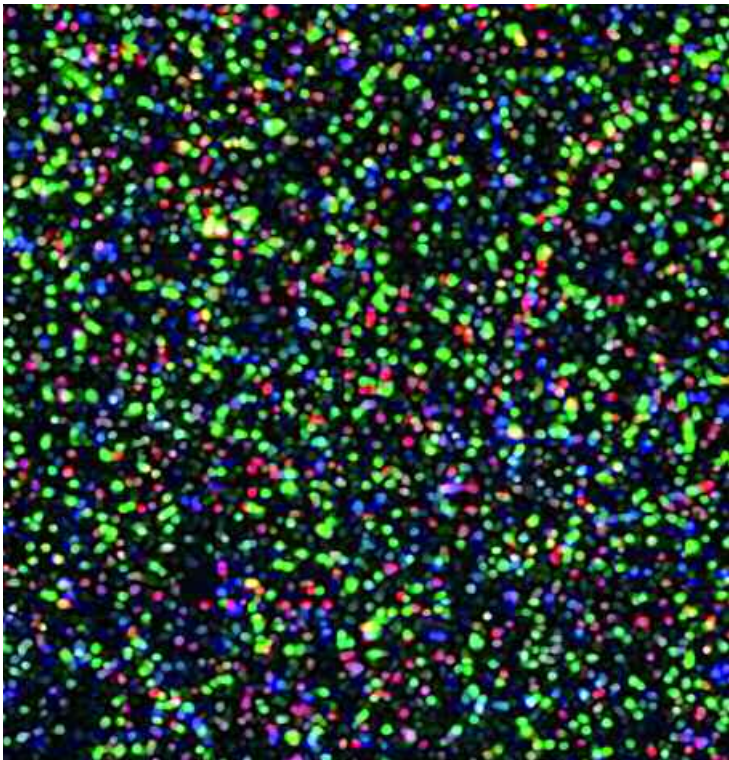


Technical Units

Microscopy and dynamic imaging

Head of Unit: *Valeria R. Caiolfa (CNIC, San Raffaele Scientific Institute, Milan)*

Support Scientists: *Moreno Zamai*
Susana Sánchez Donoso
Christian Hellriegel
Antonio Manuel Santos Beneit
Elvira Arza

Postdoctoral Researchers: *Valeria Corti*
Antonio Trullo
Giulia Ossato

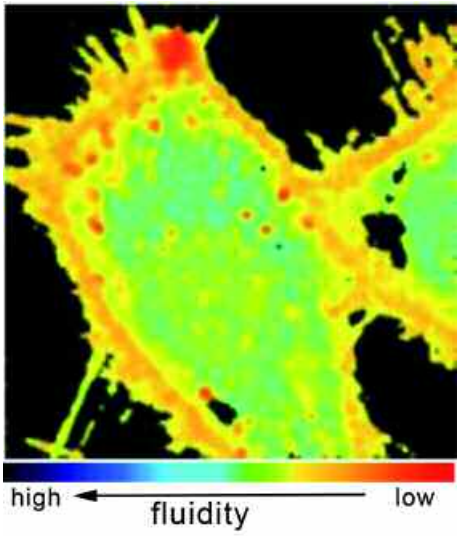
RESEARCH INTEREST

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.

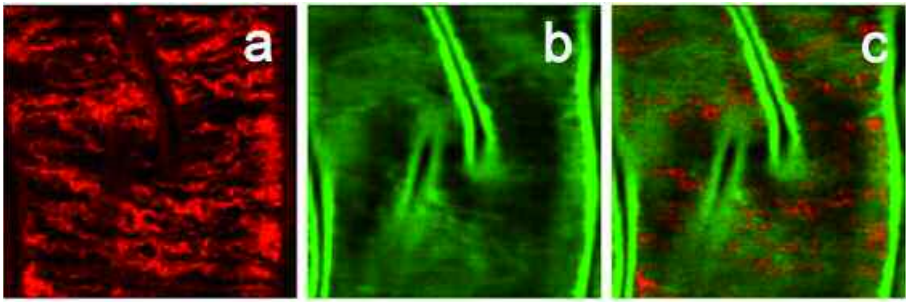
The Unit is also strongly committed to technological innovation and development of new applications of interest to scientists at the CNIC and beyond. Ongoing research collaborations with internal and international groups are developing procedures for fluorescence correlation spectroscopy, SHG, and in 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.

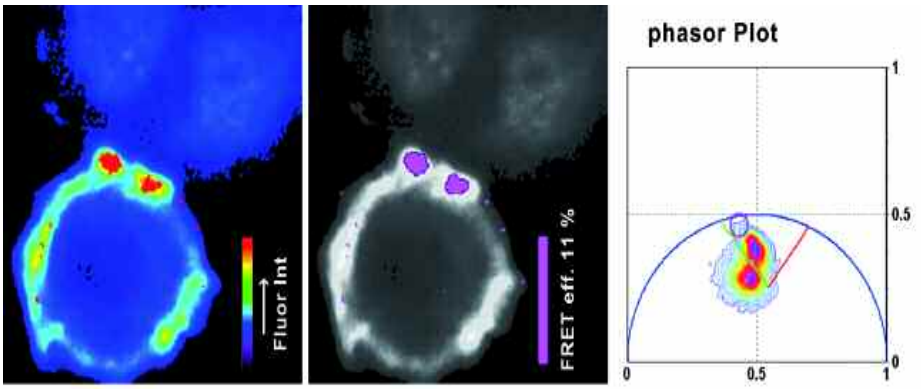


Multiphoton microscopy: Laurdan GP image of a Hela cell, showing regions mapped for different lipid fluidity.

Technical Units



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

Hellriegel C, Caiolfa VR, Corti V, Sidenius N, Zamai M. **Number and brightness image analysis reveals ATF-induced dimerization kinetics of uPAR in the cell membrane.** *FASEB J* (2011) 25: 2883-97

Sanchez SA, Gunther G, Tricerri MA, Gratton E. **Methyl-beta-cyclodextrins preferentially remove cholesterol from the liquid disordered phase in giant unilamellar vesicles.** *J Membr Biol* (2011) 241: 1-10

Sanchez S, Bakas L, Gratton E, Herlax V. **Alpha hemolysin induces an increase of erythrocytes calcium: A FLIM 2-photon phasor analysis approach.** *PLoS One* (2011) 6: e21127

Muriel O, Echarri A, Hellriegel C, Pavon DM, Beccari L, Del Pozo MA. **Phosphorylated filamin A regulates actin-linked caveolae dynamics.** *J Cell Sci* (2011) 124: 2763-76

Scielzo C, Bertilaccio MT, Simonetti G, Dagklis A, ten Hacken E, Fazi C, Muzio M, Caiolfa VR, Kitamura D, Restuccia U, Bachi A, Rocchi M, Ponzoni M, Ghia P, Caligaris-Cappio F. **HS1 has a central role in the trafficking and homing of leukemic B cells.** *Blood* (2010) 116: 3537-46

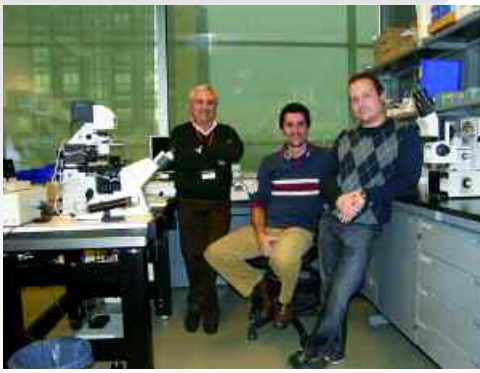
Technical Units

Transgenesis

Head of Unit: *Luis-Miguel Criado Rodríguez*

Support Scientist: *José Mª Fernández*

Technician: *David Esteban Martínez*



RESEARCH INTEREST

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.

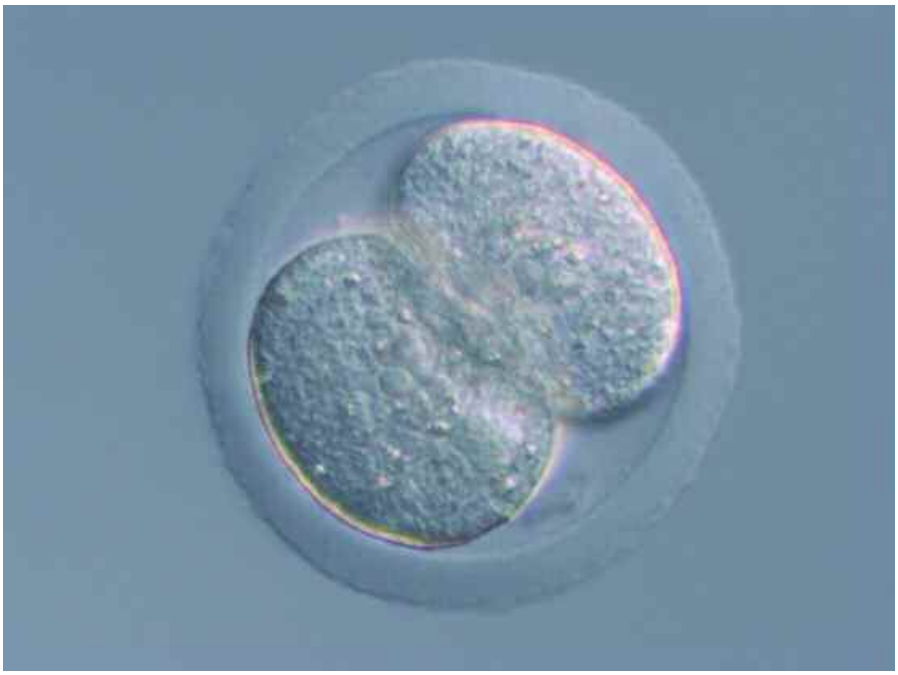
Transgenic mice are produced in the Unit by the established methodologies of microinjection of DNA in solution into zygote pronuclei (pronuclear microinjection) or of recombinant lentiviruses beneath the zygote zona pellucida (subzonal or perivitelline microinjection). Chimeric mice for the generation 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.

Zygote from a WISTAR rat

Technical Units



Two-cell embryo from a WISTAR rat

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

SELECTED PUBLICATIONS

Pallares P, Garcia-Fernandez RA, Criado LM, Letelier CA, Fernandez-Toro JM, Esteban D, Flores JM, Gonzalez-Bulnes A. **Substantiation of ovarian effects of leptin by challenging a mouse model of obesity/type 2 diabetes.** *Theriogenology* (2010) 73: 1088-95

Technical Units

Genomics

Head of Unit: *Ana Dopazo*

Support Scientists: *Sergio Callejas*
Alberto Benguría

Technician: *Rebeca Álvarez*

RESEARCH INTEREST

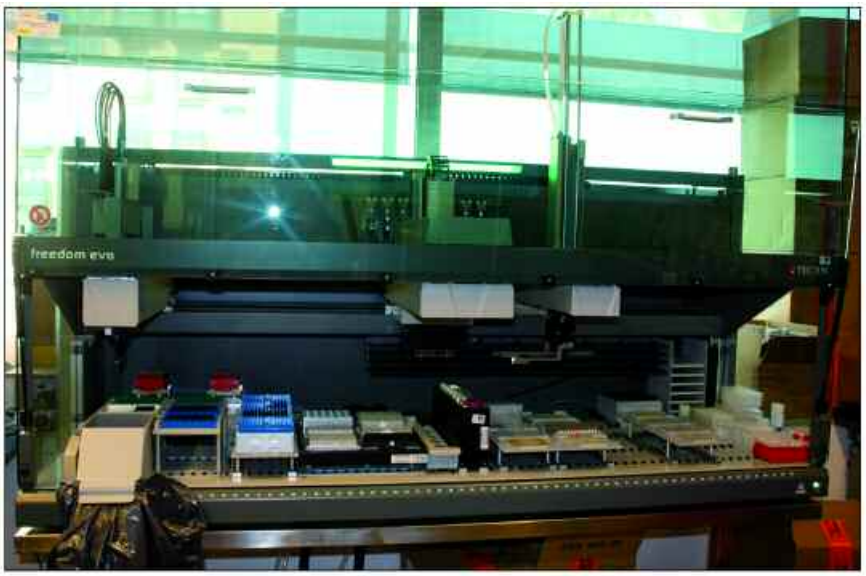
The Genomics Unit provides the latest genomic technologies to the scientific community at the CNIC and beyond, together with expert assistance with experimental design.

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 II*x*. 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.

Illumina flow cell for NGS

Technical Units



Tecan robot for automated RNA Seq sample library preparation

MAJOR GRANTS

Ministerio de Ciencia e Innovación. FIS (PI10/01124)

SELECTED PUBLICATIONS

Estrada JC, López-Romero P, Benguría A, Dopazo A, L Carrera-Quintanar, C Albo, E Roche, E Perales Clemente, Enríquez Domínguez JA, Bernad A, Samper E. **Culture of human mesenchymal stem cells at low oxygen tension improves growth and genetic stability by activating glycolysis.** *Cell Death and Differentiation* (accepted)

Sánchez-Cabo F, Rainer J, Dopazo A, Trajanoski Z, Hackl H. **Insights into global mechanisms and disease by gene expression profiling.** *Methods Mol Biol* (2011) 719: 269-98

Sanchez-Ramos C, Tierrez A, Fabregat O, Wild B, Sanchez-Cabo F, Arduini A, Dopazo A, Monsalve M. **PGC-1α regulates TLS activity: Role in oxidative stress gene expression.** *Antioxid Redox Sign* (2011) 12: 325-37

Callejas S, Alvarez R, Dopazo A. **Automatic genomics: A user-friendly program for the automatic designing and plate loading of medium-throughput qPCR experiments.** *Biotechniques* (2011) 50: 46-50

Tomé M, López-Romero P, Albo C, Sepúlveda JC, Fernández-Gutiérrez B, Dopazo A, Bernad A, González MA. **miR-335 orchestrates cell proliferation, migration and differentiation in human mesenchymal stem cells.** *Cell Death Differ* (2011) 18: 985-95

Technical Units

Pluripotent cell technology

Head of Technical Service: *Giovanna Giovinazzo*

Support Scientist: *Francisco Gutiérrez*

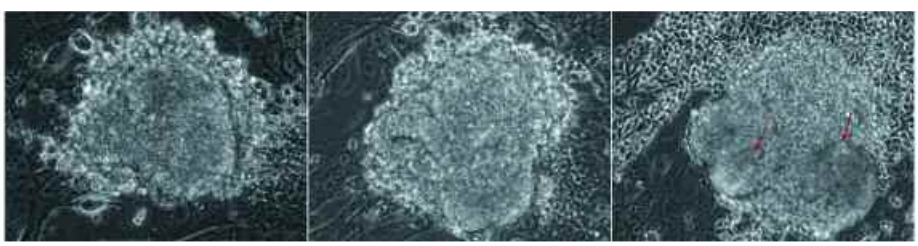
Technician: *María Ángeles Sanguino*

RESEARCH INTEREST

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.

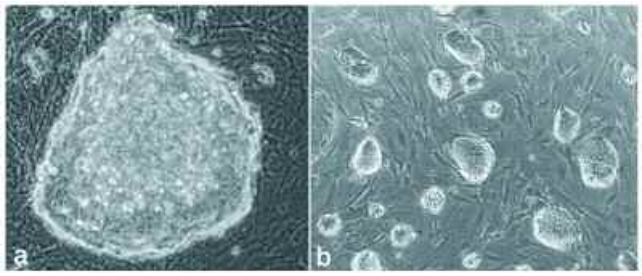
One of the unit's core tasks is to facilitate the generation of genetically-modified mice through homologous recombination in mouse embryonic stem cells (mESCs). Our staff takes charge of all the key steps of the gene targeting protocol: electroporation of the targeting vector, selection, karyotyping, 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/Bl6 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.

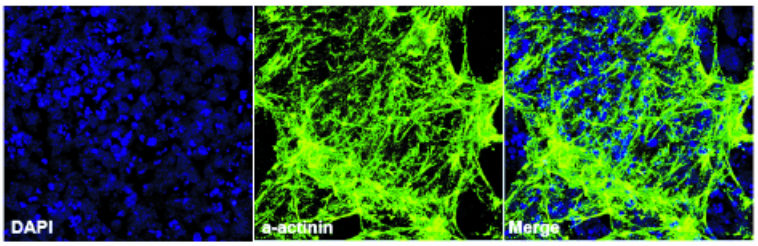


Blastocyst outgrowth and inner cell mass expansion during the derivation of a mutant mouse embryonic stem cell line. Images show an attached embryo on a feeder cell layer at day 3, day 4, and day 5 in culture (moving left to right). Arrows mark ES cell-like areas.

Technical Units



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 iPS cells.

MAJOR GRANTS

- Ministerio de Ciencia e Innovación. FIS (CTA0801)

SELECTED PUBLICATIONS

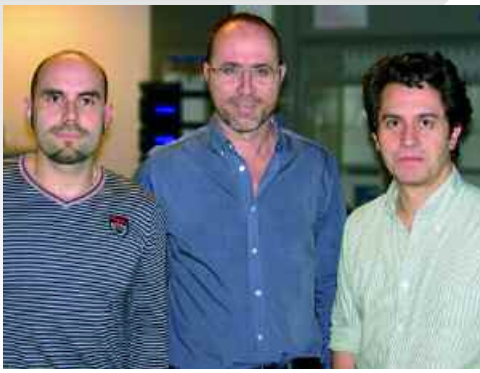
Casanova JC, Uribe V, Badia-Careaga C, Giovinazzo G, Torres M, Sanz-Ezquerro JJ. **Apical ectodermal ridge morphogenesis in limb development is controlled by Arid3b-mediated regulation of cell movements.** *Development* (2011) 138: 1195-205

Technical Units

Proteomics

Head of Unit: *Juan Antonio López*

Support Scientists: *Enrique Calvo*
Emilio Camafeita



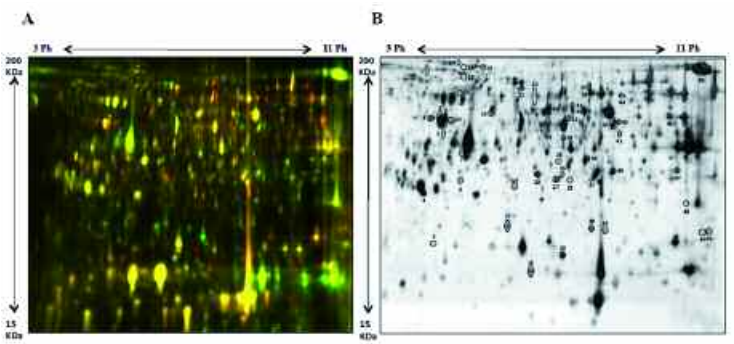
RESEARCH INTEREST

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.

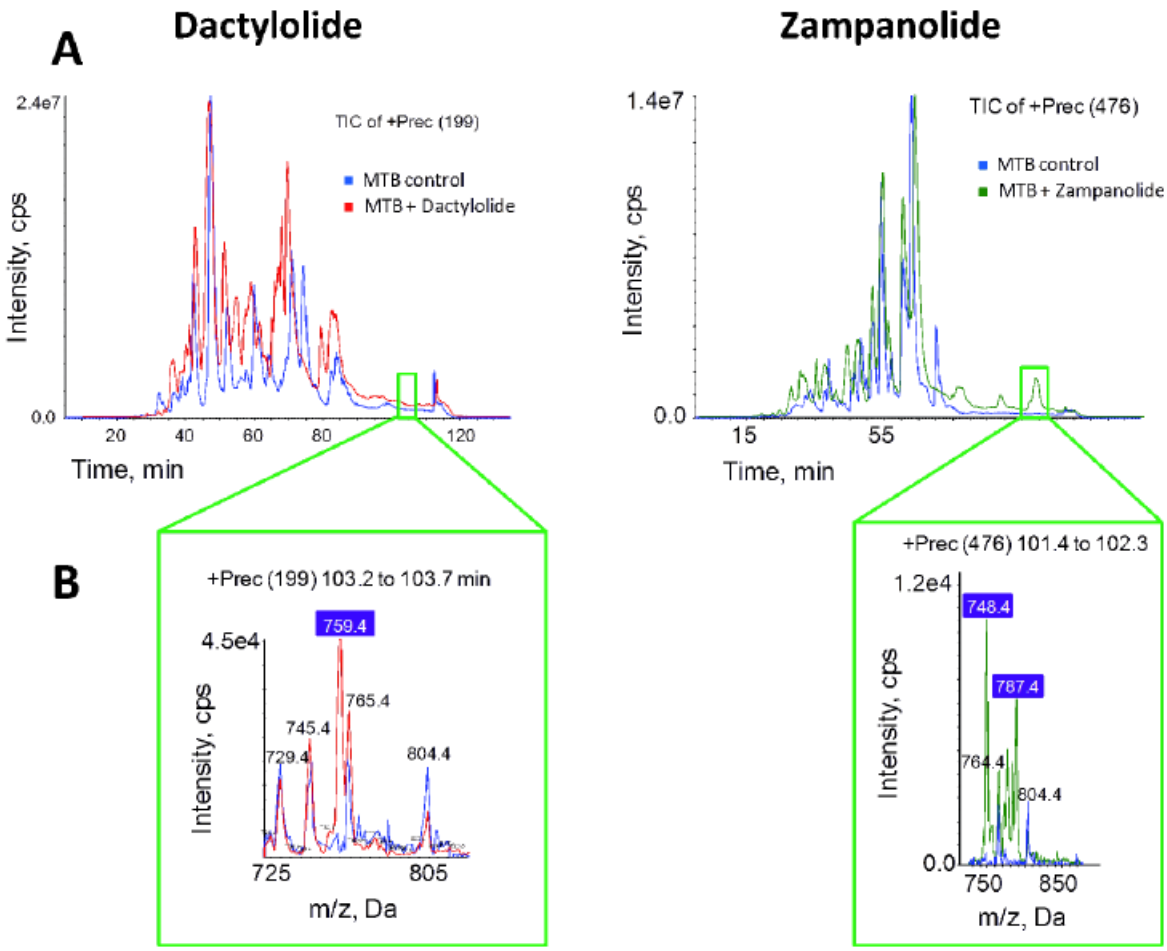
These approaches make use of shotgun and targeted proteomic analyses. By using high-throughput tandem mass spectrometry methods for global proteome profiling, we are increasing the analysis sensitivity to enable us to reliably quantify and detect low-abundance proteins in complex biological specimens, 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.*

Technical Units



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.* ***Best Poster Award.*** *4th Congress of the Spanish Proteomics Society "New Trends in Proteomics". 8-11 February, 2011. Segovia, Spain.*

SELECTED PUBLICATIONS

Goetz JG, Minguet S, Navarro-Lérida I, Lazcano JJ, Samaniego R, Calvo E, Tello M, Osteso-Ibáñez T, Pellinen T, Echarri A, Cerezo A, Klein-Szanto AJ, Garcia R, Keely PJ, Sánchez-Mateos P, Cukierman E, del Pozo MA. **Biomechanical remodeling of the microenvironment by stromal caveolin-1 favors tumor invasion and metastasis.** *Cell* (2011) 146: 148-63

Ramos-Mozo P, Madrigal-Matute J, Martínez-Pinna R, Blanco-Colio LM, López JA, Camafeita E, Meilhac O, Michel J-B, Aparicio C, Vega de Ceniga M, Egido J, Martin-Ventura JL. **Proteomic analysis of polymorphonuclear neutrophils identifies catalase as a novel biomarker of abdominal aortic aneurysm: potential implication of oxidative stress in abdominal aortic aneurysm progression.** *Arterioscler Thromb Vasc Biol* (2011) 31: 3011-19

Martinez-Pinna R, Ramos-Mozo P, Blanco-Colio LM, López JA, Calvo E, Camafeita E, Lindholt JS, Meilhac O, Michel JB, Vega de Ceniga M, Egido J, Martin-Ventura JL. **Identification of peroxiredoxin-1 as a novel biomarker of abdominal aortic aneurysm evolution.** *Arterioscler Thromb Vasc Biol* (2011) 31: 935-43

Calvo E, Camafeita E, Fernández-Gutiérrez B, López JA. **Applying selected reaction monitoring to targeted proteomics.** *Expert Rev Proteomics* (2011) 8: 165-73

Garrido T, Dominguez F, López JA, Camafeita E, Quiñonero A, Martinez-Conejero JA, Pellicer A, Conesa A, Simón C. **Modeling human endometrial decidualization from the interaction between proteome and secretome.** *J Clin Endocrinol Metabol* (2011) 96: 706-16

Technical Units

Bioinformatics

Head of Unit: *Fernando Martínez*

Technicians: *Fátima Sánchez Cabo*
Carlos Torroja

RESEARCH INTEREST

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.

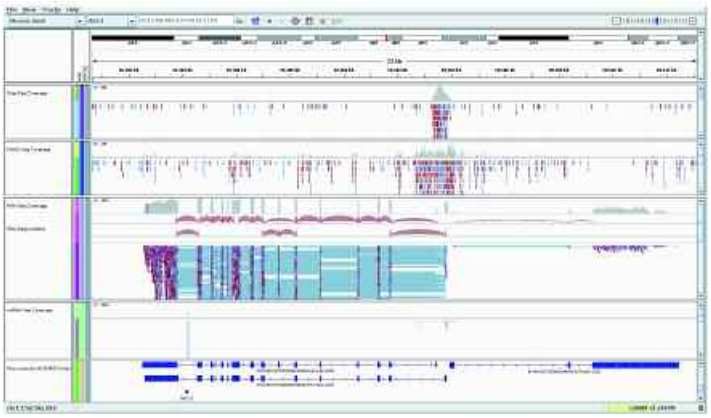
The Unit focuses on the analysis and interpretation of high-throughput biological data from CNIC research projects, with special emphasis on data generated by the Genomics, Proteomics and Cellomics Units. One of the Unit's main aims is to develop and implement analysis pipelines using state-of-the-art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

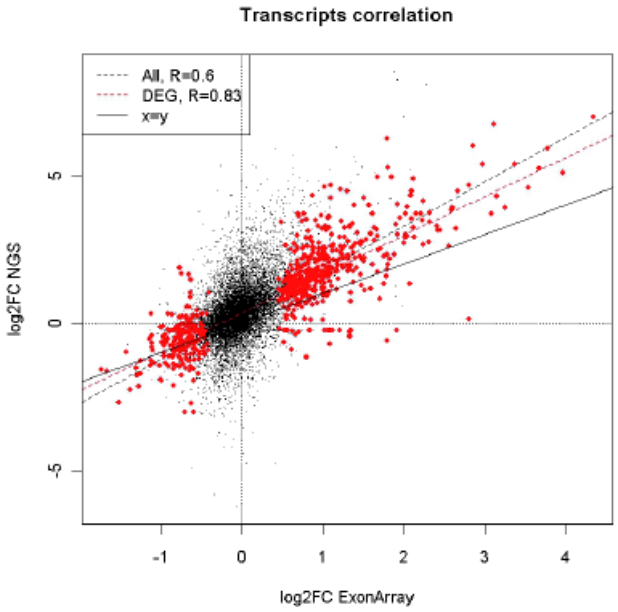
Services

- 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.

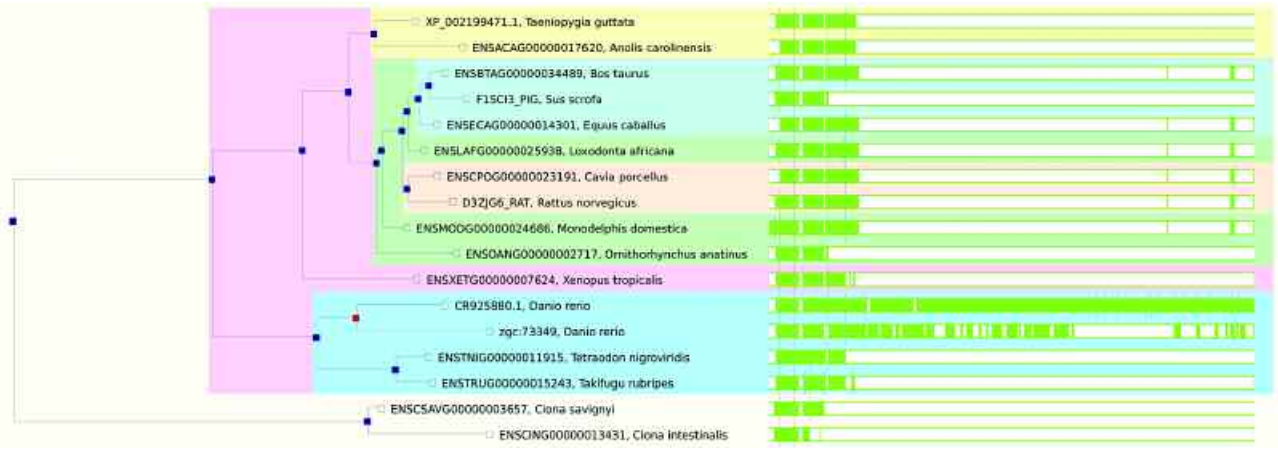


IGV Browser displaying information from different NGS experiments (Chip-Seq, FAIRE-Seq, RNA-Seq and miRNA-Seq) analyzed at the CNIC.

Technical Units



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.



Ensembl Component GeneTree and Compara-Multi-Gene Tree for zgc:73349.

SELECTED PUBLICATIONS

Dunkler D, Sánchez-Cabo F, Heinze G. Statistical analysis principles for Omics data. Methods Mol Biol. 2011;719:113-31.

Sánchez-Ramos C, Tierrez A, Fabregat-Andrés O, Wild B, Sánchez-Cabo F, Arduini A, Dopazo A, Monsalve M. PGC-1α regulates translocated in liposarcoma activity: role in oxidative stress gene expression. Antioxid Redox Signal. 2011 Jul 15;15(2):325-37. Epub 2011 May 21.

Sanchez-Cabo F, Rainer J, Dopazo A, Trajanoski Z and Hackl H. **Insights into global mechanisms and disease by gene expression profiling.** Methods Mol Biol (2011) 719: 269-98

Bayona-Bafaluy MP, Sanchez-Cabo F, Fernandez-Silva P, Perez-Martos A and Enriquez JA. **A genome-wide shRNA screen for new OxPhos related genes.** Mitochondrion (2011) 11: 467-75

Mittelbrunn M, Gutierrez-Vazquez C, Villarroya-Beltri C, Gonzalez S, Sanchez-Cabo F, Gonzalez MA, Bernad A and Sanchez-Madrid F. **Unidirectional transfer of microRNA-loaded exosomes from T cells to antigen-presenting cells.** Nat Commun (2011) 2: 282

Technical Units

Cellomics

Head of Unit:	*María Montoya*
Support Scientists:	*José Manuel Ligos* *Hind Azegrouz*
Predoctoral Researchers:	*Begoña Diez* *Carmen Muñoz*
Technicians:	*Raquel Nieto* *Mariano Vitón* *Mª Montserrat Arroyo* *Ignacio Cotillo*

RESEARCH INTEREST

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

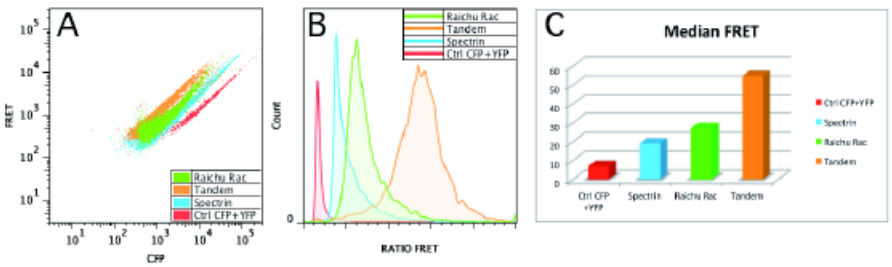
- Three latest generation digital analytical flow cytometers: two Becton Dickinson FACSCanto II machines and one Cyan (Beckman Coulter).
- Two high speed flow sorters: A MoFlo (Beckman Coulter) and a custom made FACSAria II (Becton Dickinson).
- Cytometry software (Modfit and FlowJo).

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).

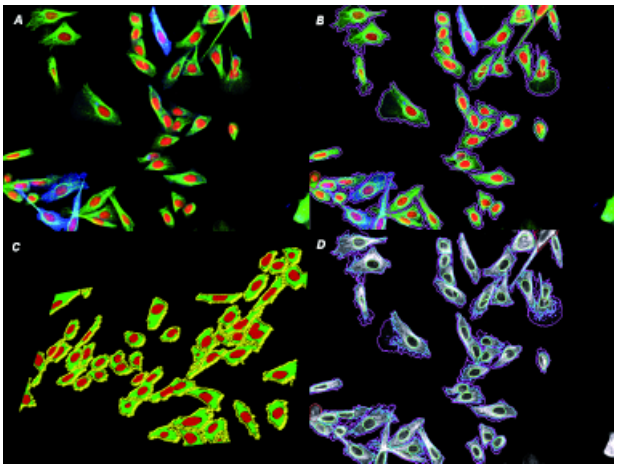
The Image Analysis Unit (IAU) was established in 2011 with the aim of providing solutions for image-based scientific applications by developing computational techiniques that extract information from biological images. The IAU is equipped with dedicated image analysis software packages (Acapella, Definiens,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



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.*

Technical Units



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.*

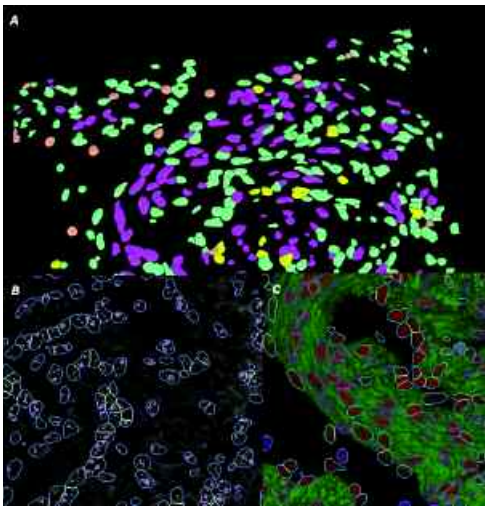


Image analysis development for quantitative cardio-imaging. *(A) 3D rendering of nucleus segmentation and classification; nuclear color coding indicates BrdU and cardiomyocyte staining classification. (B) Telomere segmentation based on local maxima seed detection and 3D constrained dilation. (C) 2D representation 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

Escobar B, de Cárcer G, Fernández-Miranda G, Cascón A, Bravo-Cordero JJ, Montoya MC, Robledo M, Cañamero M, Malumbres M. **Brick1 is an essential regulator of actin cytoskeleton required for embryonic development and cell transformation.** *Cancer Res* (2010) 70: 9349-59

Magariños M, Aburto MR, Sánchez-Calderón H, Muñoz-Agudo C, Rapp UR, Varela-Nieto I. **RAF kinase activity regulates neuroepithelial cell proliferation and neuronal progenitor cell differentiation during early inner ear development.** *PLoS One* (2010) 5: e14435

Daudén E, Pedraz J, Pérez-Gala S, Muñoz C, Vitón M, Onate MJ, García-Díez A. **Effect of mycophenolate mofetil therapy on the phenotypic profile of peripheral blood leukocyte populations in psoriatic patients.** *Eur J Dermatol* (2010) 20: 233-4

Trucco E, Azegrouz H, Dhillon B. **Modeling the tortuosity of retinal vessels: does calibre play a role?** *IEEE Trans Biomed Eng* (2010) 57: 2239-47

Technical Units

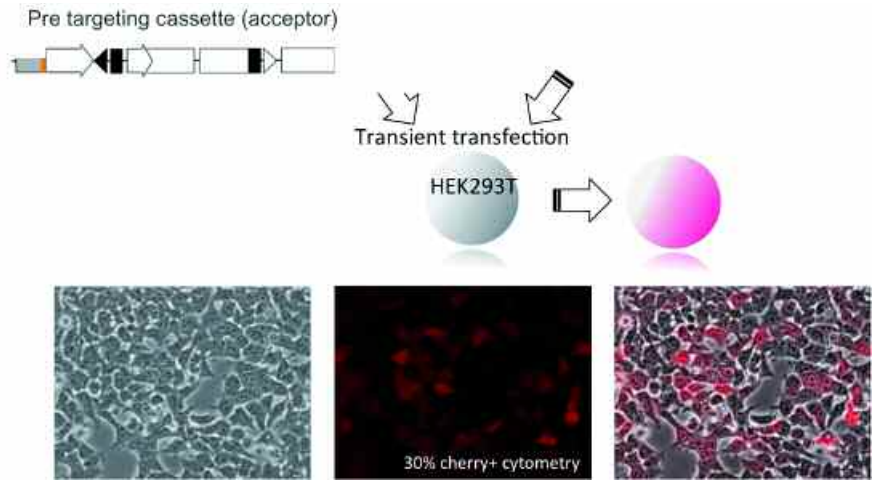
Viral Vectors

Head of Technical Service: *Juan Carlos Ramírez*
Support Scientist: *Raúl Torres*
Technician: *Aída García*

RESEARCH INTEREST

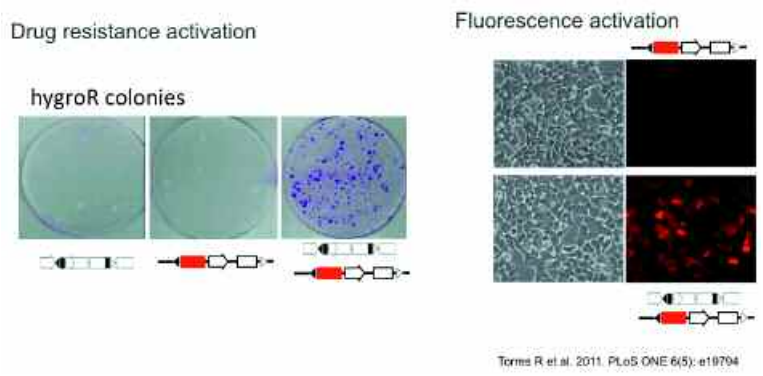
The Viral Vector facility is dedicated to providing high-quality recombinant viruses (lentivirus, adenovirus and adeno-associated virus) for preclinical studies at the CNIC and beyond. The facility's capabilities expanded in 2011 to complete a collection of more than 80 HIV-derived lentiviral backbones 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).

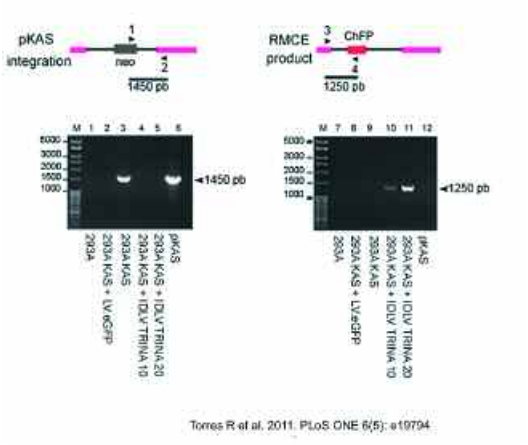


High-efficiency RMCE driven by non-integrative lentivirus.

Technical Units



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

Lonardo E, Hermann PC, Mueller MT, Huber S, Balic A, Miranda-Lorenzo I, Zagorac S, Alcala S, Rodriguez-Arabaolaza I, Ramirez JC, Torres R, Garcia E, Hidalgo M, Cebrián DA, Heuchel R, Löhr M, Berger F, Bartenstein P, Aicher A, Heeschen C. **Nodal/Activin signaling drives self-renewal and tumorigenicity of pancreatic cancer stem cells and provides a target for combined drug therapy.** *Cell Stem Cell* (2011) 9: 433-46

Torres R, García A, Ramirez JC. **Non-integrative lentivirus drives high-frequency cre-mediated cassette exchange in human cells.** P*LoS ONE* (2011) 6 (5): e19794

Alonso-Ferrero ME, Valeri A, Yañez R, Navarro S, Garin MI, Ramirez JC, Bueren JA, Segovia JC. **Immunoresponse against the transgene limits hematopoietic engraftment of mice transplanted in utero with virally transduced fetal liver.** *Gene Therapy* (2011) 18: 469-78

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

7

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

Arduini A, Serviddio G, Escobar J, Tormos AM, Bellanti F, Vina J, Monsalve M and Sastre J.
Mitochondrial biogenesis fails in secondary biliary cirrhosis in rats leading to mitochondrial DNA depletion and deletions.
Am J Physiol Gastrointest Liver Physiol (2011) 301: G119-27
IF: 3.522

Bayona-Bafaluy MP, Sanchez-Cabo F, Fernandez-Silva P, Perez-Martos A and Enriquez JA.
A genome-wide shRNA screen for new OxPhos related genes.
Mitochondrion (2011) 11: 467-75
IF: 3.238

Canon S, Fernandez-Tresguerres B and Manzanares M.
Pluripotency and lineages in the mammalian blastocyst: An evolutionary view.
Cell Cycle (2011) 10: 1731-38
IF: 4.999

Casanova JC, Uribe V, Badia-Careaga C, Giovinazzo G, Torres M and Sanz-Ezquerro JJ.
Apical ectodermal ridge morphogenesis in limb development is controlled by Arid3b-mediated regulation of cell movements.
Development (2011) 138: 1195-205
IF: 6.898

Chow A, Lucas D, Hidalgo A, Mendez-Ferrer S, Hashimoto D, Scheiermann C, Battista M, Leboeuf M, Prophete C, van Rooijen N, Tanaka M, Merad M and Frenette PS.
Bone marrow CD169+ macrophages promote the retention of hematopoietic stem and progenitor cells in the mesenchymal stem cell niche.
J Exp Med (2011) 208: 261-71
IF: 14.776

Crippa S, Cassano M, Messina G, Galli D, Galvez BG, Curk T, Altomare C, Ronzoni F, Toelen J, Gijsbers R, Debyser Z, Janssens S, Zupan B, Zaza A, Cossu G and Sampaolesi M.
miR669a and miR669q prevent skeletal muscle differentiation in postnatal cardiac progenitors.
J Cell Biol (2011) 193: 1197-212
IF: 9.921

Del Monte G, Casanova JC, Guadix JA, Macgrogan D, Burch JB, Perez-Pomares JM and de la Pompa JL.
Differential Notch Signaling in the Epicardium Is Required for Cardiac Inflow Development and Coronary Vessel Morphogenesis.
Circ Res (2011) 108: 824-36
IF: 9.504

Fabregat-Andres O, Tierrez A, Mata M, Estornell-Erill J, Ridocci-Soriano F and Monsalve M.
Induction of PGC-1alpha Expression Can Be Detected in Blood Samples of Patients with ST-Segment Elevation Acute Myocardial Infarction.
PLoS One (2011) 6: e26913
IF: 4.411

Felkin LE, Narita T, Germack R, Shintani Y, Takahashi K, Sarathchandra P, López-Olañeta MM, Gómez-Salinero JM, Suzuki K, Barton PJ, Rosenthal N and Lara-Pezzi E.
Calcineurin Splicing Variant Calcineurin AB1 Improves Cardiac Function After Myocardial Infarction Without Inducing Hypertrophy.
Circulation (2011) 123: 2838-47
IF: 14.429

Fernandez-Vizarra E, Enriquez JA, Perez-Martos A, Montoya J and Fernandez-Silva P.
Tissue-specific differences in mitochondrial activity and biogenesis.
Mitochondrion (2011) 11: 207-13
IF: 3.238

Ferraro F, Lymperi S, Mendez-Ferrer S, Saez B, Spencer JA, Yeap BY, Masselli E, Graiani G, Prezioso L, Rizzini EL, Mangoni M, Rizzoli V, Sykes SM, Lin CP, Frenette PS, Quaini F and Scadden DT.
Diabetes impairs hematopoietic stem cell mobilization by altering niche function.
Sci Transl Med (2011) 3: 104ra101
IF: 3.292

Appendix

Publications 2011

Fuster JJ, Gonzalez-Navarro H, Vinue A, Molina P, Andres-Manzano MJ, Nakayama KI, Nakayama K, Diez-Juan A, Bernad A, Rodriguez C, Martinez-Gonzalez J and Andres V.
Deficient p27 Phosphorylation at Serine 10 Increases Macrophage Foam Cell Formation and Aggravates Atherosclerosis Through a Proliferation-Independent Mechanism.
Arterioscler Thromb Vasc Biol (2011) 31: 2455-63
IF: 7.215

Galajda Z, Balla J, Szentmiklosi AJ, Biro T, Czifra G, Dobrosi N, Cseppento A, Patonay L, Roszer T, Balla G, Popescu LM, Lekli I and Tosaki A.
Histamine and H(1) -Histamine Receptors Faster Venous Circulation.
J Cell Mol Med (2011) 15: 2614-2623
IF: 4.608

Gonzalez-Rosa JM, Martin V, Peralta M, Torres M and Mercader N.
Extensive scar formation and regression during heart regeneration after cryoinjury in zebrafish.
Development (2011) 138: 1663-74
IF: 6.898

Hondares E, Rosell M, Diaz-Delfin J, Olmos Y, Monsalve M, Iglesias R, Villarroya F and Giralt M.
PPARalpha induces PGC-1alpha gene expression and contributes to the thermogenic activation of brown fat; involvement of PRDM16.
J Biol Chem (2011) 286: 43112-22
IF: 5.328

Josowitz R, Carvajal-Vergara X, Lemischka IR and Gelb BD.
Induced pluripotent stem cell-derived cardiomyocytes as models for genetic cardiovascular disorders.
Curr Opin Cardiol (2011) 26: 223-9
IF: 2.736

Marcon L, Arques CG, Torres MS and Sharpe J.
A computational clonal analysis of the developing mouse limb bud.
PLoS Comput Biol (2011) 7: e1001071
IF: 5.515

Martin D, Pantoja C, Fernandez Minan A, Valdes-Quezada C, Molto E, Matesanz F, Bogdanovic O, de la Calle-Mustienes E, Dominguez O, Taher L, Furlan-Magaril M, Alcina A, Canon S, Fedetz M, Blasco MA, Pereira PS, Ovcharenko I, Recillas-Targa F, Montoliu L, Manzanares M, Guigo R, Serrano M, Casares F and Gomez-Skarmeta JL.
Genome-wide CTCF distribution in vertebrates defines equivalent sites that aid the identification of disease-associated genes.
Nat Struct Mol Biol (2011) 18: 708-14
IF: 13.685

Monsalve M and Olmos Y.
The Complex biology of FOXO.
Curr Drug Targets (2011) 12: 1322-50
IF: 3.061

Moreno-Loshuertos R, Ferrin G, Acin-Perez R, Gallardo ME, Viscomi C, Perez-Martos A, Zeviani M, Fernandez-Silva P and Enriquez JA.
Evolution Meets Disease: Penetrance and Functional Epistasis of Mitochondrial tRNA Mutations.
PLoS Genet (2011) 7: e1001379
IF: 9.543

Nus M, Macgrogan D, Martinez-Poveda B, Benito Y, Casanova JC, Fernandez-Aviles F, Bermejo J and de la Pompa JL.
Diet-Induced Aortic Valve Disease in Mice Haploinsufficient for the Notch Pathway Effector RBPJK/CSL.
Arterioscler Thromb Vasc Biol (2011) 31: 1580-8
IF: 7.215

Palazon A, Teijeira A, Martinez-Forero I, Hervas-Stubbs S, Roncal C, Penuelas I, Dubrot J, Morales-Kastresana A, Perez-Gracia JL, Ochoa MC, Ochoa-Callejero L, Martinez A, Luque A, Dinchuk J, Rouzaut A, Jure-Kunkel M and Melero I.
Agonist anti-CD137 mAb act on tumor endothelial cells to enhance recruitment of activated T lymphocytes.
Cancer Res (2011) 71: 801-11
IF: 8.234

Perales-Clemente E, Fernandez-Silva P, Acin-Perez R, Perez-Martos A and Enriquez JA.
Allotopic expression of mitochondrial-encoded genes in mammals: achieved goal, undemonstrated mechanism or impossible task?
Nucleic Acids Res (2011) 39: 225-34
IF: 7.836

Perez-Pomares JM and de la Pompa JL.
Signaling during epicardium and coronary vessel development.
Circ Res (2011) 109: 1429-42
IF: 9.504

Pernaute B, Spruce T, Rodriguez TA and Manzanares M.
MiRNA-mediated regulation of cell signaling and homeostasis in the early mouse embryo.
Cell Cycle (2011) 10: 584-91
IF: 4.999

Appendix

Publications 2011

Prieur X, Mok CY, Velagapudi VR, Nunez V, Fuentes L, Montaner D, Ishikawa K, Camacho A, Barbarroja N, O'Rahilly S, Sethi J, Dopazo J, Oresic M, Ricote M and Vidal-Puig A.
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

Rosello-Diez A and 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
IF: 2.864

Roszer T, Menendez-Gutierrez MP, Lefterova MI, Alameda D, Nunez V, Lazar MA, Fischer T and Ricote M.
Autoimmune Kidney Disease and Impaired Engulfment of Apoptotic Cells in Mice with Macrophage Peroxisome Proliferator-Activated Receptor {gamma} or Retinoid X Receptor {alpha} Deficiency.
J Immunol (2011) 186: 621-31
IF: 5.745

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

Sanchez-Ramos C, Tierrez A, Fabregat-Andres O, Wild B, Sanchez-Cabo F, Arduini A, Dopazo A and Monsalve M.
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, Panse KD, Lara-Pezzi E, Lee SJ, Sam F and Walsh K.
Cardiac myocyte-specific ablation of follistatin-like 3 attenuates stress-induced myocardial hypertrophy.
J Biol Chem (2011) 286: 9840-8
IF: 5.328

Tello D, Balsa E, Acosta-Iborra B, Fuertes-Yebra E, Elorza A, Ordonez A, Corral-Escariz M, Soro I, Lopez-Bernardo E, Perales-Clemente E, Martinez-Ruiz A, Enriquez JA, Aragones J, Cadenas S and Landazuri MO.
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

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Appendix

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IF: 6.270

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IF: 3.569

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IF: 4.411

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IF: 3.305

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PLoS One (2011) 6: e21127
IF: 4.411

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PLoS One (2011) 6: e29278
IF: 4.411

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PLoS One (2011) 6: e21825
IF: 4.411

Tome M, Lopez-Romero P, Albo C, Sepulveda JC, Fernandez-Gutierrez B, Dopazo A, Bernad A and Gonzalez MA.
miR-335 orchestrates cell proliferation, migration and differentiation in human mesenchymal stem cells.
Cell Death Differ (2011) 18: 985-95
IF: 9.050

Torres R, Garcia A, Paya M and Ramirez JC.
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PLoS One (2011) 6: e19794
IF: 4.411

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Electrophoresis (2011) 32: 2640-7
IF: 3.569

	TOTAL (*)	CUMULATIVE IF	AVERAGE IF
TOTAL	151	1095.475	7.255
CARDIOVASCULAR DEVELOPMENT AND REPAIR	35	277.877	7.939
EPIDEMIOLOGY, ATHEROTHROMBOSIS AND IMAGING	80	575.437	7.193
VASCULAR BIOLOGY AND INFLAMMATION	17	150.909	8.877
TECHNICAL UNITS	28	194.105	6.932

(*) 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

Name	Secondary School	Autonomic Region
Andarcio Calvo, Elisabeth	I.E.S. Jesús Marín	Andalucía
Ibáñez Ajarilla, Antonio Felipe	I.E.S. sol de Portocarrero	Andalucía
Mañas Sevillano, Estebana	Sagrada Familia	Andalucía
Marín Carballo, Clara	I.E.S. José de Mora	Andalucía
Pulido Moraina, Lucía	I.E.S. Fernando III	Andalucía
Ramírez Sánchez, Sara	I.E.S Jerez y Caballero	Andalucía
Torrecillas Moreno, Beatriz	C.E.S. Almicerán	Andalucía
Valladares Millán, Ismael	I.E.S. Salmedina	Andalucía

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

Name	Degree	University
Alonso Caballero, Álvaro	Biology	Autónoma de Madrid
Alonso Merina, Elvira	Biotechnology	Universidad de León
Bascón Romero, Francisco	Biotechnology	Universidad Pablo Olavide
Bilal Álvarez, Usama	Medicine	Universidad de Oviedo
Cano Linares, María Isabel	Biology	Universidad de Jaén

Appendix

Training Programs and Courses

Fellowships in 2011

Name	Degree	University
Farrás Llobet, Alba	Medicine	Autónoma de Barcelona
Fernández Barrera, Jaime	Biochemistry	Autónoma de Madrid
García García, Andrés	Biology	Universidad de Málaga
García Rubio, Julio César	Medicine	Universidad de Oviedo
Gasparyan, Ani	Pharmacy	Universidad de Valencia
González Ramos, Silvia	Biotechnology	Politécnica de Valencia
Hernández Delso, Inmaculada	Pharmacy	Universidad de Navarra
Jaso Tamame, Angel Luis	Biology	Universidad de Sevilla
Jiménez Santaella, Laura	Chemistry	Universidad de Málaga
Lechuga Vieco, Ana Victoria	Biotechnology	Universidad Pablo Olavide
Leiva Cepas, Fernando	Medicine/ Biochemistry	Universidad de Córdoba
Lorenzo Martín, Cristina	Biology	Autónoma de Madrid
Marcin Pileki, Bartosz	Biotechnology	Jagiellonian University / Poland
Martínez Molledo, María	Biology	Universidad de Salamanca
Moreno Vicente, Roberto	Biology	Autónoma de Madrid
Motas Mallol, Sandra	Biotechnology	Autónoma de Barcelona
Muras, Aleksandra	Biotechnology	Warsaw University
Ogonek, Justina Anna	Biotechnology	Warsaw University
Orbea Sopeña, Pablo	Pharmacy	Universidad de Navarra
Osuna Gálvez, Alberto	Chemistry	Universidad de Granada
Piñera Guirao, Antonio	Medicine	Universidad de Murcia
Rodríguez Bovolenta, Elena	Biology	Complutense de Madrid
Ramírez Martínez, Andrés	Biochemistry/ Biotechnology	Universidad Rovira i Virgili
Rodríguez Arboli, Eduardo	Medicine/ Biochemistry	Universidad de Sevilla
Ruíz González, Lorena	Pharmacy	Universidad de Alcalá
Sanz García, Adriana	Biochemistry	Autónoma de Madrid
Siguero Álvarez, Marcos	Biology	Autónoma de Madrid
Suarez Velázquez, Andrés	Medicine	Universidad de Oviedo
Villahoz Lázaro, Silvia	Biotechnology	Universidad de León

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 Valentín 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

Name	Degree - University	Master	Master - University
Cano Linares, María Isabel	Universidad de Jaén	Molecular Biomedicine	Autónoma de Madrid
Castellano Castillo, Daniel	Universidad de Granada	Molecular and Cellular Biology	Autónoma de Madrid
Díaz Díaz, Covadonga	Universidad de Oviedo	Molecular Biomedicine	Autónoma de Madrid
Fernández Barrera, Jaime	Complutense de Madrid	Research in Immunology	Complutense de Madrid
Sánchez Iranzo, Hector	Universidad de Valencia	Molecular Biomedicine	Autónoma de Madrid

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

Name	Title of thesis	University	CNIC Department	Thesis Advisor (s)
Camafeíta Fernández, Emilio	Application of genomic and proteomic techniques to search for molecular alterations in mesenchimal stem cells and chondrocytes in patients with knee osteoarthitis	Autónoma de Madrid	Proteomics Unit	López del Olmo, Juan Antonio / Fernández Gutiérrez, Benjamín
Del Monte Nieto, Gonzalo	Expression and functional analysis of notch signalling during cardiac development with special focus on the epicardium and coronary vasculature	Autónoma de Madrid	Cardiovascular Developmental Biology	De la Pompa Mínguez, José Luis
Escudero González, Beatriz	Analysis of DNA Polymerase MU deficiency in non homologous end joining. Aging and tumor development implications	Autónoma de Madrid	Regenerative Cardiology	Bernad Miana, Antonio / Samper, Enrique

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Training Programs and Courses

Name	Title of thesis	University	CNIC Department	Thesis Advisor (s)
Fernández-Tresguerres, Beatriz	Evolution of embryonic pluripotency	Autónoma de Madrid	Cardiovascular Developmental Biology	Manzanares, Miguel
García Andrés, Clara	Isolation and characterisation of new genes implicated in the development of vertebrate extremities	Autónoma de Madrid	Cardiovascular Developmental Biology	Torres Sánchez, Miguel
Grande García, Araceli	Role of caveolin-1 in cell polarization and directional migration	Autónoma de Madrid	Vascular Biology and Inflammation	Del Pozo Barriuso, Miguel Ángel
Marcos Contreras, Óscar Armando	Development of new streptavidin-plaminogen activator conjugates that bind erythrocytes in vivo: direct formation of thromboprophylactic agents in the circulation	Universidad de Alcalá	Epidemiology, Atherothrombosis and Imaging	Murciano, Juan Carlos
Muriel López, Olivia	Identification of regulators of the loss of cell adhesion induced caveolin1 internalization. Regulation by filamin A	Autónoma de Madrid	Vascular Biology and Inflammation	Del Pozo, Miguel Ángel

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

Name	Funding Agency	University	CNIC Department	Joined previously through another Training Program
Aix Sacido, Esther	FPU (Spanish Ministry of Education and Science)	Autónoma de Madrid	Cardiovascular Development and Repair	BMM9 2009-2010 / MASTER Program 2009
Alameda Serrano, Daniel	FPI (Spanish Ministry of Education and Science)	Autónoma de Madrid	Regenerative Cardiology	CICERONE Program 2007
Bednareck, Dorota	FPI (Spanish Ministry of Education and Science)	Autónoma de Madrid	Cardiovascular Development and Repair	No
Bergacín Liberman, Gabriel	CNIC contract	Autónoma de Madrid	Cardiovascular Development and Repair	No
Blanco Menéndez, Noelia	CNIC contract	Autónoma de Madrid	Vascular Biology and Inflammation	No
Casanova Acebes, María	FPI (Spanish Ministry of Education and Science)	Autónoma de Madrid	Epidemiology, Atherothrombosis and Imaging	No
Cedenilla Horcajuelo, Marta	FPU (Spanish Ministry of Education and Science)	Autónoma de Madrid	Cardiovascular Development and Repair	CICERONE Program 2008 /Cardiovascular Postgraduate Program 2008-2009 / MASTER Program 2008

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Training Programs and Courses

Name	Funding Agency	University	CNIC Department	Joined previously through another Training Program
D'Amato, Gaetano	Marie Curie Initial Training Network (NotchIT)	Autónoma de Madrid	Cardiovascular Development and Repair	No
Díez Cabezas, Begoña	FPU (Spanish Ministry of Education and Science)	Autónoma de Madrid	Vascular Biology and Inflammation	No
Escolano Artigas, Amelia	FPI (Spanish Ministry of Education and Science)	Autónoma de Madrid	Vascular Biology and Inflammation	No
García-Prieto Cuesta, Jaime	CNIC contract	Autónoma de Madrid	Epidemiology, Atherothrombosis and Imaging	No
Gómez Velázquez, Melisa	CNIC contract	Autónoma de Madrid	Cardiovascular Development and Repair	MASTER Program 2009/Cardiovascular Postgraduate Program 2009-2010
González Rosa, Juan Manuel	FPU (Spanish Ministry of Education and Science)	Autónoma de Madrid	Cardiovascular Development and Repair	CICERONE Program 2008 / MASTER Program 2008
Guadamilla Mora, Marta C.	FPI (Spanish Ministry of Education and Science)	Complutense de Madrid	Vascular Biology and Inflammation	No
Gutiérrez Vázquez, Cristina	CAM (Madrid Autonomic Region)	Autónoma de Madrid	Vascular Biology and Inflammation	CICERONE Program 2007 /Cardiovascular Postgraduate Program 2008-2009
Hamczyk, Magda	FPI (Spanish Ministry of Education and Science)	Autónoma de Madrid	Epidemiology, Atherothrombosis and Imaging	CICERONE Program 2010
Hernández de Riquer, Mª Victoria	FPI (Spanish Ministry of Education and Science)	Autónoma de Madrid	Vascular Biology and Inflammation	No
Herrera Merchan, Antonio	Human Frontier Science Foundation	Autónoma de Madrid	Cardiovascular Development and Repair	No
Izarra Pérez, Alberto	FPI (Spanish Ministry of Education and Science)	Autónoma de Madrid	Cardiovascular Development and Repair	No
Izquierdo Hernández, Helena	FPI (Spanish Ministry of Education and Science)	Autónoma de Madrid	Vascular Biology and Inflammation	CICERONE Program 2008 and 2009 / PRACTICAL Program 2009-10
Koziol, Agnieszka	FPU (Spanish Ministry of Education and Science)	Autónoma de Madrid	Vascular Biology and Inflammation	No

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Training Programs and Courses

Name	Funding Agency	University	CNIC Department	Joined previously through another Training Program
Latorre Pellicer, Ana	Diputación General de Aragón	Universidad de Zaragoza	Cardiovascular Development and Repair	No
Lavín Plaza, Begoña	FPI (Spanish Ministry of Education and Science)	Autónoma de Madrid	Epidemiology, Atherothrombosis and Imaging	No
López Fontal, Raquel	FIS (National Institute of Health Carlos III)	Autónoma de Madrid	Cardiovascular Development and Repair	No
Lozano Vidal, Noelia	FPU (Spanish Ministry of Education and Science)	Autónoma de Madrid	Vascular Biology and Inflammation	Cardiovascular Postgraduate Program 2009-2010 / MASTER Program 2009
Luna Zurita, Luis	CNIC contract	Autónoma de Madrid	Cardiovascular Development and Repair	No
Luxán García, Guillermo	FPI (Spanish Ministry of Education and Science)	Autónoma de Madrid	Cardiovascular Development and Repair	No
Marco Lázaro, Ricardo	CNIC contract	Universidad de Zaragoza	Cardiovascular Development and Repair	No
Martín Alonso, Mara	FPI (Spanish Ministry of Education and Science)	Autónoma de Madrid	Vascular Biology and Inflammation	CICERONE Program 2008 /Cardiovascular Postgraduate Program 2009-2010
Martín Pérez, Lara	FPI (Spanish Ministry of Education and Science)	Autónoma de Madrid	Cardiovascular Development and Repair	CICERONE Program 2008 /Cardiovascular Postgraduate Program 2009-2010
Mateos San Martín, Daniel	CAM (Madrid Autonomic Region)	Autónoma de Madrid	Cardiovascular Development and Repair	No
Matesanz Marín, Adela	FPI (Spanish Ministry of Education and Science)	Autónoma de Madrid	Vascular Biology and Inflammation	No
Méndez Barbero, Nerea	FPU (Spanish Ministry of Education and Science)	Autónoma de Madrid	Vascular Biology and Inflammation	Cardiovascular Postgraduate Program 2008-2009 / MASTER Program 2008
Molina Sánchez, Pedro	FPU (Spanish Ministry of Education and Science)	Universidad de Valencia	Epidemiology, Atherothrombosis and Imaging	No
Moreno Rodríguez, Vanessa	CAM (Madrid Autonomic Region)	Autónoma de Madrid	Vascular Biology and Inflammation	No

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Training Programs and Courses

Name	Funding Agency	University	CNIC Department	Joined previously through another Training Program
Munch, Juliane	Notch IT, Marie Curie	Autónoma de Madrid	Cardiovascular Development and Repair	Cardiovascular Postgraduate Program 2009-2010
Muñoz Agudo, Carmen	FPI (Spanish Ministry of Education and Science)	Autónoma de Madrid	Vascular Biology and Inflammation	No
Núñez Andrade, Norman	FPI (Spanish Ministry of Education and Science)	Autónoma de Madrid	Vascular Biology and Inflammation	No
Olmos Buchelt, Yolanda	SAF (Spanish Ministry of Science and Innovation)	Complutense de Madrid	Cardiovascular Development and Repair	No
Peralta López, Marina	CNIC contract	Autónoma de Madrid	Cardiovascular Development and Repair	CICERONE Program 2009 /PRACTICALS Program 2008-9 /Cardiovascular Postgraduate Program 2009-20010 / MASTER Program 2009
Rayón Alonso, Teresa	FPU (Spanish Ministry of Education and Science)	Autónoma de Madrid	Cardiovascular Development and Repair	No
Rodríguez, Juan Camilo Estrada	Red TERCEL (La Paz Hospital)	Autónoma de Madrid	Cardiovascular Development and Repair	No
Roselló Díez, Alberto	FPI (Spanish Ministry of Education and Science)	Autónoma de Madrid	Cardiovascular Development and Repair	No
Sala Valdés, Mónica	FIS (National Institute of Health Carlos III)	Autónoma de Madrid	Vascular Biology and Inflammation	No
Sánchez Ramos, Cristina	FPI (Spanish Ministry of Education and Science)	Autónoma de Madrid	Cardiovascular Development and Repair	No
Silvestre Roig, Carlos	Mariano Losantos del Campo Foundation	Universidad de Valencia	Epidemiology, Atherothrombosis and Imaging	No
Tarín Cerezo, Carlos A.	FPI (Spanish Ministry of Education and Science)	Universidad de Alcalá	Epidemiology, Atherothrombosis and Imaging	No
Tejera Puente, Emilio	FIS (National Institute of Health Carlos III)	Autónoma de Madrid	Vascular Biology and Inflammation	No
Tomé Pizarro, María	CNIC contract	Autónoma de Madrid	Cardiovascular Development and Repair	CICERONE Program 2008 /Cardiovascular Postgraduate Program 2008-2009

Appendix

Training Programs and Courses

Name	Funding Agency	University	CNIC Department	Joined previously through another Training Program
Travisano, Stanislao Igor	FPI (Spanish Ministry of Education and Science)	Universidad de Zaragoza	Cardiovascular Development and Repair	No
Uribe Sokolov, Verónica	FPU (Spanish Ministry of Education and Science)	Autónoma de Madrid	Cardiovascular Development and Repair	CICERONE Program 2007 and 2008 / MASTER Program 2008
Urso, Katia	FIS (National Institute of Health Carlos III)	Autónoma de Madrid	Vascular Biology and Inflammation	No
Valiente Alandí, Iñigo	FPU (Spanish Ministry of Education and Science)	Autónoma de Madrid	Cardiovascular Development and Repair	CICERONE Program 2008 /Cardiovascular Postgraduate Program 2008-2009 / MASTER Program 2008
Villa del Campo, Cristina	FPI (Spanish Ministry of Education and Science)	Autónoma de Madrid	Cardiovascular Development and Repair	CICERONE Program 2007-09 /Cardiovascular Postgraduate Program 2009-20010 / MASTER Program 2009
Wild, Brigitte	Spanish Ministry of Science and Innovation contract	Autónoma de Madrid	Cardiovascular Development and Repair	No

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

Name	Institution
Arias, Teresa	Centro de Investigación Aplicada, Navarra
García Lunar, Inés	Hospital Puerta de Hierro, Madrid
Mateo de Castro, Jesús	Centro Nacional de Investigaciones Cardiovasculares - Madrid
Pazos, Pablo	Hospital de Vigo

Appendix

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

Name	Hospital	CNIC Department
Fernández Jiménez, Rodrigo	Hospital Clínico San Carlos - Madrid	Epidemiology, Atherothrombosis and Imaging

CARDIOVASCULAR PATHOPHYSIOLOGY Course: "From symptoms to genes"

SOCIEDAD ESPAÑOLA DE Cardiología

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

7 **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 **Alvaro 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 Díez-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

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**

20-23 **European Meeting on Mitochondrial Pathology**

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

> 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

22 **Jesús Ruíz-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

> 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

Appendix

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

Award: Extraordinary PhD Thesis prize from the Universidad Autónoma de Madrid
Awarded to: **Alberto Roselló**

Award: Extraordinary PhD Thesis prize from the Universidad Autónoma de Madrid
Awarded to: **Luis Luna**

Award: rize for the best publication in basic research. VIII National Research Prize, Fundación Mutua Madrileña
Awarded to: **José Luis de la Pompa**

Award: Fellowship to participate in the 2012 Keystone Meeting on Cardiovascular Development, Taos, USA
Awarded to: **Verónica Uribe**

Award: Poster presentation prize at the ESC Meeting 2011, Praga, Czech Republic
Awarded to: **Verónica Uribe**

Award: Poster presentation prize at the ESC Meeting 2011, Praga, Czech Republic
Awarded to: **Guillermo de Luxán**

Award: Oral presentation prize at the ESC Meeting 2011, Praga, Czech Republic
Awarded to: **Nadia Mercader**

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 Argentinean 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
Awarded to: **Vicente Andrés's Group. Authors: C.Silvestre, P.Fernández, Ó.Pello, R.Viana, C.Indolfi, C.Rodríguez, R.Rodríguez-Calvo, P.Martín-Fuentes, M.Solanas-Barca, F.Civeira, G.Bauriedel, R.Hutter, V.Fuster, B.Ibáñez, FJ.Chaves, J.Martínez-González, V.Andrés**

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**

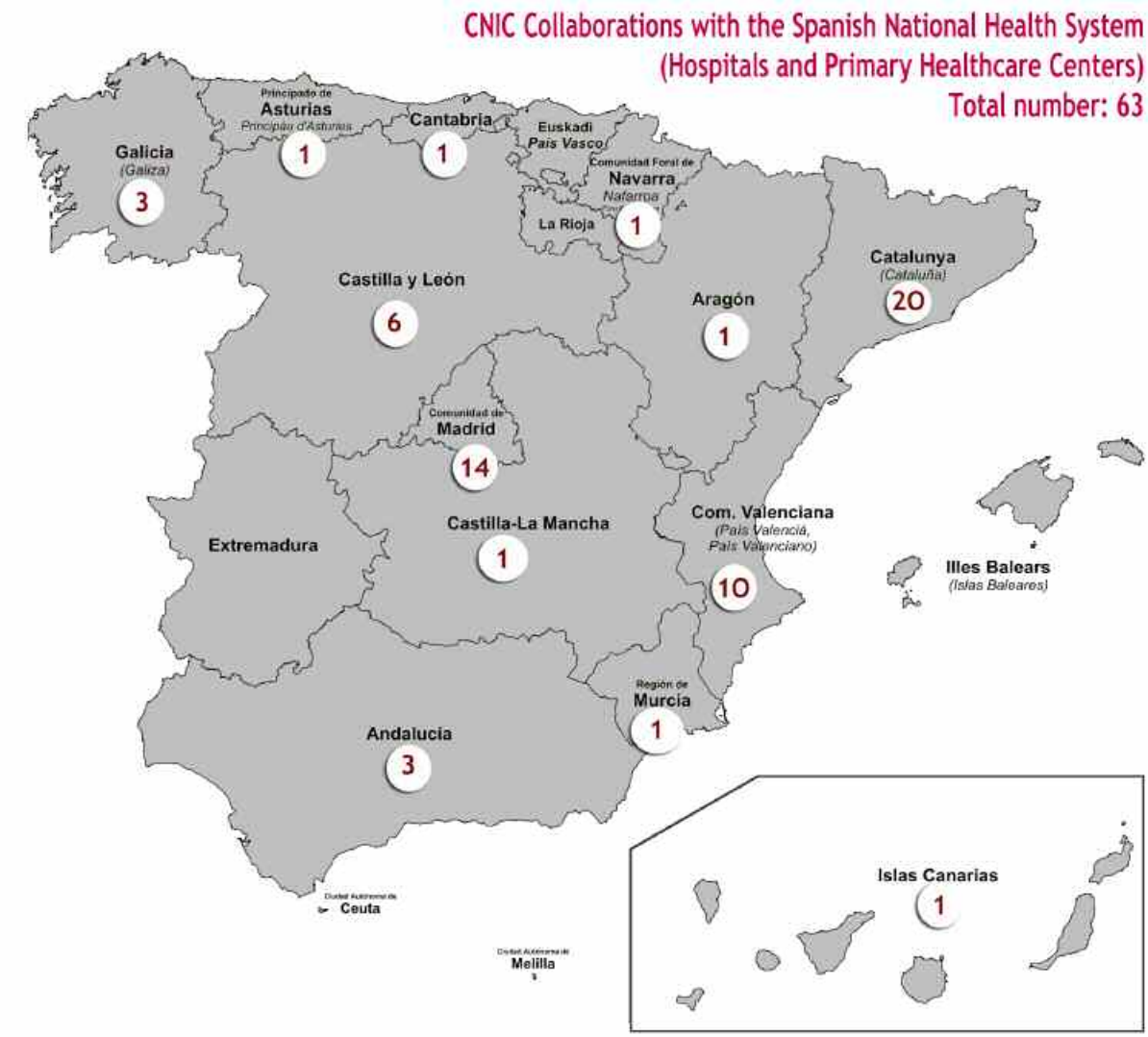
Award: Panamerican Nutrition and Food Research Award from Grupo Bimbo
Awarded to: **José Mª Ordovás**

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**

Appendix

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

GOBIERNO DE ESPAÑA
MINISTERIO DE ECONOMÍA Y COMPETITIVIDAD
Instituto de Salud Carlos III

Private Funding

Fundación procnic
acciona
BBVA
fundación abertis
endesa
FUNDACIÓN MUTUAMADRILEÑA
FUNDACIÓN BOTÍN
FUNDACIÓN ARAGÓN
gasNatural fenosa
INDITEX
"la Caixa"
PRISA
Fundación REPSOL
Telefónica

Appendix

Funding

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 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.
- Mechanisms of MTOC guidance and Genetic Transfer at the Immune Synapse: novel modes of Immuno-modulation (GENTRIS) – Dr. Francisco Sánchez-Madrid.
- 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):

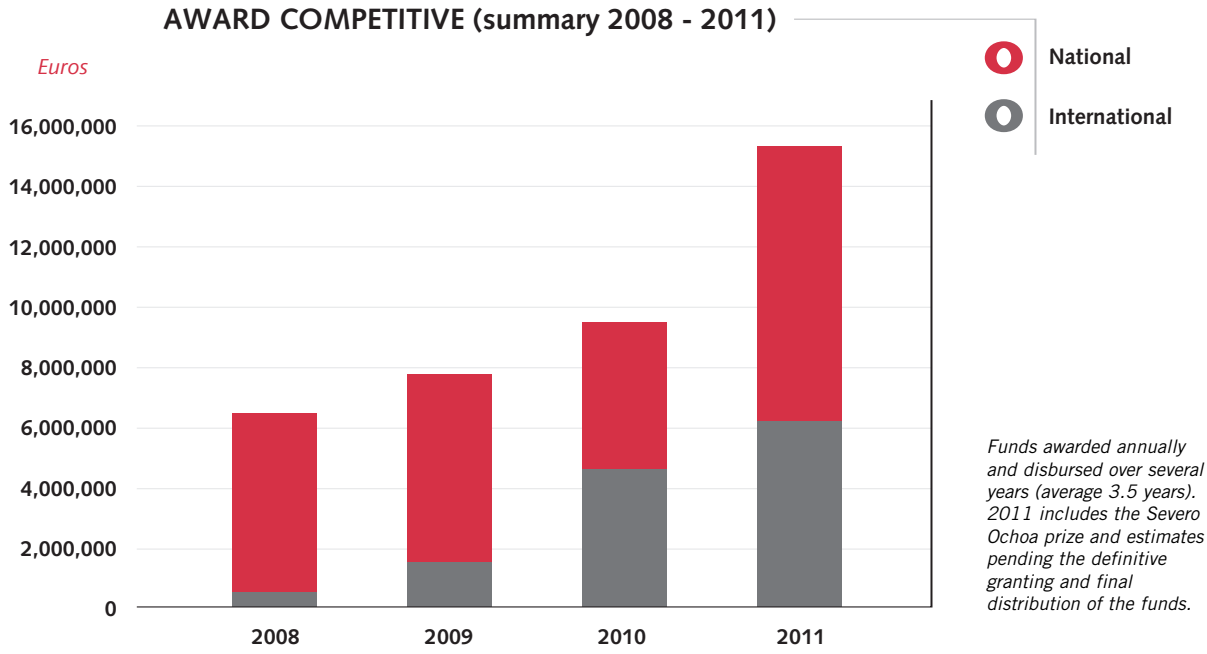
- From 2008 to 2011 the CNIC won about 150 grants (projects, grants and other subsidies) on a competitive basis.

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.

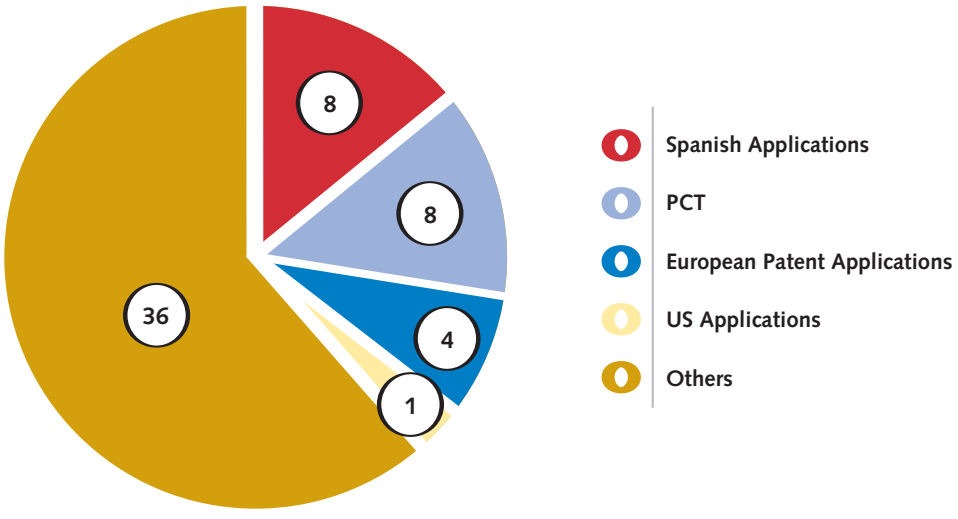


Funds awarded annually and disbursed over several years (average 3.5 years). 2011 includes the Severo Ochoa prize and estimates pending the definitive granting and final distribution of the funds.

The 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.



Appendix

Funding

Encouraging a culture of innovation

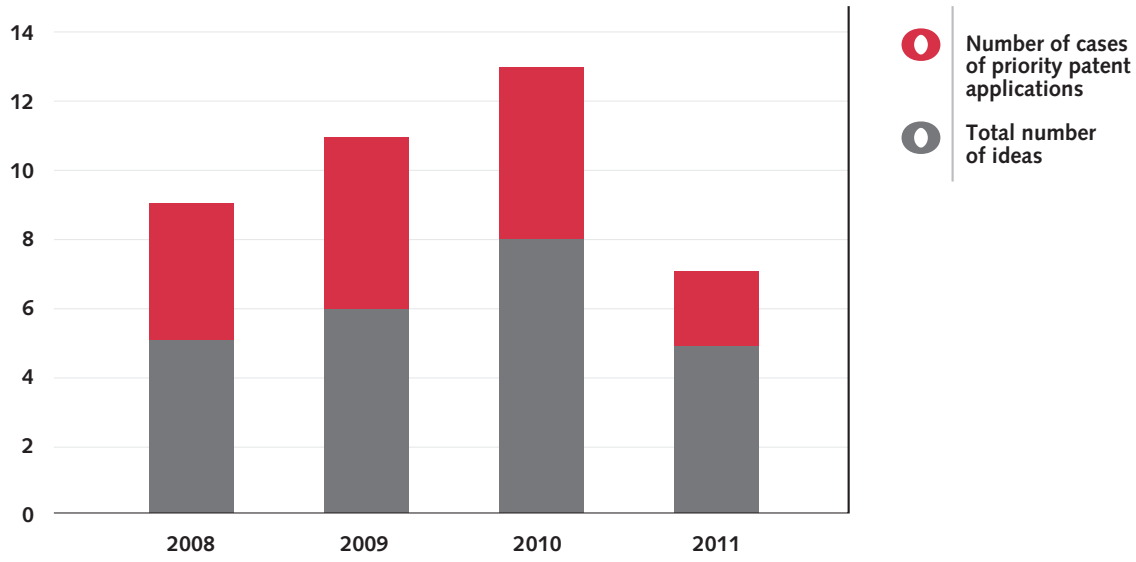
- Our researchers are increasingly involved in generating inventions, protected by the Center. In recent years interest has grown in cooperation with business and technological development.
- To increase the number of researchers participating in these initiatives, a strategy is being designed for communication 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.

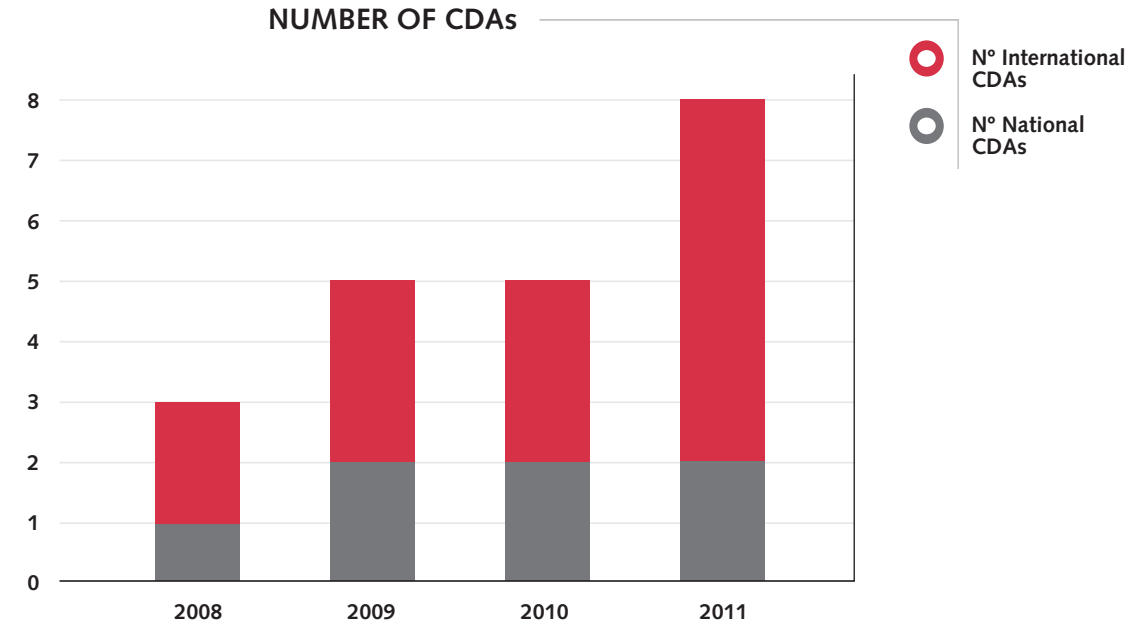
Proactive management: essential for working with third parties

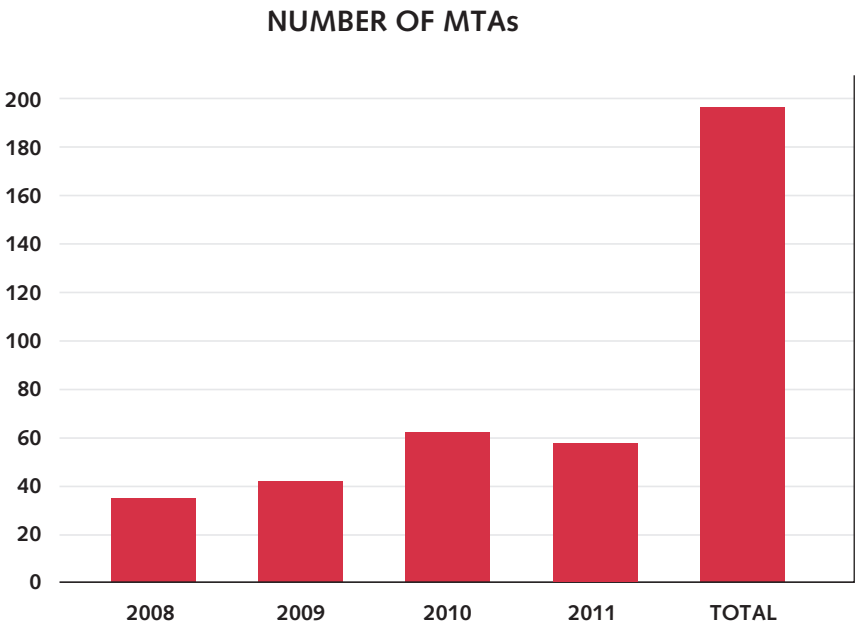
- The CNIC offers its researchers active and continued monitoring in their relations with all players in the science and technology sectors.
- The protection of results with patents is managed in collaboration with industrial property agencies specializing in biomedicine.
- The CNIC uses standard models 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



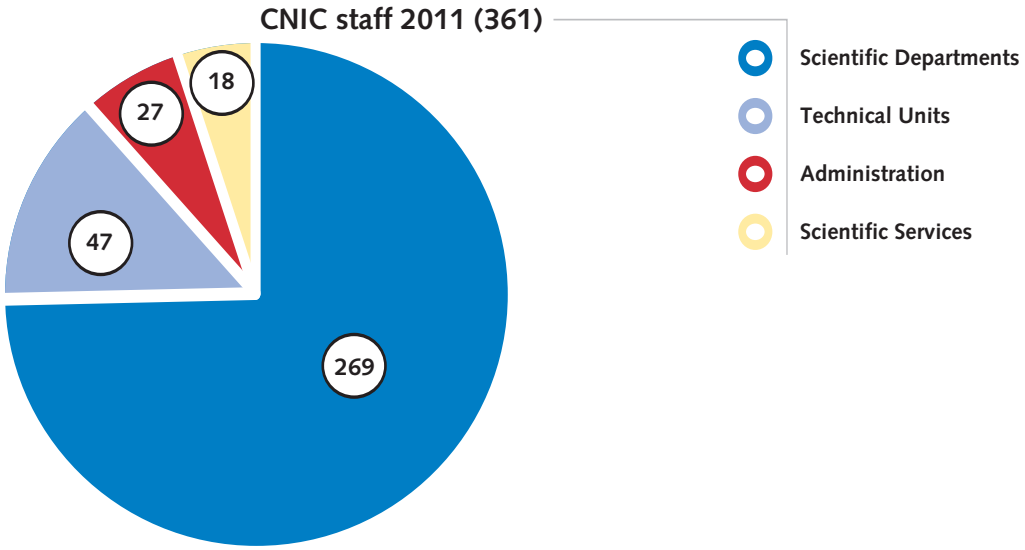


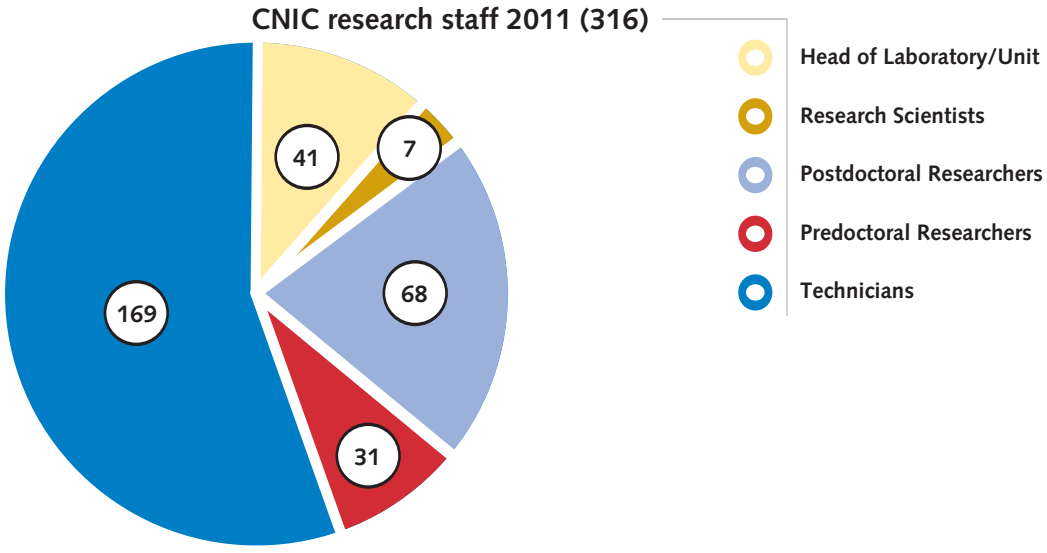
Innovation: a priority for the CNIC

- 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

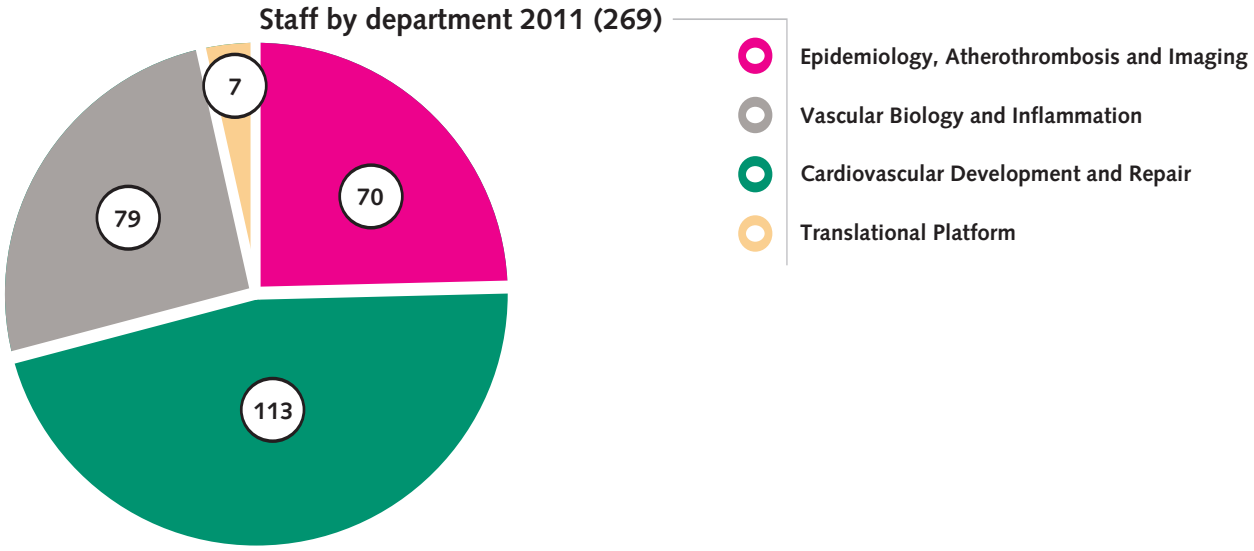
Staff Figures

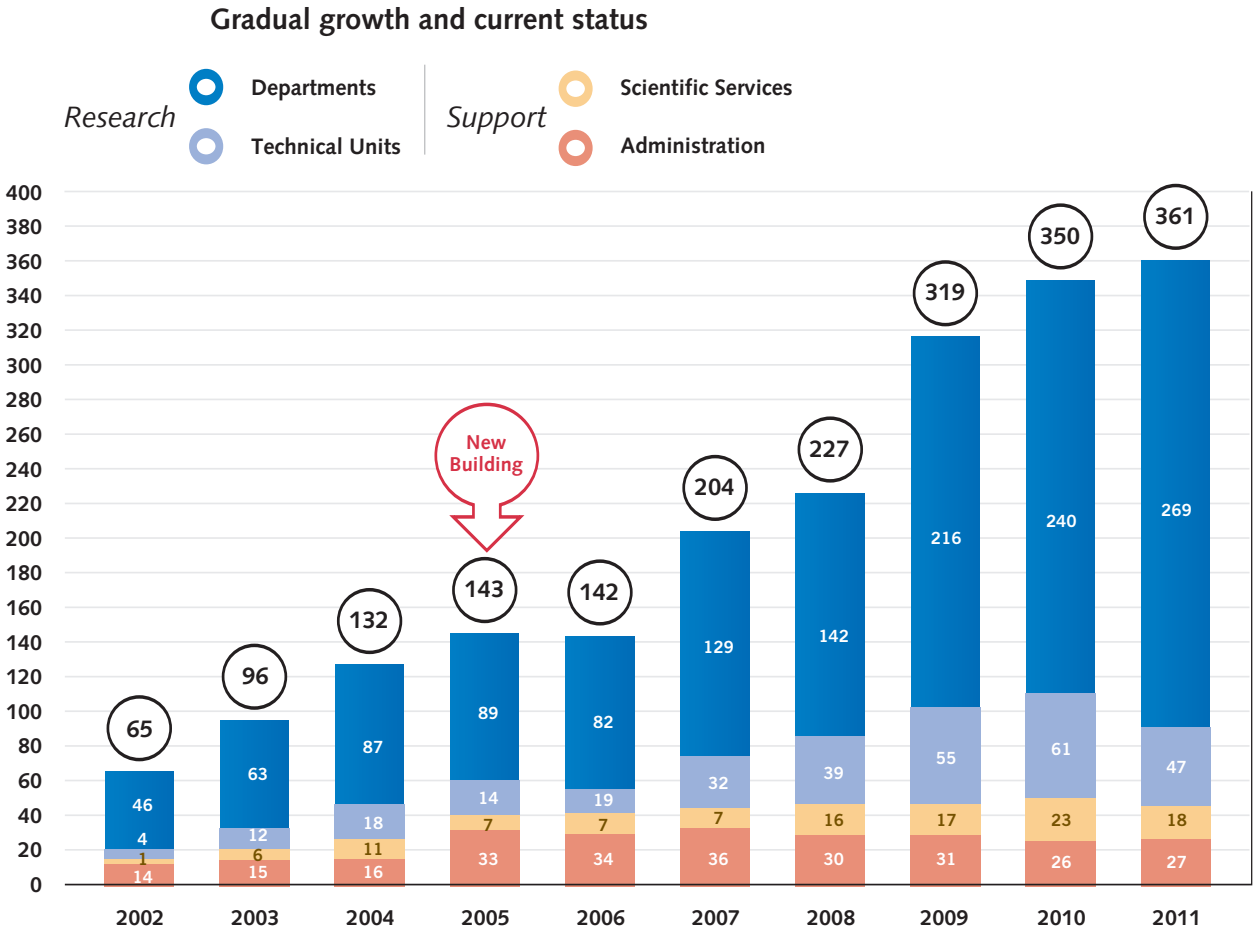




Appendix

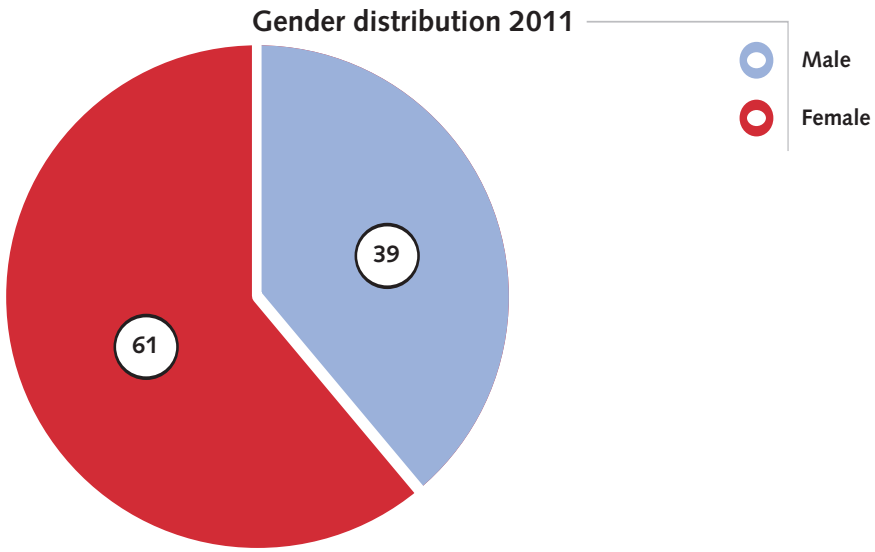
Staff Figures

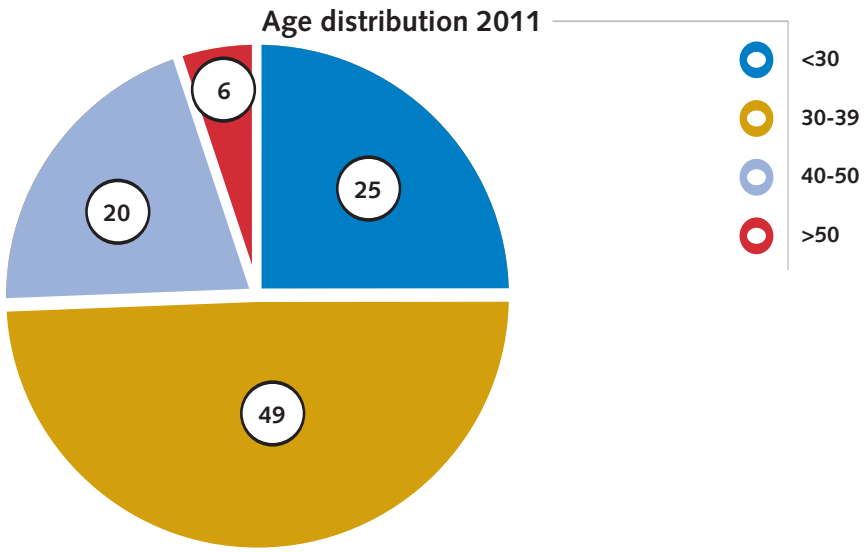




Appendix

Staff Figures





Fundaciónprocnic

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