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Cardiovasculares
Carlos III

SCIENTIFIC REPORT 2010

SCIENTIFIC REPORT

2010

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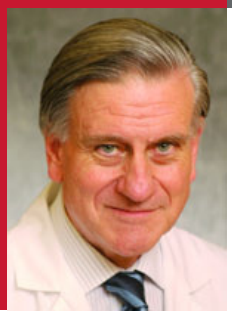

Valentín Fuster. *General Director*

The CNIC exists to generate new knowledge about cardiovascular diseases and to work to translate that knowledge into improved health outcomes for patients. Making this bold vision a reality requires an exquisitely coordinated effort involving not just our teams of basic and clinical researchers, but also a comprehensive support network that includes specialists with dedicated expertise in maintaining and adapting the Center's infrastructure, securing finance, generating and perfecting specialized reagents, providing tailored training programs, pioneering the development of new technologies, and a host of other strategic and scientific activities.

Much of the work of the early phase of the CNIC project was concerned with putting these diverse elements in place, but now, with staffing levels approaching two-thirds of full capacity, and after several years in which this network has underpinned strong and continued growth in the Center's scientific productivity, it is clear that it is no longer appropriate to talk of a new institution, and that we are now entering a period of consolidation and maturement.

This is abundantly evident in the CNIC's performance across the range of its activities last year. The quality of the Center's publications improved significantly, reaching an average impact factor of 8. Moreover, 30% of CNIC publications last year had an impact factor above 9, and 10% of publications were above 14. Last year also saw significant progress in relation to intellectual property, with five new patent applications filed, four others at an advanced stage, and one application filed in 2008 awarded. Currently efforts are underway to register and commercialize the CNIC Polypill.

The development of the Polypill is underpinned by two important clinical studies. One, studying the pharmacodynamic interaction with symvastatin in 340 patients, started in October. The other is the FOCUS project, a European Commission funded study that will test the effectiveness of the Polypill at improving treatment adherence in a cohort of 4000 patients across 80 centers and five countries. Recruitment to the FOCUS trial will commence in March 2011. Other ongoing translational projects are IMJOVEN, which examines the excess risk in young women with myocardial infarction, and the Aragon Workers Health Study (AWHS), a study of cardiovascular risk factors in workers at the General Motors car plant in Zaragoza. Recruitment for IMJOVEN is well advanced, and clinical examinations and laboratory tests have commenced for AWHS. New translational projects launched last year include METOCARD, an examination of the cardioprotective effects of metoprolol run through strategic alliances with major Spanish hospitals and emergency services.

Also started last year was the CNIC-Santander PESA study. This highly innovative trial applies modern non-invasive imaging techniques to the detection of subclinical vascular lesions in 4500 middle-aged workers. PESA, a longitudinal study run in partnership with Banco Santander and the Marcelino Botín Foundation, provides a unique opportunity to examine the association of subclinical parameters with the presence of genetic, epigenetic, metabolomic, proteomic and environmental factors. The study will also provide important information about the prevalence of unrecognized myocardial infarction. Participants are assessed with a range of imaging techniques, including magnetic resonance imaging and positron emission tomography, to determine their atherosclerotic burden and monitor its progression. The study will 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.

During 2010 the CNIC increased its international funding by more than 5.5 fold above that secured in 2009. More than half of this funding comes from the European Commission, and one of our proudest achievements last year was the award of €2.4 m European funding for our COFUND Programme. This flagship training program will ensure that the CNIC retains a competitive edge in the recruitment of the brightest young group leaders to the Center.

Our across-the-board commitment to training was much in evidence last year. We now have 72 predoctoral researchers signed up to our PhD training program, and more than 400 participants benefited from the diverse courses and training awards on offer at the CNIC. Many of these programs, for example the Cicerone program which provides practical laboratory training to final year undergraduates, attract participants from outside Spain, further cementing our international links and building a network of goodwill that we hope will carry forward into long-lasting collaborations.

Miguel Torres. *Associate Director*

Perhaps the most visible sign of the vibrant scientific activity at the Center last year was our busy seminar program. More than 30 invited speakers presented in the CNIC seminar series, sharing their international expertise in diverse areas of biomedicine and translational research. Numerous departmental seminars and technical workshops were also held, helping to place the CNIC on the map as an important venue for knowledge and training.

Despite the continuing economic difficulties in Spain and elsewhere, the CNIC continued to grow in 2010. This growth included the incorporation of two new groups, led by Simón Méndez Ferrer in the Department of Cardiovascular Developmental Biology and by Guadalupe Sabio in the Department of Vascular Biology and Inflammation. The continued development of the groups already established at the Center is also a top priority, and to ensure that this is based on scientific excellence CNIC scientists are periodically assessed by the CNIC Scientific Advisory Board. Nine group leaders were evaluated last year, and the program of evaluations will continue in 2011.

Growth in the Center's support infrastructure included the creation of the Bioinformatics Unit and the consolidation of Gene Targeting Unit as two linked services, Pluripotent Cell Technology and Viral Vectors. But undoubtedly the most significant development was the establishment of the Imaging Facility. This major installation houses state-of-the-art imaging equipment for animal studies at the main CNIC site, and further equipment for studies with patients is installed at the nearby Hospital Carlos III. The facility is devoted to the latest multimode imaging techniques for precise anatomic and functional cardiovascular studies, and covers echocardiography, computed tomography and magnetic resonance imaging, and will also be equipped with technology for magnetic particle imaging, a tomographic imaging technique developed by Philips that achieves resolutions finer than one millimeter. The Imaging Facility is fundamental to the CNIC project, and its launch marks a step-change in the CNIC's activities, as many of the Center's translational and basic research programs depend on the advanced imaging procedures it makes possible.

As any center such as ours develops, there is clearly a need to adapt the scientific organization to meet changing needs. An important change last year was the incorporation of the Epidemiology area within the Atherothrombosis Imaging department, now named Epidemiology, Atherothrombosis and Imaging. This reflects the need for ever closer coordination between translational research and advances in the laboratory: the imaging technologies developed in the department are providing detailed information about the early development of atherosclerotic plaques, and sophisticated epidemiologic analyses are needed to rapidly convert these findings into new diagnostic and prognostic tools.

The CNIC's stature within the international scientific community and wider society was cemented through numerous strategic alliances that were either initiated or consolidated last year. One of the key events was the formation of the new Strategic Advisory Board (SAB), which held its first meeting in November. The SAB is a 13-strong panel of internationally renowned scientists chaired by Professor Thomas Lüscher, director of cardiovascular research at the Institute of Physiology, University of Zurich. The SAB provides essential objective assessment of the Center's performance, to ensure that the CNIC's strategic direction maintains a clear, competitive focus. Our translational projects have allied us with several hospitals of the Spanish National Health System, and our partnerships with industry support our pioneering work with new imaging technologies (Philips) and our clinical trial of the CNIC Polypill (Ferrer). Alliances with academic institutions extend the support network for our training initiatives. At the national scale, we established partnerships with the university system for postgraduate training. And internationally, CNIC scientists and clinicians receive training through our partnerships with the Mount Sinai School of Medicine and Johns Hopkins University. Above all, we continue to enjoy the generous support of the ProCNIC Foundation, and our collaboration with one partner—Banco Santander—led to an agreement, signed April 7, with Banco Santander and the Marcelino Botín Foundation to provide financial and logistical support for the PESA CNIC-Santander study.

The achievements of the last year are testament to the continuing growth in the CNIC's scientific productivity, our increasing international profile and the ongoing diversification of our translational and training programs. With the main elements of the CNIC project now in place or due to commence activity soon, we feel confident that the Center will play an ever more important role at the forefront of research into the causes and treatment of cardiovascular disease.

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Basic Research Departments

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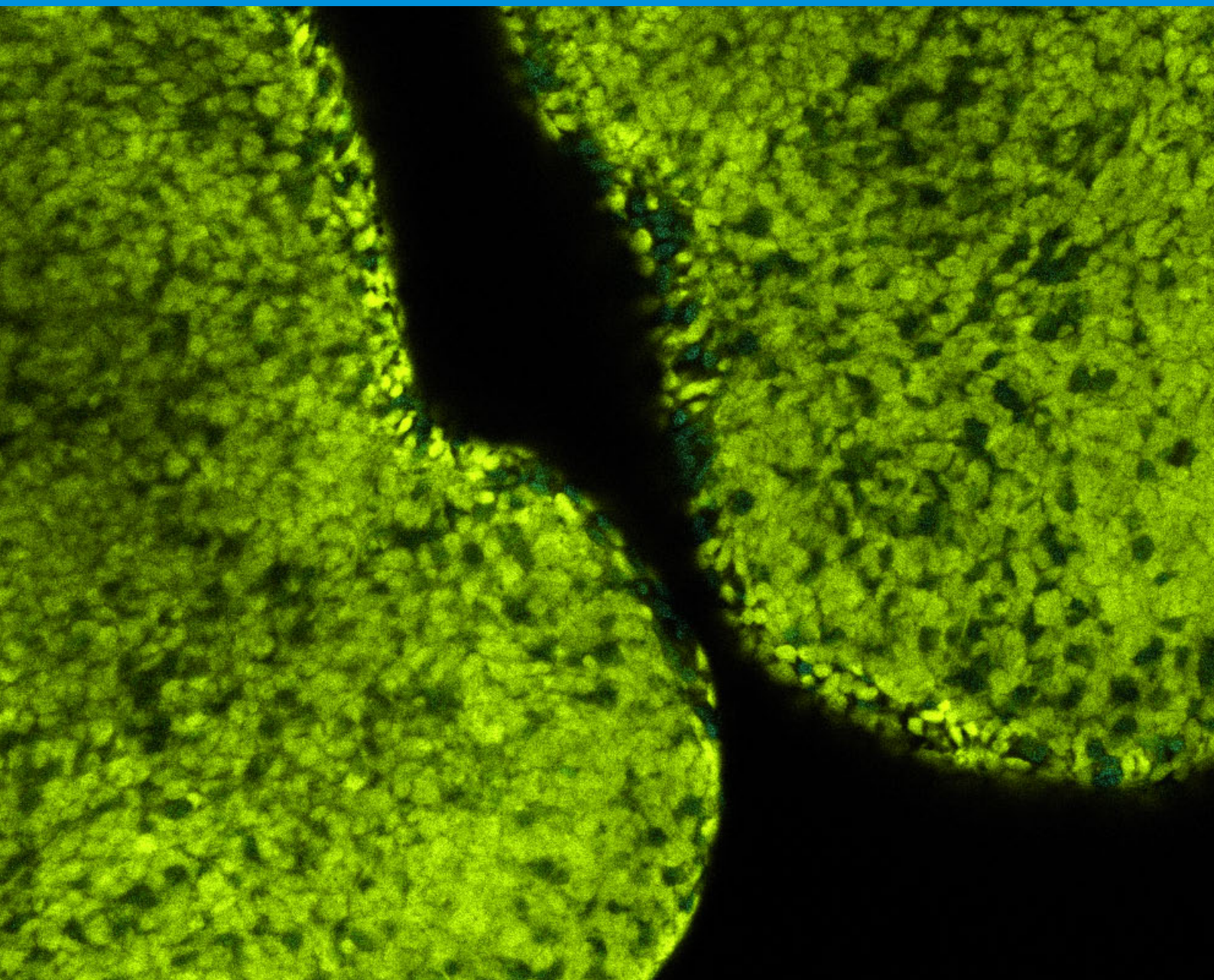
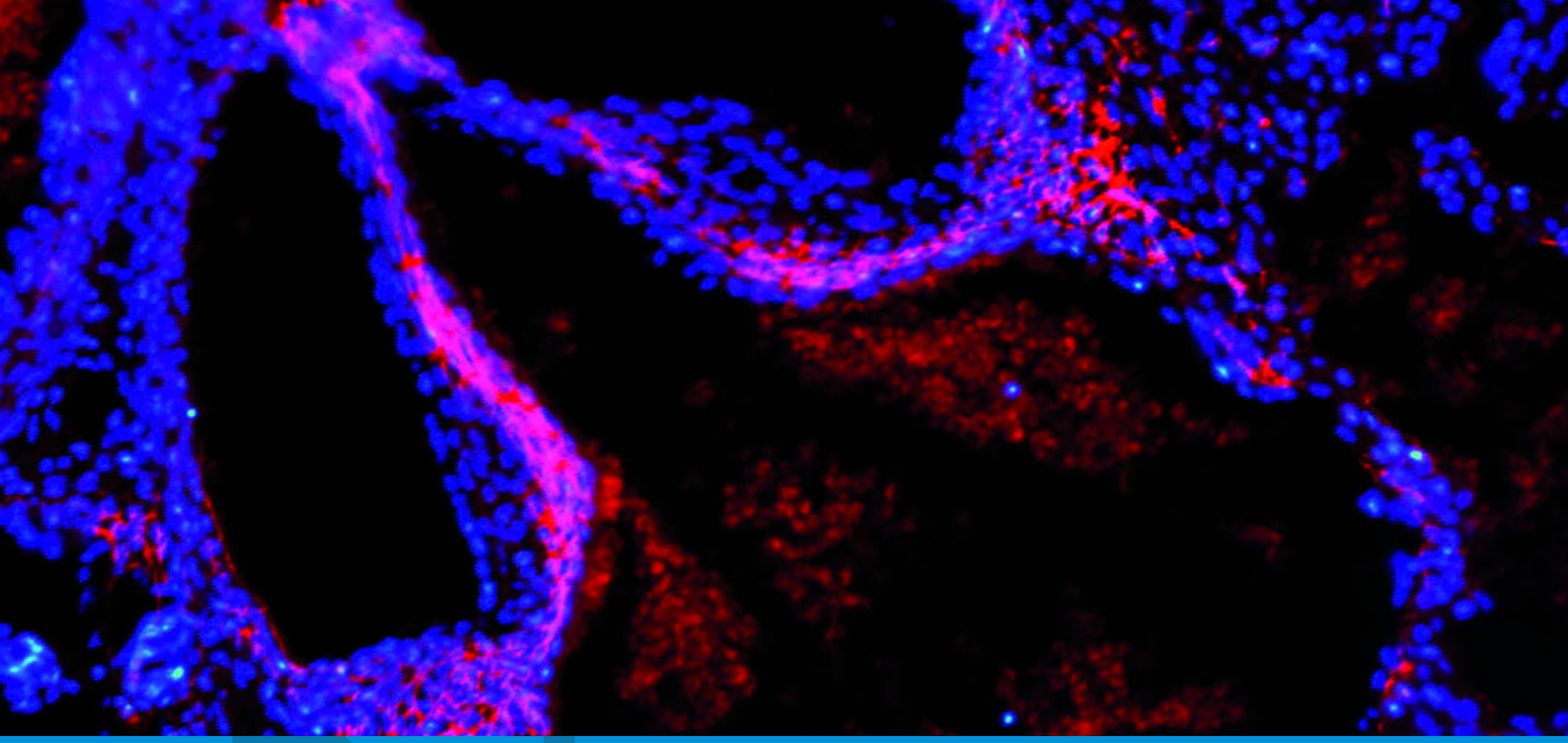
Cardiovascular Developmental Biology





Basic Research Departments

1 Cardiovascular Developmental Biology

Research in the CDB department is structured into three strategic areas:

- the early steps in the establishment of the embryonic lineages,
- the origin, differentiation and patterning of cardiovascular cell lineages,
- the integration of signaling pathways in cardiac development, homeostasis and disease.

These processes are studied in chick, mouse and zebrafish animal models through a variety of complementary experimental approaches including cell biology, imaging, global gene expression analysis and biochemistry.

DEPARTMENT DIRECTOR:	*Miguel Torres*
DEPARTMENT MANAGEMENT:	*Beatriz Ferreiro* *Ángel Ciprés*
LOGISTICS:	*Teresa Casaseca*
ADMINISTRATIVE SUPPORT:	*Sandra Cillero*

Basic Research Departments 1

Cardiovascular Developmental Biology

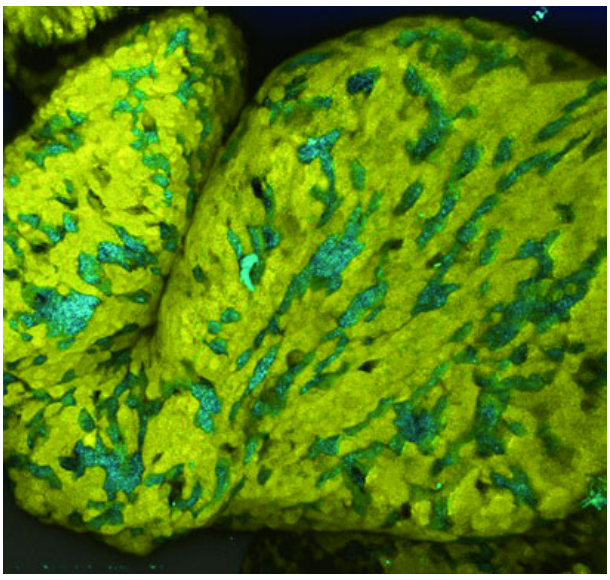
Genetic control of organ development and regeneration

Head of Laboratory:	*Miguel Torres*
Research Scientists:	*Nadia Mercader* *Laura Carramolino*
Postdoctoral Researchers:	*Cristina Clavería* *Ricardo Costa* *Mónica González-Lázaro* *Adrian Grimes* *Silvia Martín-Puig* *Laura Padrón-Barthe* *Catalina Ana Rosselló*
Predoctoral Researchers:	*Juan Manuel González-Rosa* *Daniel Mateos* *Marina Peralta* *Alberto Roselló* *Cristina Villa*
Technicians:	*Joana Fuentes* *Rocío Sierra* *Susana Temiño*
Visiting Scientist:	*Clara García-Andrés*

RESEARCH INTEREST

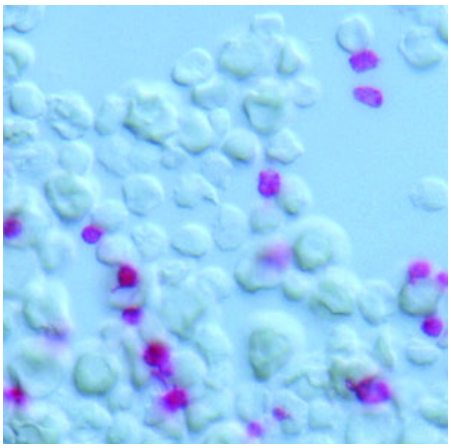
We aim to understand the cellular basis of cardiovascular development, homeostasis and regeneration. A main focus of the laboratory is the role of regionally-expressed transcription factors in cardiovascular development. In this area, we have generated gain- and loss-of-function mouse models of the homeodomain trancription factors Meis and Pbx. This work has revealed new roles for these factors in cardiovascular development, and also demonstrated the involvement of platelets in lymphangiogenesis, which suggests a general role of this blood lineage in vascular morphogenesis and remodeling that might be relevant to vascular disease. We have also developed new genetic mosaic mouse models that allow in vivo clonal analysis and random mosaic gene manipulation. These approaches are being used to investigate cell lineage relationships and topology during cardiovascular development and to explore the role of cell competition in the mouse embryo.

Our work on heart regeneration concentrates on the epicardium, which is the outermost layer of the vertebrate heart and plays an important role during cardiac development as a source of progenitor cells and signals controlling myocardial proliferation. Recently a role for the epicardium has been suggested during regeneration, but its exact function in this setting is still unkown. Using the zebrafish model we are analyzing the formation of the epicardium in vivo and studying the fate of epicardially derived cells and their role during cardiac regeneration.

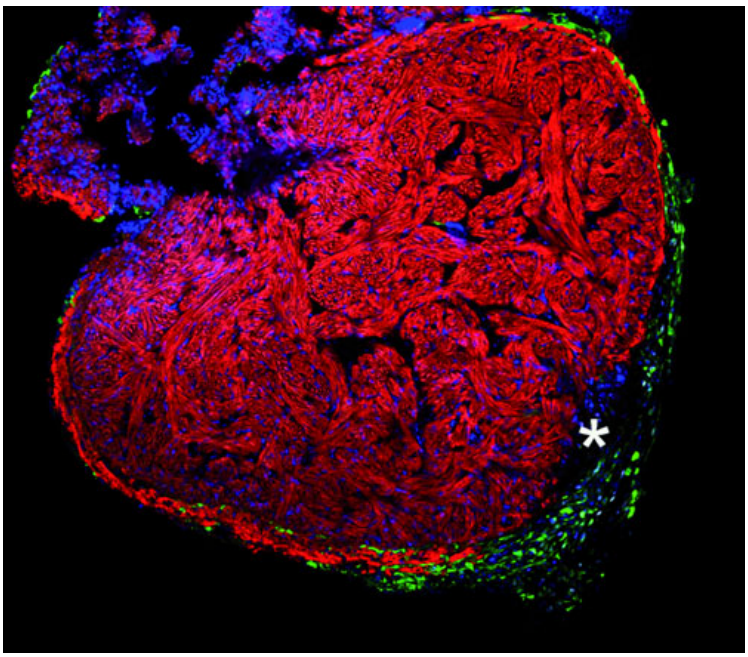


Amira 3D reconstruction compiled from a confocal Z-stack of a random mosaic E9.5 heart. The image shows a ventral view of the heart tube, encompassing the outflow tract and the right and left ventricles. The cell distribution in the mosaic reveals the regional tissue deformation occuring during heart morphogenesis

1 Cardiovascular Developmental Biology



Circulating platelets in the mid-gestation mouse embryo revealed by staining for CD41



Regenerating adult zebrafish heart, with a newly-formed epicardial layer (green cells) covering the injured area (asterisk)

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). PI: M.Torres**
- **Ministerio de Ciencia e Innovación (BFU2009-08331). PI: M.Torres**
- **Ministerio de Ciencia e Innovación (BFU2008-00212/BMC). PI: N.Mercader**
- **Ministerio de Ciencia e Innovación. (RYC-2006-001694). PI: N.Mercader**
- **Ministerio de Ciencia e Innovación. FIS (CP09/00100). PI S. Martin Puig**
- **Comunidad de Madrid (CM S-SAL0190-2006). PI: M.Torres**
- **EU Marie Curie (FP7-IEF-GA-2009-251226). PI: R. Costa.**

SELECTED PUBLICATIONS

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García-Andrés C, Torres M. **Comparative expression pattern analysis of the highly conserved chemokines SDF1 and CXCL14 during amniote embryonic development.** *Dev Dyn* (2010) 239: 2769-77

Rosello CA, Torres M. **Gene transfer by electroporation into hemogenic endothelium in the avian embryo.** *Dev Dyn* (2010) 239: 1748-54

Grimes AC, Durán AC, Sans-Coma V, Hami D, Santoro MM, Torres M. **Phylogeny informs ontogeny: a proposed common theme in the arterial pole of the vertebrate heart.** *Evol Dev* (2010) 12: 555-67

González-Rosa JM, Padrón-Barthe L, Torres M, Mercader N. **Lineage tracing of epicardial cells during development and regeneration.** *Rev Esp Cardiol* (2010) 63: 36-48

Basic Research Departments 1

Cardiovascular Developmental Biology

Intercellular signaling in cardiovascular development and disease

Head of Laboratory:	*José Luis de la Pompa*
Postdoctoral Researchers:	*Jesús Chamorro* *Luis Luna Zurita* *Donal Macgrogan* *Beatriz Martínez* *Meritxell Nus* *Belén Prados*
Predoctoral Researchers:	*Gonzalo del Monte* *Gaetano D'Amato* *Álvaro González* *Guillermo Luxán* *Juliane Münch*
Technicians:	*Vanesa Bou* *Ana Cabrero* *Eva García* *Patricia Martínez*
Visiting Scientist:	*José María Pérez-Pomares*

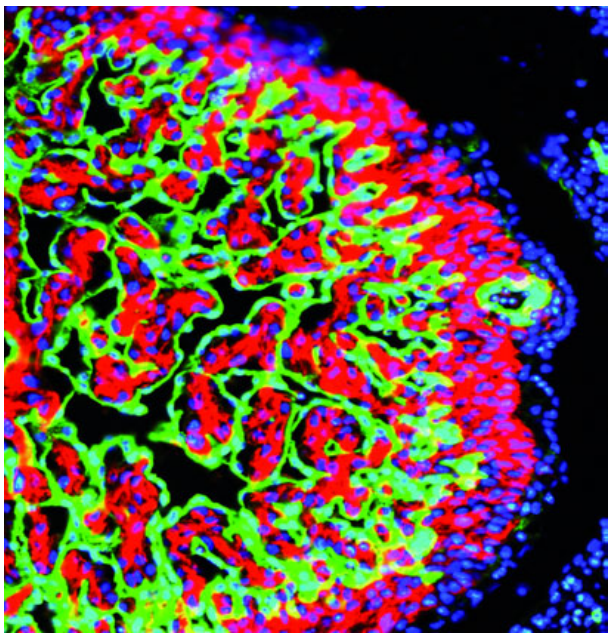
RESEARCH INTEREST

We are interested in the signaling mechanisms that regulate cardiac development and homeostasis and how these may be altered in the diseased heart. During the last year our efforts have centered on the role of Notch signaling in the development of the epicardium and coronary vasculature, the development and function of the heart valves and chambers, and the implication of Notch in zebrafish heart and fin regeneration.

The epicardium, the epithelial covering of the heart, is involved in coronary vessel and cardiac interstitium development and in myocardial growth and maturation. We have found that Notch inactivation in the epicardium impairs coronary artery differentiation and severely reduces ventricular myocardium thickness. We also study *mind bomb1 (Mib1)*, which encodes a ubiquitin ligase essential for Notch signaling. *Mib1* inactivation in cardiac endothelium and myocardium causes valve prolapse and impairs ventricle development. We are currently generating new mouse models to manipulate the expression of other molecules that interact with Notch in the embryonic or adult heart.

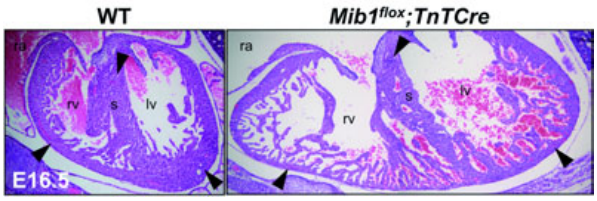
We have established an in vitro model of aortic valve disease using porcine valve cells, in which we can inhibit Notch signaling. Notch inhibition downregulates the Notch target *Hey1* and concomitantly upregulates the osteogenic signal Bmp2, suggesting that Notch represses osteoblast differentiation in the healthy valve. This approach is complemented by work with double *ApoE*- and *Notch*-deficient mice, in which we study the combined action of endothelial dysfunction and Notch deficiency in valve disease. We are also analyzing the correlation between NOTCH pathway alterations and disease severity in samples from valve disease patients.

The role of Notch in heart regeneration is being studied in the zebrafish ventricular ablation model. Notch is reactivated after cardiac damage and its activity is sustained throughout the repair process. Ectopic Notch activation impairs cardiac repair, and we are studying whether defective epicardial signaling is involved.

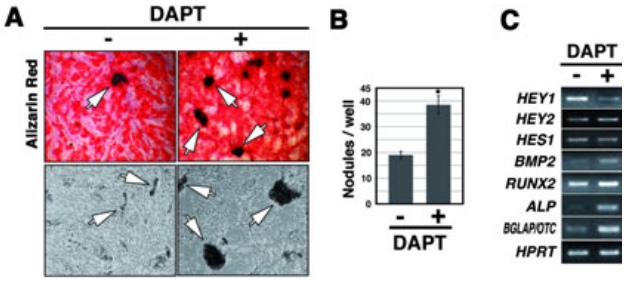


CD31/α-SMA staining in a transverse section of the left ventricle of an E13.5 WT1;N1^{flox} mutant mouse embryo, showing a fistula formed by endothelium surrounded by SMCs. CD31 (green), α-SMA (red), DAPI (blue).

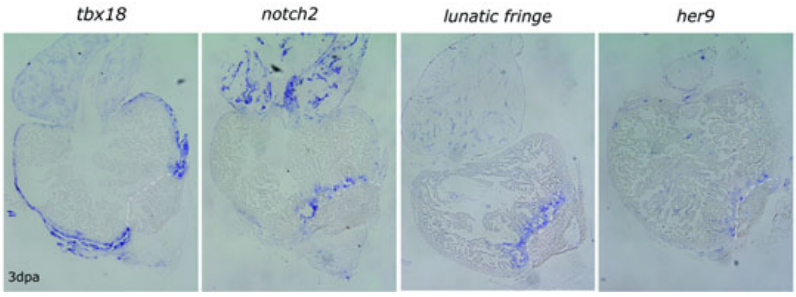
1 Cardiovascular Developmental Biology



Cross-section of the heart at E16.5. Left, wild type; right, Mib1; cTnT-Cre *mutant. Note the reduction in compact zone myocardium (arrowheads) and the increased ventricular trabeculation. lv, left ventricle; s, septum; rv, right ventricle; ra, right atrium.*



Notch inactivation leads to decreased Hey1 *expression in porcine aortic valve cells, activation of osteoblast-specific genes and increased calcification* in vitro. ***(A)*** *Alizarin Red staining and DIC images of cultured porcine aortic valve interstitial cells showing calcification foci induced by the Notch inhibitor DAPT (arrows).* ***(B)*** *Quantification shows a two-fold increase in calcification foci in Notch-inhibited cells.* ***(C)*** *RT-PCR analysis showing reduced* Hey1 *expression and increased osteogenic gene expression in Notch-inhibited cells.*



Expression of Notch signaling elements (blue) in the regenerating zebrafish heart. The dotted line delineates the amputation plane.

MAJOR GRANTS

- European Commission FP6 (LSHM-CT-2005-018630)

- European Commission FP7, Initial Training Network (215761)

- Ministerio de Ciencia e Innovación (SAF 2007-62445)

- Ministerio de Ciencia e Innovación. FIS RETICS (Recava II: RD06/0014/0038)

- Ministerio de Ciencia e Innovación. FIS RETICS (TERCEL: RD06/0010/1013)

- Ministerio de Ciencia e Innovación. FIS (CD08/00257). PI: B. Prados

- Ministerio de Ciencia e Innovación. FIS (CD09/00452). PI: M. Nus

- Comunidad de Madrid (P-2006/BIO-194). PI and coordinator of the five groups of the Network: J.L. de la Pompa

- Centro Nacional de Investigaciones Cardiovasculares (FPIT CNIC-09)

- Fundació La Marató de TV3 (081731)

- Junta de Castilla y León, Grupos de Excelencia (GR-176)

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Moncho-Amor V, Ibañez de Cáceres I, Bandres E, Martínez-Poveda B, Orgaz JL, Sánchez-Pérez I, Zazo S, Rovira A, Albanell J, Jiménez B, Rojo F, Belda-Iniesta C, García-Foncillas J, Perona R. **DUSP1/MKP1 promotes angiogenesis, invasion and metastasis in non-smallcell lung cancer.** *Oncogene* (2011) 30, 668-678.

Martínez-Poveda B, Verotta L, Bombardelli E, Quesada AR, Medina MA. **Tetrahydrohyperforin and octahydrohyperforin are two new potent inhibitors of angiogenesis.** *PLoS One* (2010) 5: e9558

Basic Research Departments 1

Cardiovascular Developmental Biology

Stem cells in organ generation, regeneration and aging

Head of Laboratory: *Ignacio Flores*

Postdoctoral Researcher: *Tania Aguado*

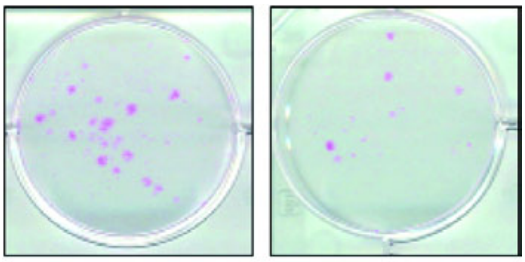
Predoctoral Researchers: *Esther Aix*
Dorotha Bednarek
María del Mar de Miguel

Technician: *Irene de Diego*

RESEARCH INTEREST

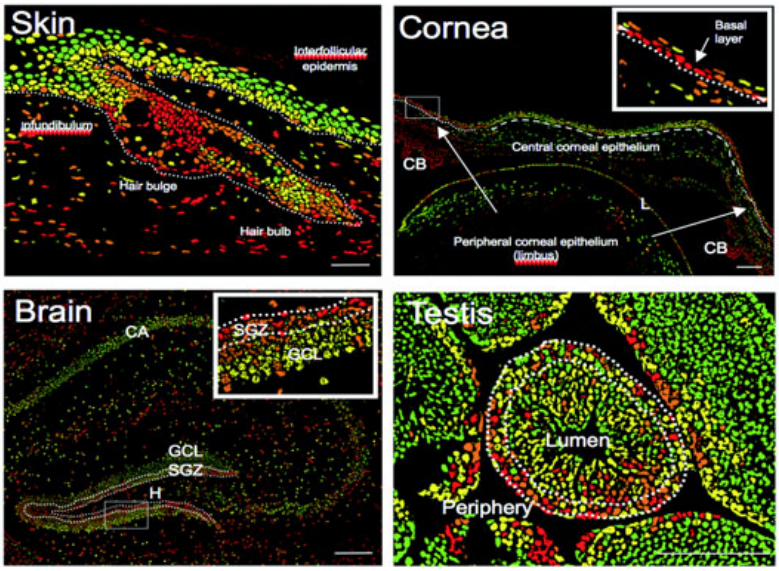
How an organ develops and persists during adult life is a fundamental question in biology. One hypothesis of organ maintenance is that stem cell functionality determines the ability of tissues to replace worn-out or injured parts. However, for most tissues the nature of the organ-forming cells and stem cells is poorly defined. We recently showed that within tissues there is a gradient in the length of telomeres, the physical chromosome ends. Given that telomeres shorten with each cell division, we hypothesize that the most primitive cells will be those cells harboring the longest telomeres.

We are currently conducting a high-content telomere length analysis to study the location, prevalence and status of putative cardiac stem cells and their progeny during organogenesis and aging. We are also examining the relationship between telomere length and the ability of cardiac cells to generate new cardiac tissue. Finally, we are investigating the factors that regulate telomere length, with the aim of defining their contribution to cell differentiation. Through these approaches, we hope to obtain 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.



Telomere attrition diminishes the proliferation potential of stem cells ex vivo. *Representative images showing the number and size of macroscopic colonies formed by keratinocytes isolated from telomerase-deficient G1Terc$^{-/-}$ mice (relatively long telomeres) and G3Terc$^{-/-}$ mice (relatively short telomeres).*

1 Cardiovascular Developmental Biology



Cells with the longest telomeres locate to stem cell compartments in mouse tissues. *Representative topographic telomere length maps generated from confocal telomere Q-FISH images. Nuclei are pseudo-colored according to their average telomere fluorescence, from the longest telomeres (red) to the shortest (green).*

MAJOR GRANTS

- **Ministerio de Ciencia e Innovación (SAF2009-10480)**
- **Ministerio de Ciencia e Innovación (RYC-2006-3067)**
- **Asociación Española contra el Cáncer. AECC (2009). PI: T. Aguado**

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Garcia-Lavandeira M, Quereda V, Flores I, Saez C, Diaz-Rodriguez E, Japon MA, Ryan AK, Blasco MA, Dieguez C, Malumbres M, Alvarez CV. **A GRFa2/Prop1/stem (GPS) cell niche in the pituitary.** *PLoS One* (2009) 4: e4815

Basic Research Departments 1

Cardiovascular Developmental Biology

Molecular regulation of heart development and disease

Head of Laboratory: *Enrique Lara-Pezzi*

Technician: *Marina Mercedes López Oñaleta*

Master Student: *Jesús María López Salinero*

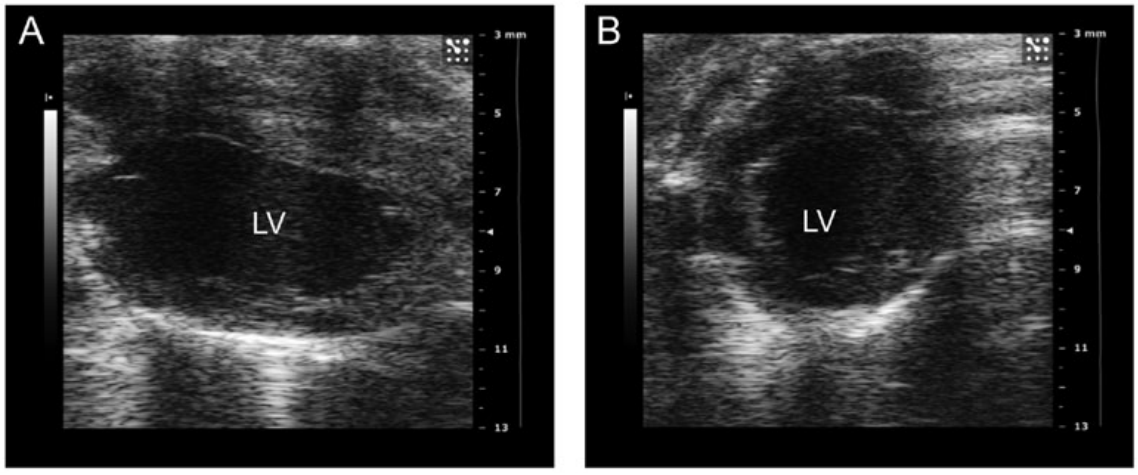
Visiting Scientist: *María Villalba Orero*

RESEARCH INTEREST

Our lab studies the molecular mechanisms that regulate cardiac development and heart disease. One of our major interests is 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.

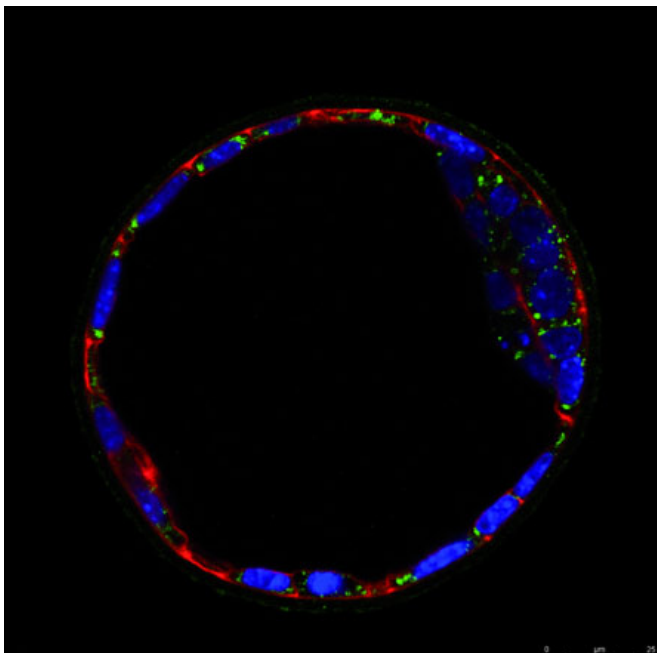
Together with the Genomics and Bioinformatics Units at the CNIC, we have used high density exon microarrays and RNA-Seq to create a global map of AS isoforms expressed during heart failure. This map has enabled us to identify cis-regulatory sequences and trans-regulatory splicing factors associated with AS, and we are analyzing the roles of these factors in the heart through knockdown and knockout 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. We are now exploring the role of CnAβ1 in stem cells and in the developing embryo, where it is strongly expressed.



Ultrasound analysis of an infarcted mouse heart. *The figure shows short-axis (A) and long-axis (B) views of the heart of a CnAβ1 transgenic mouse 28 days after the induction of myocardial infarction by permanent ligation of the left coronary artery. LV, left ventricle. Images were obtained in diastole.*

1 Cardiovascular Developmental Biology



CnAβ1 distribution in the early embryo. *The image shows a confocal immunofluorescence image of a mouse blastocyst stained with an anti-CnAβ1 antibody (green). Cell membrane is stained with rhodamine-labeled phalloidin (red) and nuclei are counterstained with DAPI (blue).*

MAJOR GRANTS

- **European Commission FP7. Marie Curie European Reintegration Grant (239158)**
- **Ministerio de Ciencia e Innovación (BFU2009-20016)**
- **Ministerio de Ciencia e Innovación. FIS (CP08/00144)**
- **British Heart Foundation (PG/08/084/25827). co-PI, E. Lara-Pezzi. Funds held at Imperial College London, UK**
- **British Heart Foundation (PG/07/020/22503). co-PI, E. Lara-Pezzi. Funds held at Imperial College London, UK**

SELECTED PUBLICATIONS

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

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

Brand NJ, Lara-Pezzi E, Rosenthal N, Barton PJ. **Analysis of cardiac myocyte biology in transgenic mice: a protocol for preparation of neonatal mouse cardiac myocyte cultures.** *Methods Mol Biol* (2010) 633: 113-24

Lara-Pezzi E, Cesare M, Terracciano CM, Soppa GK, Smolenski RT, Felkin LE, Yacoub MH, Barton PJ. **A gene expression profile of the myocardial response to clenbuterol.** *J Cardiovasc Transl Res* (2009) 2: 191-7

Oshima Y, Ouchi N, Shimano M, Pimentel DR, Papanicolaou KN, Panse KD, Tsuchida K, Lara-Pezzi E, Lee SJ, Walsh K. **Activin A and follistatin-like 3 determine the susceptibility of heart to ischemic injury.** *Circulation* (2009) 120: 1606-15

Basic Research Departments 1

Cardiovascular Developmental Biology

Functional genomics of embryonic pluripotency and heart development

Head of Laboratory: *Miguel Manzanares*

Postdoctoral Researchers: *Luis Aguirre*
Eva Alonso
Cristina Arias
Susana Cañón

Predoctoral Researchers: *Beatriz Fernández Tresguerres*
Teresa Rayón

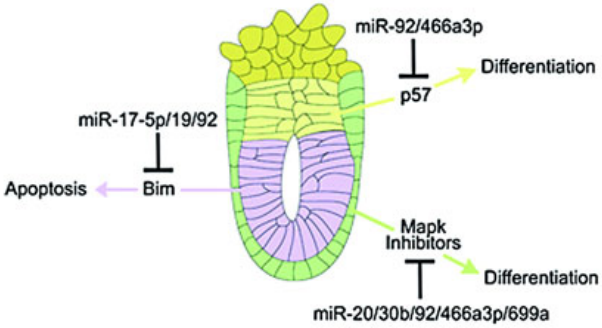
Masters Student: *Melisa Gómez*

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 screen for and identify distal acting cis-regulatory sequences, and study how they act on their target genes and how these targets are organized into gene regulatory networks underlying a specific biological state. 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.

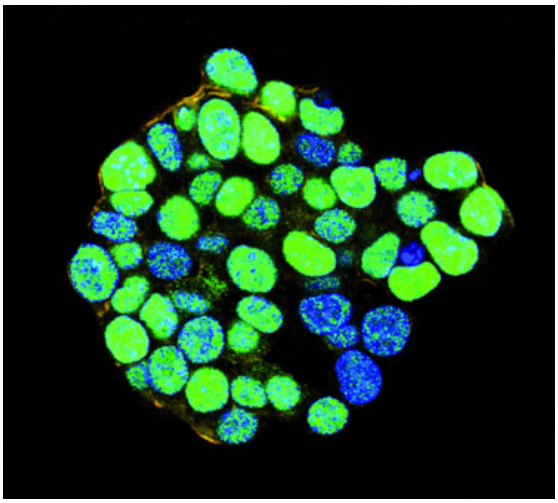
We recently showed that the sustained pluripotency of embryonic cells is an evolutionary novelty in mammals. The core transcription factors that establish embryonic pluripotency in mammals are not expressed in the early chick embryo in a manner compatible with them having this role. Using bioinformatics tools we found that the regulatory elements through which these core factors control their downstream targets are not present in the chick genome, and thus appeared de novo in the mammalian lineage. We have also analyzed the role of miRNAs in the early mouse embryo by using a Dicer loss-of-function model. Using extraembryonic stem cells to characterize phenotypes in detail, we find that the three blastocyst-derived stem cell populations have different requirements for miRNAs. Our study highlights how 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.

Another area of interest is the role of genomic regions associated with increased risk of human diseases such as diabetes or colorectal cancer. Genome-wide association studies show that many of these associations fall in intergenic regions, and, through a combination of comparative genomics, transgenic assays and studies of chromatin structure, we have found that many of these the risk-associated regions contain cis-regulatory elements. These studies highlight the gene-regulatory basis for many human diseases, and open an important area of research that we will be actively pursuing in the future.



Distinct roles of miRNAs in the early mouse embryo. In the epiblast (purple) miRNAs inhibit the pro-apoptotic factor Bim, thus preventing cell death. In contrast, miRNAs in extraembryonic tissues act to prevent differentiation. In the trophoblast (yellow), this is achieved by inhibition of the cell cycle regulator p57, while in the extraembryonic endoderm (green) miRNAs inhibit negative regulators of the Mapk signaling pathway.

1 Cardiovascular Developmental Biology



A colony of mouse ES cells showing the expression of the pluripotency factor Oct4 (green). Nuclei are counterstained in blue.

MAJOR GRANTS

- **European Commission FP7. EuroSyStems (200720)**
- **Ministerio de Ciencia e Innovación (BFU2008-00838)**
- **Ministerio de Ciencia e Innovación. CONSOLIDER Project (CSD2007-0008)**
- **Ministerio de Ciencia e Innovación (JCI-2008-2980). PI: C. Arias**
- **Centro Nacional de Investigaciones Cardiovasculares. CNIC-08-2009. PI and coordinator of four groups: M. Manzanares**

SELECTED PUBLICATIONS

Ragvin A, Moro E, Fredman D, Navratilova P, Drivenes O, Engström PG, Alonso ME, de la Calle Mustienes E, Gómez-Skarmeta JL, J Tavares MJ, Casares F, Manzanares M, van Heyningen V, Molven A, Njølstad PR, Argenton F, Lenhard B, Becker TS. **Long-range gene regulation links genomic type 2 diabetes and obesity risk regions to *HHEX*, *SOX4* and *IRX3*.** *Proc Natl Acad Sci U S A* (2010) 107:775-80

Pernaute B, Cañon S, Crespo M, Fernandez-Tresguerres B, Rayon T, Manzanares M. **Comparison of extraembryonic expression of *Eomes* and *Cdx2* in pre-gastrulation chick and mouse embryo unveil regulatory changes along evolution.** *Dev Dyn* (2010) 239: 620-9

Spruce S#, Pernaute B#, Di-Gregorio A, Cobb B, Merkenschlager M, Manzanares M*, Rodriguez TA*. **An early developmental role for miRNAs in the maintenance of extra-embryonic stem cells in the mouse embryo.** *Dev Cell* (2010) 19: 207-19 (Evaluated by Faculty of 1000 Biology).
#Joint 1st authors; *Corresponding authors

Pittman AM, Naranjo S, Jalava SE, Twiss P, Ma Y, Olver B, Lloyd A, Vijayakrishnan J, Qureshi M, Broderick P, van Wezel T, Morreau H, Tuupanen S, Aaltonen LA, Alonso ME, Manzanares M, Gavilán A, Visakorpi T, Gómez-Skarmeta JL, Houlston RS. **Allelic variation at the 8q23.3 colorectal cancer risk locus functions as a cis-acting regulator of EIF3H.** *PLoS Genetics* (2010) 6: e1001126. (Comment in *Cell* 2010: 143, 179)

Fernandez-Tresguerres B, Cañon S, Pernaute B, Rayon T, Crespo M, Torroja C, Manzanares M. **Evolution of the mammalian embryonic pluripotency gene regulatory network.** *Proc Natl Acad Sci U S A* (2010) 107: 19955-60. (Comments in *PNAS* 2010: 107, 19606; *Nat Rev Genet* 2010: 11, 818)

Basic Research Departments 1

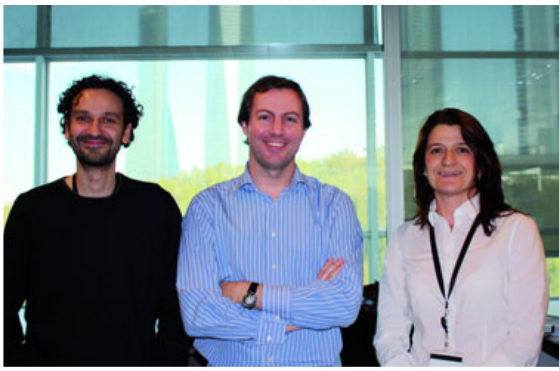
Cardiovascular Developmental Biology

Stem cell niche pathophysiology

Head of Laboratory: *Simón Méndez Ferrer*

Postdoctoral Researcher: *Joan Isern Marín*

Technician: *Ana María Martín de Ana*

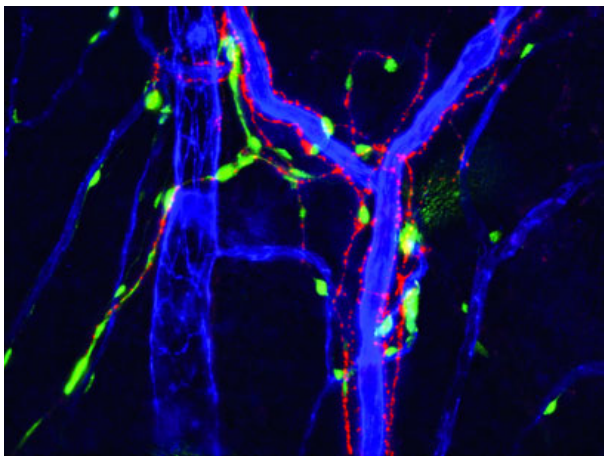


RESEARCH INTEREST

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

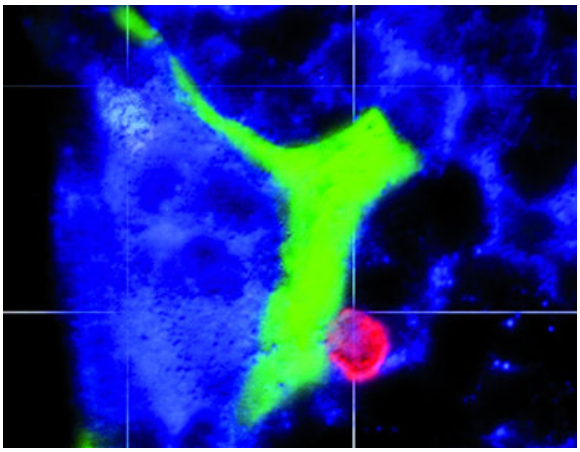
Our earlier work described a tight regulation of the bone marrow stem cell niche by circadian oscillations of sympathetic activity. Light onset induces noradrenaline release from bone marrow varicosities, leading to rapid downregulation of CXCL12/SDF-1, the only chemokine known to direct hematopoietic 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 peri-vascular nestin$^+$ cells. These cells are true niche cells: they colocalize with HSCs, express high levels of core HSC maintenance genes, selectively downregulate these genes during HSC mobilization by granulocyte colony-stimulating factor (G-CSF) or β_3-adrenergic stimulation, and their deletion triggers significant alterations in bone marrow HSC homing and content. Interestingly, peri-vascular nestin$^+$ cells are also functional mesenchymal stem cells (MSC): they account for all mesenchymal activity (fibroblastic colony-forming units), show clonal multilineage differentiation toward the three major mesenchymal lineages, display robust self-renewal in serial transplantations and contribute to osteochondral lineages in vivo. These findings suggest that the bone marrow stem cell niche is composed of a unique pairing of MSCs and HSCs, tightly regulated by local input from the microenvironment and by long-distance cues from hormones and the autonomic nervous system.

Our investigation of this sympathetic regulation has revealed that β_2- and β_3-adrenergic receptors have different functions in bone marrow, but cooperate during G-CSF-induced HSC mobilization. This process is not the mirror image of that which triggers HSC homing to the bone marrow. We have gained insight into differential homing pathways—some of which are shared with leukocytes—by showing redundant and nonredundant roles for the adhesion molecules ICAM-1, ICAM-2, and VCAM-1 in lymphocyte homing. We have also uncovered some of the mechanisms by which the niche regulates progenitor cell differentiation during development, showing that the transcription factor Eklf critically regulates the formation of primitive erythroid cells and their maturation in a dose-dependent manner.

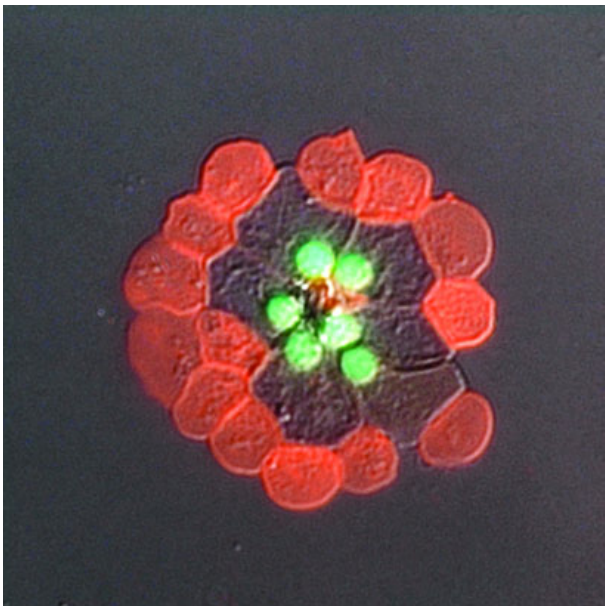


Peri-vascular nestin$^+$ mesenchymal stem cells are innervated by sympathetic fibers in the bone marrow. Projection stack (~100 μm) of fluorescent images showing the distribution of Nestin-*GFP$^+$ cells (green), CD31/PECAM$^+$ vascular endothelial cells (blue) and tyrosine hydroxylase$^+$ sympathetic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

1 Cardiovascular Developmental Biology



A bone marrow stem cell niche made for two. Projection stack (~15 μm) of fluorescent images showing a CD150⁺ (red) CD48⁻, CD3e⁻, Ter119⁻, Gr1⁻, B220⁻ and CD11b⁻ (antigens labeled in blue) hematopoietic stem cell adjacent to a nestin-GFP⁺ mesenchymal stem cell (green) in the bone marrow (from Nature 466: 829-34). Grid, 50 μm



The image shows a fluorescence overlay of Ter119-stained cytocentrifuged embryonic blood cells from E13.5 ε-globin::H2B-GFP;Eklf+/- transgenic mouse embryos. Ter119 (red) is expressed on the enucleated definitive erythrocytes at the periphery of the cluster but not on the larger, nucleated primitive erythroblasts (EryP) in the center (green nuclei). From: Blood, 116:3972-80 (Front cover)

MAJOR GRANTS

- **ASH Scholar Award. American Society of Hematology**
- **Ministerio de Ciencia e Innovación. (RYC-2009-04703)**

SELECTED PUBLICATIONS

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*

Méndez-Ferrer S, Battista M, Frenette PS. **Cooperation of β_2- and β_3-adrenergic receptors in hematopoietic progenitor cell mobilization.** *Ann N Y Acad Sci* (2010) 1192: 139-44

Méndez-Ferrer S, Frenette PS. **$G\alpha_s$ uncouples haematopoietic stem cell homing and mobilisation.** *Cell Stem Cell* (2009) 4: 379-80

Boscacci RT, Pfeiffer F, Gollmer K, Sevilla AI, Martín AM, Soriano SF, Natale D, Henrickson S, von Andrian UH, Fukui Y, Mellado M, Deutsch U, Engelhardt B, Stein JV. **Comprehensive analysis of lymph node stroma-expressed Ig superfamily members reveals redundant and nonredundant roles for ICAM-1, ICAM-2, and VCAM-1 in lymphocyte homing.** *Blood* (2010) 116: 915-25

Isern J, Fraser ST, He Z, Baron MH. **Dose-dependent regulation of primitive erythroid maturation and identity by the transcription factor Eklf.** *Blood* (2010) 116: 3972-80 (Front cover)

Basic Research Departments 1

Cardiovascular Developmental Biology

Role of new genes in cardiovacular development

Head of Laboratory: *Juan José Sanz Ezquerro*

Predoctoral Researchers: *Verónica Uribe Sokolov*

Masters Student: *Laura González Calero*

Technician: *Claudio Badía Careaga*

RESEARCH INTEREST

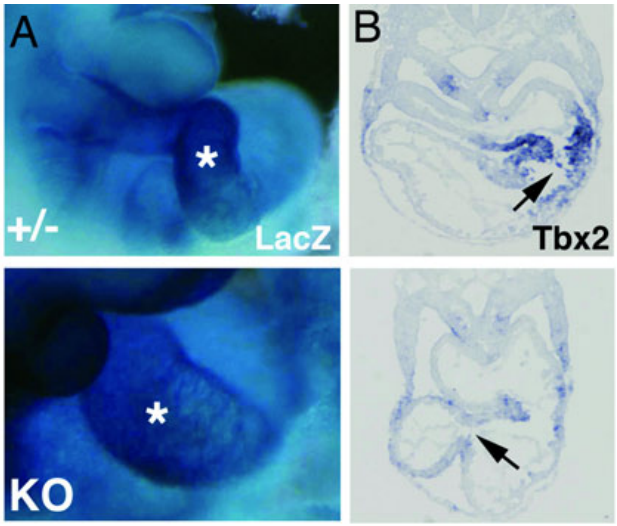
Our group investigates the molecular and cellular basis of organogenesis during embryonic development. Our approach combines studies in chick and mouse embryos with in vitro cell culture models to dissect the role of new genes in the morphogenesis of the heart and other aspects of cardiovascular development.

Much of our recent work focuses on the role during embryogenesis of Arid3b, a transcription factor of the highly conserved ARID family. Arid3b-null embryos die early in development and present with severe heart defects, but the exact roles of Arid3b in development remain unclear.

During the last year we advanced our analysis of these heart defects. The most severe alterations occur at the poles of the heart, especially a noticeable shortening of the outflow tract, a reduction in the size of the inflow region, and abnormal patterning and maturation of the AV canal. Mutant embryos show abnormal expression of several molecular markers of both the secondary heart field and chambers, pointing to important roles for Arid3b in several aspects of heart development. We also observed defects in the cytoskeleton and motility of mutant cells in vitro. Based on these findings, we believe that Arid3b might control the regulated addition and differentiation of heart precursor cells from the second heart field to the heart.

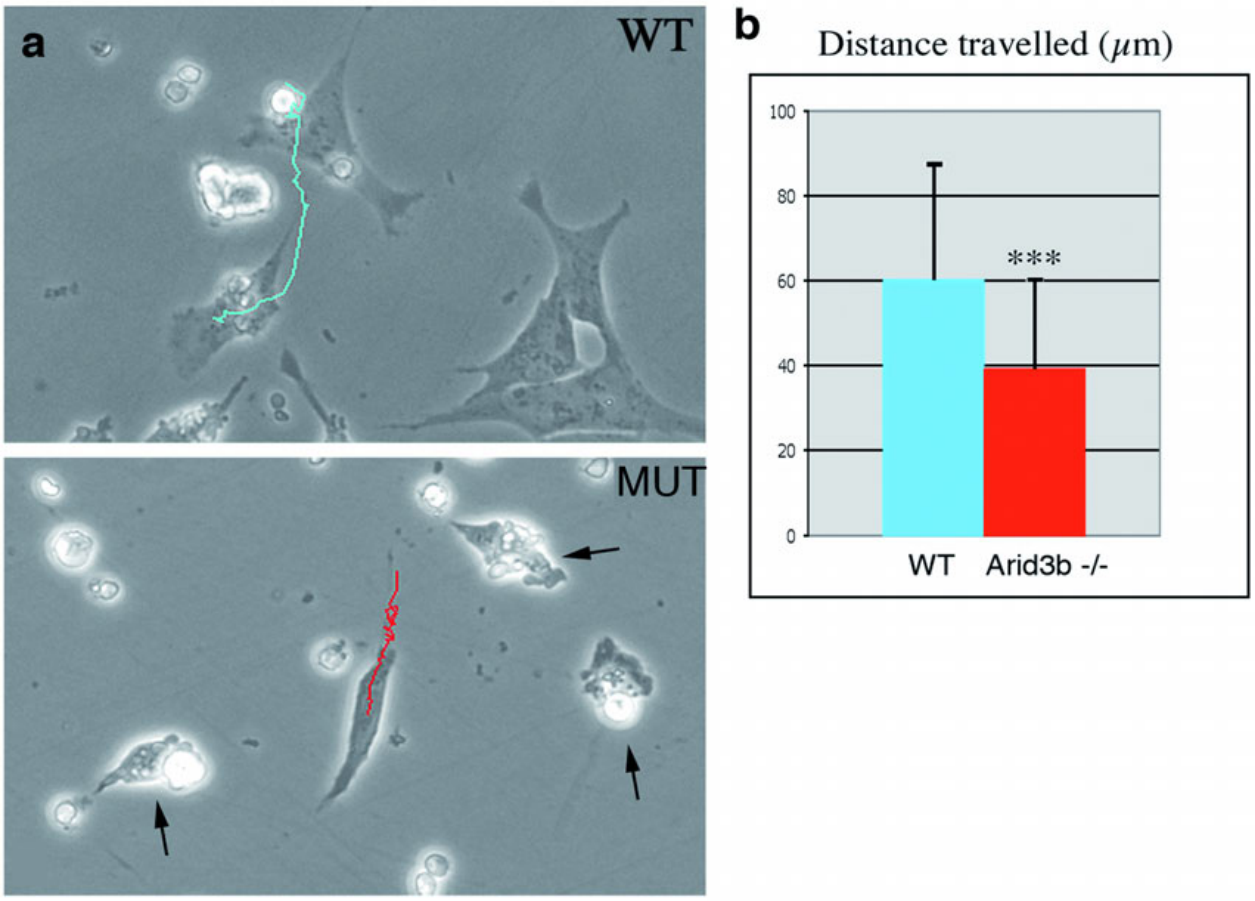
Our plans include an analysis of the cellular basis of Arid3b functions (for example its involvement in cell migration and epithelial to mesenchymal transition) and the identification of its molecular targets by microarray analysis.

Another area of interest is the role of norrin during chick embryo development. Mutations in norrin cause Norrie disease in humans, a retinal dysplasia characterized by abnormal vascularization of the retina. We have found that chicken norrin is expressed in tissues besides the eye during embryogenesis and we are characterising its expression pattern and biological functions.



Heart development defects in Arid3b knockout (KO) embryos. ***A,*** *LacZ staining of heterozygous and KO embryos at E9.5. Note the shortening and widening of the outflow tract (asterisks).* ***B,*** *In situ hybridisation for Tbx2 at E9.5 shows abscence of expression in the atrioventicular canal (arrows) of KO hearts.*

1 Cardiovascular Developmental Biology



Arid3b mutant embryonic fibroblasts show defective motility in vitro. ***A,*** *Example of the path traveled by cells after 3 hours in culture (blue and red lines mark trajectories and images correspond to the last frames of the time-lapse sequence).* ***B,*** *Quantification of the data in A, showing that Arid3b cells travel significantly shorter distances than wild type cells.*

MAJOR GRANTS

- **Fundació La Marató TV3 (082031)**
- **Ministerio de Ciencia e Innovación. FIS (CP07/251)**

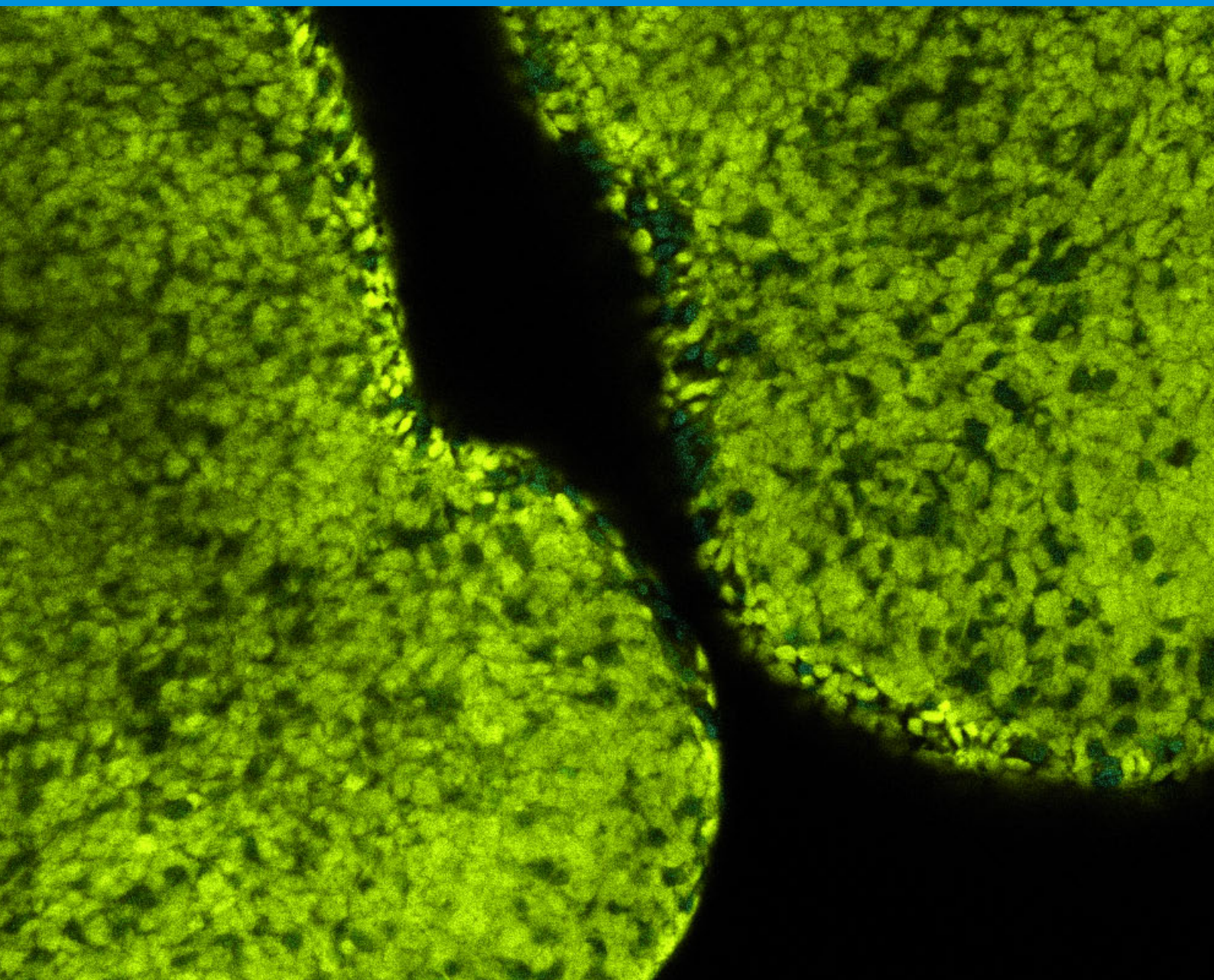
SELECTED PUBLICATIONS

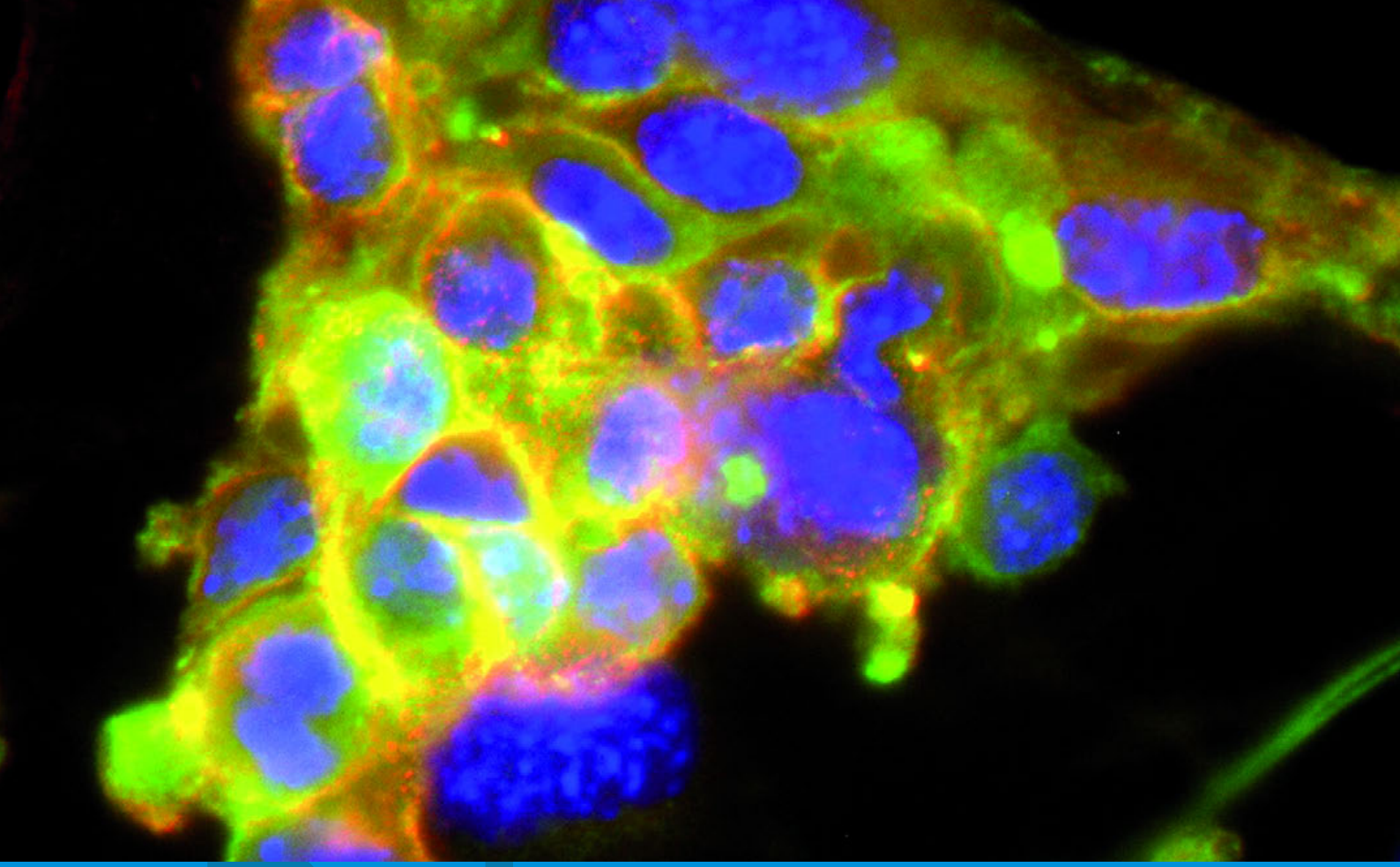
Casanova JC, Uribe V, Badia-Careaga C, Giovinazzo G, Torres M, Sanz-Ezquerro JJ. **Apical Ectodermal Ridge morphogenesis in limb development is controlled by Arid3b-mediated regulation of cell movements.** *Development* (accepted)

Basic Research Departments

2

Regenerative Cardiology





Basic Research Departments

2 Regenerative Cardiology

The RC department's activities center on the characterization of stem cell populations associated with cardiovascular system homeostasis, the interdependence of the cardiovascular and immune-inflammatory systems, and the roles of oxidative stress, cell cycle alterations and stem cell dysfunction in tissue aging.

DEPARTMENT DIRECTOR: *Antonio Bernad*

DEPARTMENT MANAGER: *Isabel Barthelemy*

SUPPORT SCIENTIST: *Carmen Albo*

ADMINISTRATIVE SUPPORT: *Marta Ramón*

Basic Research Departments 2

Regenerative Cardiology

Gene expression and genetic stability in adult stem cells

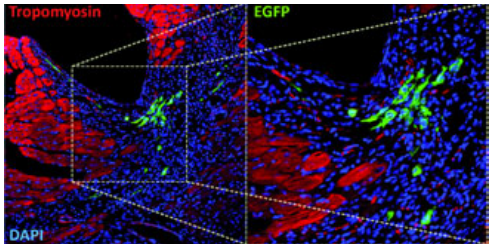
Head of Laboratory:	*Antonio Bernad*
Research Scientists:	*Manuel A. González* *Enrique Samper*
Postdoctoral Researchers:	*Xonia Carvajal* *Antonio Díez-Juan* *Marta B. Evangelista* *Silvia García* *M. Paz Moreno* *Isabel Moscoso* *Kausalia Vijayaragavan*
Predoctoral Researchers:	*Beatriz Escudero* *J. Camilo Estrada* *David Horna* *Alberto Izarra* *David Lara* *María Tomé* *Íñigo Valiente*
Masters Student:	*Francisco M. Cruz-Uréndez*
Support Scientist:	*Candelas Carreiro*
Technicians:	*Vanessa Blanca* *Ana Calvo* *Rosa M. Carmona* *Juan Carlos Sepulveda* *Yaima Torres* *Virginia Zorita*

RESEARCH INTEREST

Adult stem cells (aSCs) are crucial for the maintenance of organ homeostasis throughout life. As somatic stem cells age and accumulate damage they no longer fulfill their roles efficiently. Such cells may initiate a program of senescence or apoptosis, or alternatively can become genetically unstable, posing a danger to the organism. To understand how stem cells control the processes of self-renewal and differentiation, we focus on several related areas, working mainly with mouse and human mesenchymal stem cells (MSCs) and cardiac progenitor cells (CPCs) isolated from adult hearts.

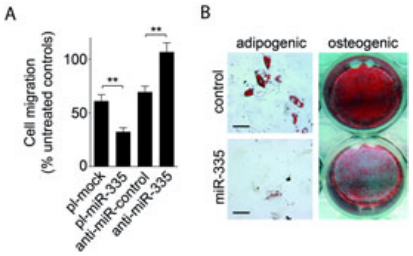
Analysis of the expression of microRNAs (miRNAs) revealed that miR-335 is significantly downregulated upon hMSC differentiation and also in other compatible differentiation models. Additionally, we found that miR-335 is upregulated in hMSCs by the canonical Wnt signaling pathway and downregulated by interferon gamma (IFN-γ), important signaling pathways that control the activation of hMSCs. These results strongly suggest that miR-335 downregulation is critical for the acquisition of reparative MSC phenotypes. In parallel studies we have analyzed the influence of ex vivo cell culture conditions on genome stability in hMSCs. Our results (Estrada et al., submitted) indicate that hMSCs are less genetically stable when cultured at high oxygen tension (21%), and that this instability is associated with dysregulation of several cancer-related genes and with radically altered metabolic parameters.

We have also initiated the molecular charaterizaction of CPCs, through a comparative study of these MSC-like populations from mouse, pig and humans. Early results indicate that BMI-1 might be an important CPC marker and that the muscle-specific miRNAs miRNA-1 and miRNA-133a modulate the ability of adult and embryonic stem cells to respond to cardiomyogenic signals.

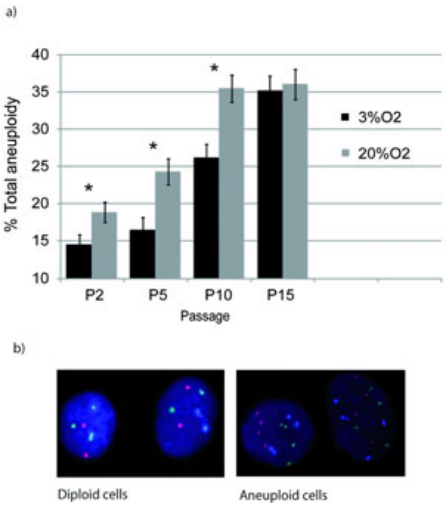


Detection of Sca-1+ CPCs transplanted into a mouse heart after acute myocardial infarction. Cells injected intramyocardially after induction of myocardial infarction by left coronary artery ligation. Sca-1+ CPCs were detected by GFP fluorescence after one week.

2 Regenerative Cardiology



Exogenous miR-335 overexpression impairs hMSC proliferation, migration and differentiation. *Bone marrow-derived hMSCs were transduced with the lentiviral vectors pLV-EmGFP-MIR335 or pLV-EmGFP-mock (encoding a negative control shRNA). Transduced (GFP+) cells were purified by FACS, and used in gain-of-function studies.* ***(A)*** *hMSCs (10^4) transduced with the indicated lentiviral vector or transfected with the indicated miRNA inhibitor were used in trans-well migration assays.* ***(B)*** *hMSCs transduced with pLV-EmGFP-MIRN335 or pLV-EmGFP-mock (control) were cultured for 3 weeks in medium containing adipogenic or osteogenic factors, followed by staining with Oil Red O or Alizarin Red S, respectively.*



Supraphysiological oxygen tension increases double strand breaks, chromosomal aberrations and aneuploidy in ex vivo cultured hMSCs. ***(A)*** *Mean % aneuploidy detected in 100-200 nuclei per hMSC cell line for each oxygen concentration. Aneuploidy was detected by FISH with centromeric probes for chromosomes 8, 13, and 17. Black bars represent hMSCs grown at 3% O_2; gray bars represent cells grown at 20% O_2. Growth at 3% O_2 significantly reduced the incidence of aneuploidy from passage 2 -10 ($p<0.05$).* ***(B)*** *Representative images of hMSC nuclei hybridized with CEP probes for chromosomes 8 (red), 11 (green) and 17 (pale blue) in diploid cells grown at 3% O_2 (left panel) and in highly aneuploid cells grown at 20% O_2 cell (right panel). Nuclei are stained with DAPI (blue).*

MAJOR GRANTS

- **European Commission FP7. European Multidisciplinary Initiative (FP7-HEALTH -2009 CAREMI). PI, Dr. Antonio Bernad (coordinator)**
- **Ministerio de Ciencia e Innovación. Programa Nacional de Internacionalización de la I+D (PLE 2009/0147). Sub-project coordinator: A. Bernad**
- **Ministerio de Ciencia e Innovación. FIS (CP07/00306). PI: M. A. González de la Peña**
- **Ministerio de Ciencia e Innovación. Programa Nacional de Internacionalización de la I+D (PLE2009 2009/0112). PI: M. A. González de la Peña**
- **Ministerio de Ciencia e Innovación (SAF2008-02099). PI, A. Bernad**
- **Mantenimiento de la estabilidad genómica en células madre. Implicación en la bioseguridad en medicina regenerativa. (S-BIO-0306-2006) PI: Dr. A. Bernad (coordinator)**
- **Ministerio de Ciencia e Innovación. FIS RETICS (TERCEL: RD06/0010/0018). Subproject Coordinator: A. Bernad**
- **Comunidad de Madrid: Plan de Innovación Empresarial (CEIT06). PI: E. Samper**

SELECTED PUBLICATIONS

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* (accepted).
* Corresponding authors

García S, Bernad A, Martín MC, Cigudosa JC, Garcia-Castro J, de la Fuente R. **Pitfalls in spontaneous in vitro transformation of human mesenchymal stem cells.** *Exp Cell Res* (2010). 316:1648-50

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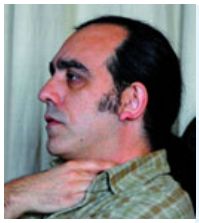
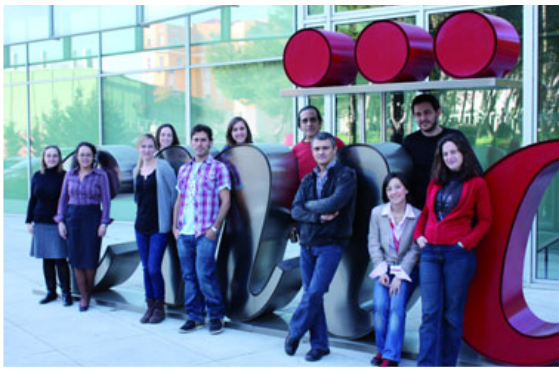
Functional genetics of the oxidative phosphorylation system



Head of Laboratory:	*José Antonio Enríquez*
Research Scientists:	*Acisclo Pérez Martos* *Patricio Fernández Silva* *Erika Fernández -Vizarra* *Nuria Garrido Pérez* *Pilar Bayona Bafaluy*
Postdoctoral Researchers:	*Carmen Colás Esteban* *Ester Perales Clemente* *Patricia Meade Huerta* *Raquel Moreno Loshuertos*
Predoctoral Researchers:	*Ricardo Marco Lázaro* *Adela María Guaras Rubio* *Ana Latorre Pellicer* *Esther Lapuente Brun* *Elena de Tomás Mateo*
Support Scientists:	*Mª Concepción Jiménez Gómez* *Nieves Movilla Meno* *Marta Roche Molina*
Visiting Scientists:	*Eduardo Balsa Martinez* *Sara Cogliati*

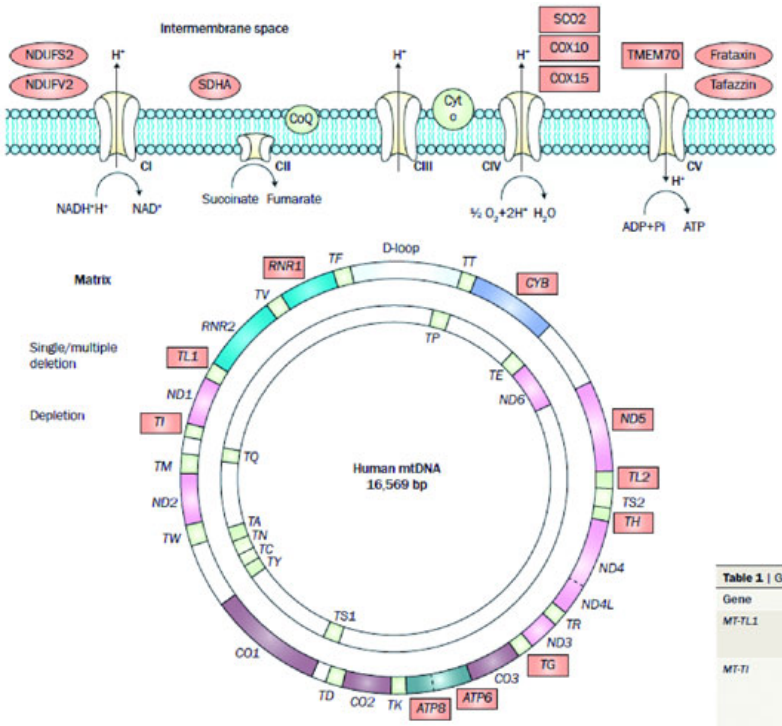


RESEARCH INTEREST

Disorders of the mitochondrial oxidative phosphorylation (OXPHOS) system are among the most common inherited metabolic disorders, affecting an estimated 1 in every 5000 live births. The mitochondrial OXPHOS system comprises the four respiratory chain complexes (complexes I, II, III and IV) and ATP synthase (complex V), and is the major source of ATP in eukaryotic cells. The mtDNA mutations associated with the clinical phenotypes of these diseases fall into three main categories: 1) Point mutations affecting either protein synthesis genes (rRNA and tRNA) or mtDNA-encoded structural OXPHOS subunits;2) Rearrangements, which can be single or multiple deletions or partial duplications; 3) Depletions, in which the amount of mtDNA is significantly reduced. Although these mtDNA defects are seldom associated solely with cardiomyopathy, they have serious effects on cardiac tissue because OXPHOS-derived ATP is essential for the heart's contractile activity. After encephalo-neuromuscular syndromes, cardiomyopathy, predominantly cardiac hypertrophy, is the most common pathology associated with mtDNA point mutations.

We use a range of approaches to investigate the role of the OXPHOS system in health and disease. One reason for the current limited knowledge in this area is that established models of electron transport chain organization are flawed. To address this, we are implementing high-throughput strategies to catalogue the set of the genes whose products participate in the biogenesis and regulation of the OXPHOS system (which we call the OXPHOME). We are also determining the factors that regulate the structural organization of the electron transport chain and the role that this superstructural organization plays in the production of reactive oxygen species (ROS). This area is linked to our interest in the role of ROS as mitochondrial second messengers and to our aim to deconstruct, in cellular models, the mammalian OXPHOS system into its functional components (electron transport, proton pumping and ATP synthesis).

2 Regenerative Cardiology



Schematic representation of the OXPHOS system embedded in the mitochondrial inner membrane (top) *and the genes encoded by human mtDNA* (bottom). *ND genes, the seven mtDNA-endoded structural subunits of complex I; CYB, the complex III structural subunit; CO genes, the three subunits of complex IV; ATP subunits, the two subunits of complex V; RNR1 and RNR2, 12S rRNA and 16S rRNA; Tx genes, the twenty-two tRNAs. Sequences of the genes are contiguous and even overlapping in the case of ND4L/ND4 and ATP8/ATP6. Mitochondrial and nuclear gene products whose mutation is associated with mitochondrial cardiomyopathy are highlighted in red.*

Table 1 | Genetic mtDNA alterations associated with mitochondrial cardiomyopathy

Gene	Mutation	Amino acid change	Main clinical phenotype	Cardiac phenc
MT-TL1	m.3243A>G	–	MELAS	HCM
	m.3260A>G	–	MiMyCa, myopathy	HCM, WPW
	m.3303C>T	–	Cardiomyopathy, myopathy	HCM, FICM
MT-TI	m.4269A>G	–	Multisystem	DCM
	m.4295A>G	–	Cardiomyopathy	HCM
	m.4300A>G	–	Cardiomyopathy	HCM
	m.4317A>G	–	Multisystem	FICMv
	m.4320A>G	–	Encephalopathy, cardiomyopathy	HCM
MT-TK	m.8334A>G	–	MERRF	HCM
	m.8348A>G	–	Cardiomyopathy	HCM, DCM
	m.8363G>A	–	Multisystem, hearing loss	HCM
MT-TG	m.9997T>C	–	Bowel dysmotility	HCM
MT-TH	m.12192A>G	–	Cardiomyopathy	DCM, HCM
MT-TL2	m.12297T>C	–	Cardiomyopathy	DCM
MT-RNR1	m.1555A>G	–	Deafness	RCM
MT-ND5	m.13513G>A	D393N	Leigh Syndrome, MELAS	HCM, WPW
MT-CYB	m.14849T>C	S35P	Multisystem	HCM
	m.15243G>A	G166E	Cardiomyopathy	HCM
	m.15498G>A	G251D	Cardiomyopathy	HCM
MT-ATP6	m.8993T>C	L156R	Leigh Syndrome, NARP	HCM
	m.8528T>C	M1T	Infantile cardiomyopathy	HCM
	m.8528T>C	W55R		
MT-ATP8	m.8529G>A	W55X	Cardiomyopathy, neuropathy	HCM

DCM: dilated cardiomyopathy; FICM: fatal infantile cardiomyopathy; HCM: hypertrophic cardiomyopathy; RCM: restrictive cardiomyopathy. See abbreviations.

Model of complex I assembly, showing ND subunit entry points. The model unites previous knowledge and the new contributions from our group (Mol Cell Biol 2010).

MAJOR GRANTS

- **Ministerio de Ciencia e Innovación (SAF2009-08007)**
- **Ministerio de Ciencia e Innovación CONSOLIDER Project (CSD2007-00020)**

SELECTED PUBLICATIONS

Perales-Clemente E, Fernández-Vizarra E, Acín-Pérez R, Movilla N, Bayona-Bafaluy MP, Moreno-Loshuertos R, Pérez-Martos A, Fernández-Silva P, Enríquez JA. **Five entry points of the mitochondrially encoded subunits in mammalian complex I assembly.** *Mol Cell Biol* (2010) 30: 3038-47

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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

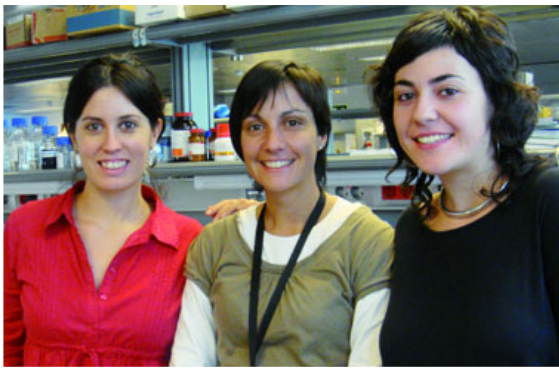
Cardiovascular related risks of obesity

Head of Laboratory: *Beatriz G. Gálvez*

Masters Students: *Aurora Bernal*
María Fernández

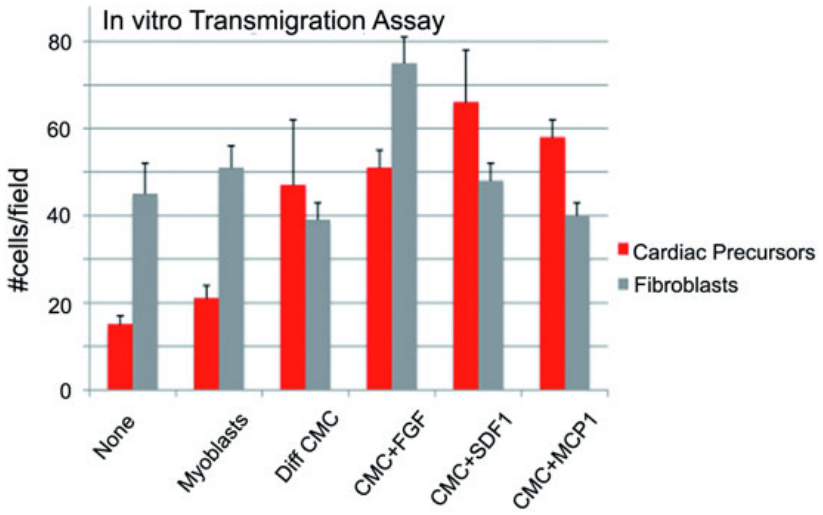
Technician: *Nuria San Martín*

Visiting Scientist: *Claudia Cordova*



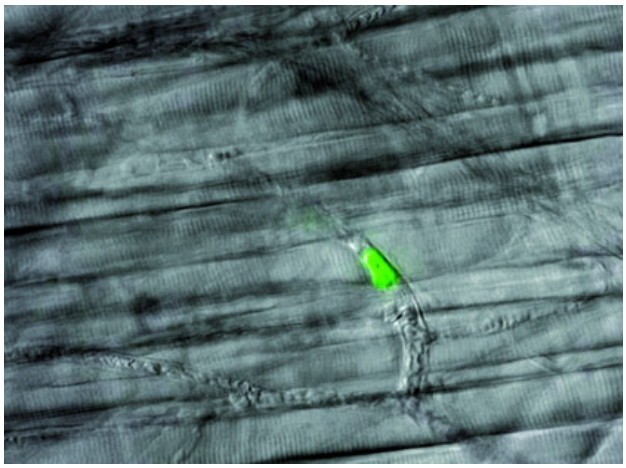
RESEARCH INTEREST

Efficient delivery of cells to heart regions is a major problem for cell therapy. Our research focuses on improving migration of mouse and human cardiac precursors to damaged heart tissue. We have found that cardiac precursors are induced to transmigrate through the endothelium by cytokines and other factors released by cardiomyocytes, among which SDF-1 is the most potent. In vivo, unstimulated GFP-tagged cardiac precursors are delivered through the femoral artery to regenerating damaged heart tissue of normal mice and mice subjected to coronary artery ligation (CAL) Quantitative PCR indicates that in vivo homing of unstimulated cardiac precursors, but pretreatment with SDF-1 increases homing threefold. Furthermore, transmigration and homing is also increased by transient expression of various surface molecules. After combined pretreatment with cytokines and surface molecules, around 50% of cardiac precursors home directly to damaged heart after intra-artery injection in CAL-treated mice. We are conducting long term experiments to assess the capacity of these modified cardiac precursors to regenerate the surface of the ventricle wall after injection into the left ventricular chamber. By defining the requirements for efficient homing of cardiac precursors to damaged heart, we aim to provide tools that will optimize cell therapy protocols for the treatment of cardiovascular diseases.



Transwell assay: Migration of stimulated cardiac precursors across the endothelium

2 Regenerative Cardiology



Intra-vital microscopy: SDF-1–stimulated GFP-tagged cardiac precursor crossing the endothelium.

MAJOR GRANTS

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

SELECTED PUBLICATIONS

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

Galvez BG, Martín NS, Salama-Cohen P, Lazcano JJ, Coronado MJ, Lamelas ML, Alvarez-Barrientes A, Eiró N, Vizoso F, Rodríguez C. **An adult myometrial pluripotential precursor that promotes healing of damaged muscular tissues.** *In Vivo* (2010) 24 :431-41

Koyanagi M, Iwasaki M, Rupp S, Tedesco FS, Yoon CH, Boeckel JN, Trauth J, Schütz C, Ohtani K, Goetz R, Iekushi K, Bushoven P, Momma S, Mummery C, Passier R, Henschler R, Akintuerk H, Schranz D, Urbich C, Galvez BG, Cossu G, Zeiher AM, Dimmeler S. **Sox2 transduction enhances cardiovascular repair capacity of blood-derived mesoangioblasts.** *Circ Res* (2010) 106: 1290-302

Barbuti A, Galvez BG, Crespi A, Scavone A, Baruscotti M, Brioschi C, Cossu G, DiFrancesco D. **Mesoangioblasts from ventricular vessels can differentiate in vitro into cardiac myocytes with sinoatrial-like properties.** *J Mol Cell Cardiol* (2010) 48: 415-23

Stem cell aging

Head of Laboratory: *Susana González*

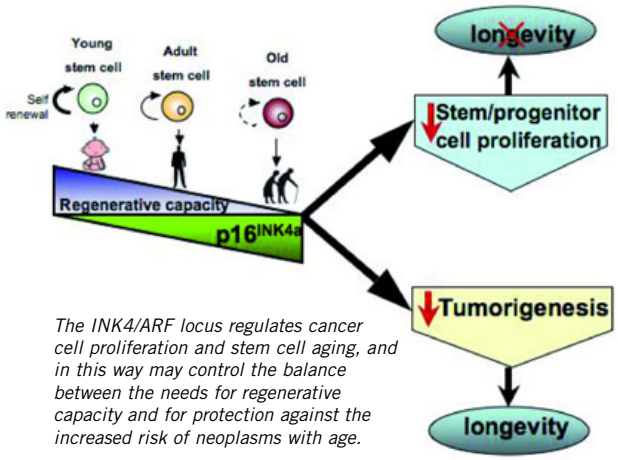
Postdoctoral Researcher: *Lorena Arranz*

Predoctoral Researchers: *Antonio Herrara Merchán*
Patricia Giraldo

RESEARCH INTEREST

The INK4b-ARF-INK4a locus encodes three tumour 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 tumour 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 tumour 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 tumour 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 neoplasms with age.

MAJOR GRANTS

- **Human Frontier Science Program Organization (HFSPO). Career Development Award**
- **Ministerio de Ciencia e Innovación. FIS (PI060627)**
- **Ministerio de Ciencia e Innovación (SAF2010-15386)**

SELECTED PUBLICATIONS

Herrera-Merchan A, Cerrato C, Luengo G, Dominguez O, Piris MA, Serrano M, González S. **miR-33-mediated downregulation of p53 controls hematopoietic stem cell self-renewal.** *Cell Cycle* (2010) 9: 3277-85

Daroca PM, Herrera-Merchán A, González S. **Insights into stem cell aging.** *Nat Rev Cardiol (CNIC Edition)* (2010) 7: 11-5

Basic Research Departments 2

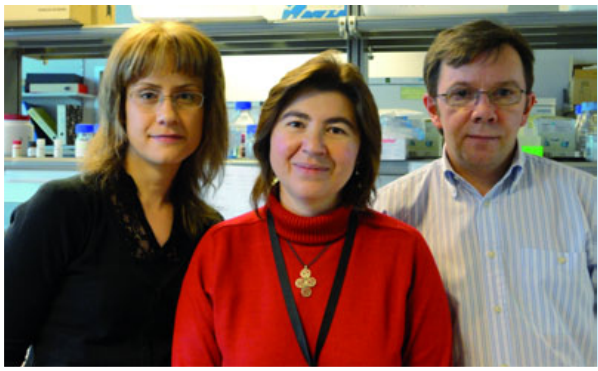
Regenerative Cardiology

Stem cell signaling

Head of Laboratory: *Kenneth J. McCreath*

Research Scientist: *Ana M. Cervera*

Postdoctoral Researcher: *Sandra Espada*

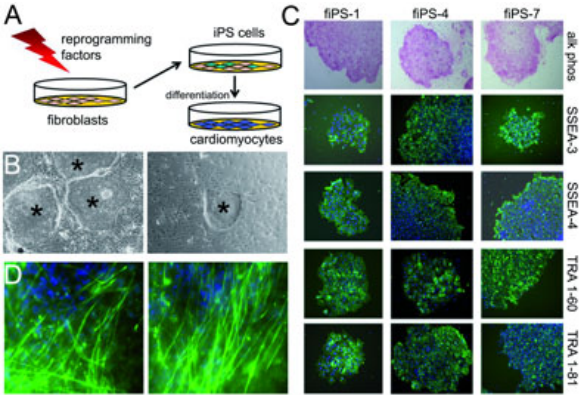


RESEARCH INTEREST

The prevailing view of mitochondria as bioenergetic facilitators has been extended by the observations that these organelles play critical roles in many cellular events. Using embryonic stem (ES) and induced pluripotential stem (iPS) cells as in vitro models we are investigating the participation of mitochondria in the maintenance of stem cell pluripotency and the capacity for differentiation. We recently showed that mitochondrial ROS, produced in high glucose cultures, are required for the differentiation of ES cell-derived cardiomyocytes. Building on these results, we are examining the regulation of microRNA (miRNA) expression during ES cell differentiation, and we are characterizing several miRNAs that are differentially expressed upon ROS depletion. In addition, we are devising protocols for the directed differentiation of human iPS cells to cardiac progenitor populations, in the hope that these cells can be used in a therapeutic context. Moreover, we are examining the possibility that stem cells derived from patients with congenital defects could provide valuable models of cardiovascular disease.

Tissue hypoxia, during cardiovascular disease, leads to increased levels of mitochondrial metabolites. Using novel loss-of-function mouse models, we are currently examining the potential of these metabolites to act as signaling molecules for cellular restoration.

Together, we expect these approaches will aid in the understanding of mitochondrial participation during both cardiovascular development and disease.



Reprogramming of fibroblasts to induced pluripotent stem (iPS) cells. *(A) Schematic representation of the reprogramming process and subsequent differentiation of iPS cells to cardiomyocytes. (B) iPS cells in culture after fibroblast reprogramming. (C) Undifferentiated surface marker expression in iPS cells. (D) Serum-driven differentiation of iPS cells leads to cardiomyocyte formation, revealed by cardiac α-actinin staining (green).*

MAJOR GRANTS

Ministerio de Ciencia e Innovación (SAF2009-07965)

SELECTED PUBLICATIONS

Luna-Crespo F, Sobrado VR, Gomez L, Cervera AM, McCreath KJ. **Mitochondrial Reactive Oxygen Species Mediate Cardiomyocyte Formation from ES Cells in High Glucose.** *Stem Cells* (2010) 28: 1132-1142

Cervera AM, Bayley J-P, Devilee P, McCreath KJ. **Inhibition of succinate dehydrogenase dysregulates histone modifications in mammalian cells.** *Mol Cancer* (2009) 8:89

Hernandez C, Santamatilde E, McCreath KJ, Cervera AM, Diez I, Ortiz-Masia D, Martinez N, Calatayud S, Esplugues JV, Barrachina MD. **Induction of trefoil factor (TFF) 1, TFF2 and TFF3 by hypoxia is mediated by hypoxia inducible factor-1: implications for gastric mucosal heaing.** *Br J Pharmacol* (2009) 156: 262-272

Basic Research Departments 2

Regenerative Cardiology

Transcriptional regulation of oxidative stress protection systems

Head of Laboratory:	*María Monsalve*
Postdoctoral Researchers:	*Nieves García-Quintáns* *Alberto Tierrez*
Predoctoral Researchers:	*Yolanda Olmos* *Cristina Sánchez* *Brigitte Wild*
Undergraduate Students:	*Sofía Cabezudo* *Javier Laso*

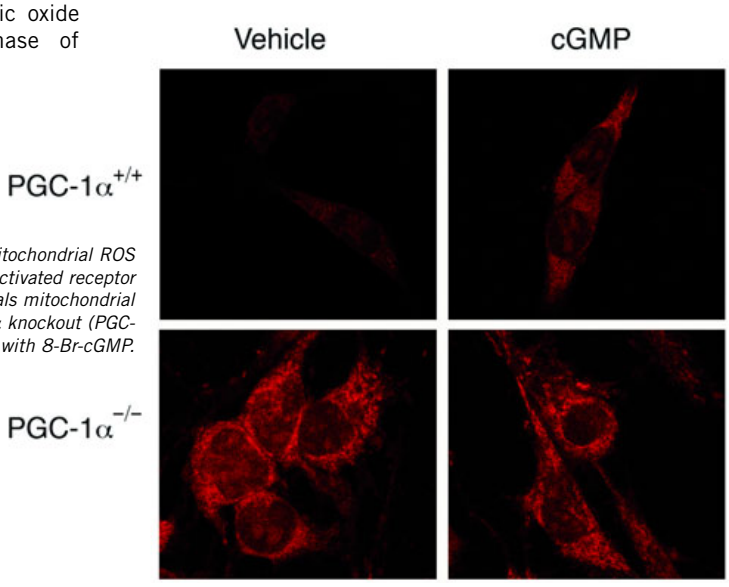
RESEARCH INTEREST

Our group studies the transcriptional mechanisms that regulate oxidative stress protection in mammals. Metabolic dysfunction and associated mitochondrial oxidative stress are emerging as primary risk factors for several major diseases, and a precise understanding of the mechanisms that control ROS detoxification will therefore be crucial for the development of new treatment strategies. We research the transcription factors involved is the regulation of the the ROS detoxification system and the impact this regulation has on human diseases, with particular emphasis on those affecting the cardiovascular system.

Our work over the last year focused on two key areas:

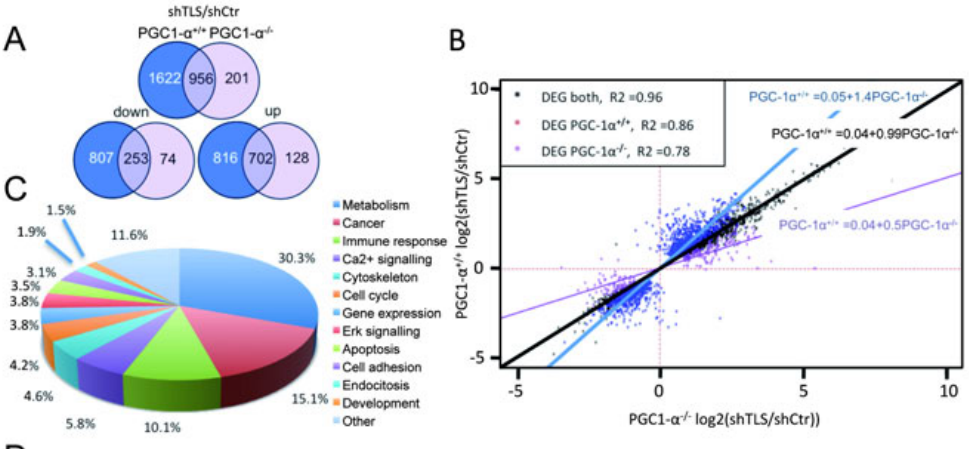
1.- Modulation of angiogenesis by ROS. We have found that when the cells lose their cell-cell contacts and produce nitric oxide, this downregulates PGC-1α levels via activation of the PI3K/AKT pathway and inactivation of the transcription factor FoxO3. The outcome is increased mitochondrial superoxide production, which is necessary for nitric oxide induced cell migration during the initial phase of angiogenesis.

2.- ROS-mediated DNA damage triggered by cell cycle arrest. The mechanisms linking ROS levels to the control of the cell cycle are still very poorly understood. We have identified the genotoxic sensor TLS (translocated in lipsarcoma) as a key regulatory component of the transcriptional complex that modulates oxidative stress protection systems via interaction with PGC-1α.



The nitric oxide/cGMP pathway cannot regulate mitochondrial ROS production in the absence of peroxisome proliferator activated receptor γ-coactivator 1α (PGC-1α). MitoSOX Red labeling reveals mitochondrial superoxide levels in wild-type (PGC-1α$^{+/+}$) and PGC-1α knockout (PGC-1α$^{-/-}$) mouse lung endothelial cells treated with 8-Br-cGMP.

2 Regenerative Cardiology



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Top 20 down regulated genes shTLS/shControl

Gene	Protein	PGC1-α+/+ Log2 (shTLS/shCtr)	Adj *p* value	PGC1-α-/- Log2 (shTLS/shCtr)	Adj *p* value
Cldn2	claudin-2	-4.7625	0.004847	-3.6807	0.022208
Hsd3b5	3-beta-hydroxy-5-ene steroid dehydrogenase	-4.1193	0.018085	-3.3091	0.060249
Anks1b	AIDA-1	-4.0950	0.000960	-2.2200	0.032002
Ubie	Ubiquinone	-3.5113	0.001586	-2.9366	0.005086
C230035I16Rik	unknown	-3.4785	0.011074	-2.6545	0.046782
Mlph	melanophilin1	-3.2921	0.006520	-3.5228	0.005622
Xpnpep2	X-prolyl aminopeptidase	-3.2513	0.020283	0.3243	0.812333
Gzmd	granzime D	-3.2398	0.038417	-1.0964	0.462563
9930111J21Rik	unknown	-3.1368	0.040939	-0.8222	0.579582
9530096D07Rik	unknown	-3.1364	0.031196	-1.4899	0.276342
NAP034039-1	unknown	-3.1317	0.016547	-1.7915	0.141157
TC1600999	unknown	-3.0842	0.015944	-2.5310	0.050064
LOC620079	unknown	-3.0761	0.023329	0.5971	0.634634
Lin54	lin-54 homolog (C. elegans)	-3.0692	0.021766	0.7468	0.133538
BC106179	unknown	-3.0238	0.014602	-2.6104	0.038926
Mrgprb3	MAS-related GPR. member B3	-3.0181	0.018809	-2.8091	0.037460
Ptscr3	Phospholipid scramblase 3	-3.0156	0.003453	-1.1615	0.101653
Entpd8	ectonucleoside triphosphate diphosphohydrolase 8	-2.9935	0.004123	-1.6588	0.068656
Il3	interleukin-3	-2.9876	0.042027	0.0169	0.992510
Anp32a	acidic nuclear phasphoprotein 32 family member A	-2.9588	0.029677	-1.2300	0.332180

Top 20 up regulated genes shTLS/shControl

Gene	Protein	PGC1-α+/+ Log2 (shTLS/shCtr)	Adj *p* value	PGC1-α-/- Log2(shTLS/shCtr)	Adj *p* value
Sox12	Transcription factor SOX	9.5002	0.326 10^{-3}	9.1412	0.411 10^{-3}
Cxxc6	TET1	6.9569	0.097 10^{-3}	6.5895	0.134 10^{-3}
Cck	Cholecystokinin	6.8334	0.123 10^{-3}	0.4568	0.781714
A_52_P877097	unknown	6.0632	0.037 10^{-3}	6.1319	0.030 10^{-3}
Olfr738	olfactory receptor 738	6.0477	0.016 10^{-3}	6.1156	0.013 10^{-3}
Fndc8	fibronectin type III domain containing 8	5.9647	0.207 10^{-3}	6.2843	0.127 10^{-3}
TC1719751	unknown	5.8899	0.063 10^{-3}	5.3262	0.119 10^{-3}
Xcr1	Chemokine XC receptor 1	5.8869	0.010 10^{-3}	6.0151	0.006 10^{-3}
Gtpbp3	GTP binding protein 3	5.8698	0.037 10^{-3}	-0.4511	0.548513
Unc5d	unc-5 homolog D	5.8242	0.505 10^{-3}	5.5297	0.710 10^{-3}
Tbc1d2	TBC1 domain family. member 21	5.7955	0.026 10^{-3}	5.7119	0.027 10^{-3}
Arl10	ADP-ribosylation factor-like 101	5.7743	0.026 10^{-3}	-1.0458	0.105760
Asb7	ankyrin repeat and SOCS box-containing protein 7	5.7627	0.016 10^{-3}	-1.0685	0.140550
AK036326	unknown	5.5209	0.044 10^{-3}	5.7643	0.030 10^{-3}
CA481501	unknown	5.5100	0.318 10^{-3}	4.6868	0.975 10^{-3}
E230015B07Rik	unknown	5.5002	0.010 10^{-3}	5.4391	0.009 10^{-3}
Adprhl1	ADP-ribosylhydrolase like 1	5.4203	0.016 10^{-3}	5.6044	0.013 10^{-3}
Terc	telomerase RNA component	5.3626	0.016 10^{-3}	-0.6817	0.611241
B230396O12Rik	unknown	5.3510	0.016 10^{-3}	5.0655	0.020 10^{-3}
A_51_P516033	unknown	5.2432	0.065 10^{-3}	5.3998	0.043 10^{-3}

TLS transcriptional activity is dependent on PGC-1α. Whole genome expression and function analysis of primary PGC-1α$^{+/+}$ and PGC-1α$^{-/-}$ hepatocytes infected with shTLS or control adenovirus.

MAJOR GRANTS

- **Ministerio de Ciencia e Innovación (SAF2009-07599)**
- **Ministerio de Ciencia e Innovación. CONSOLIDER Project (CSD2007-00020)**

SELECTED PUBLICATIONS

Monsalve M, Olmos Y. **The complex biology of FoxO.** *Current Drug Targets* (accepted)

Borniquel S, García-Quintáns N, Valle I, Olmos Y, Wild B, Martínez-Granero F, Soria E, Lamas S, Monsalve M. **Inactivation of Foxo3a and subsequent downregulation of PGC-1α mediates nitric oxide induced endothelial cell migration.** *Mol Cell Biol* (2010) 30: 4035-44

Valle I, Olmos Y, Borniquel S, Tierrez A, Soria E, Lamas S and Monsalve M. **Foxo3a is both upstream and downstream of PGC-1α in the induction of oxidative stress genes.** *J Biol Chem* (2009) 284: 14476-84.

Nuclear receptor signaling

Head of Laboratory:	*Mercedes Ricote*
Postdoctoral Researchers:	*Piedad Menéndez* *Tamás Rõszer* *Lucía Fuentes*
Predoctoral Researchers:	*Daniel Alameda* *Marta Cedenilla*
Technician:	*Vanessa Nuñez*

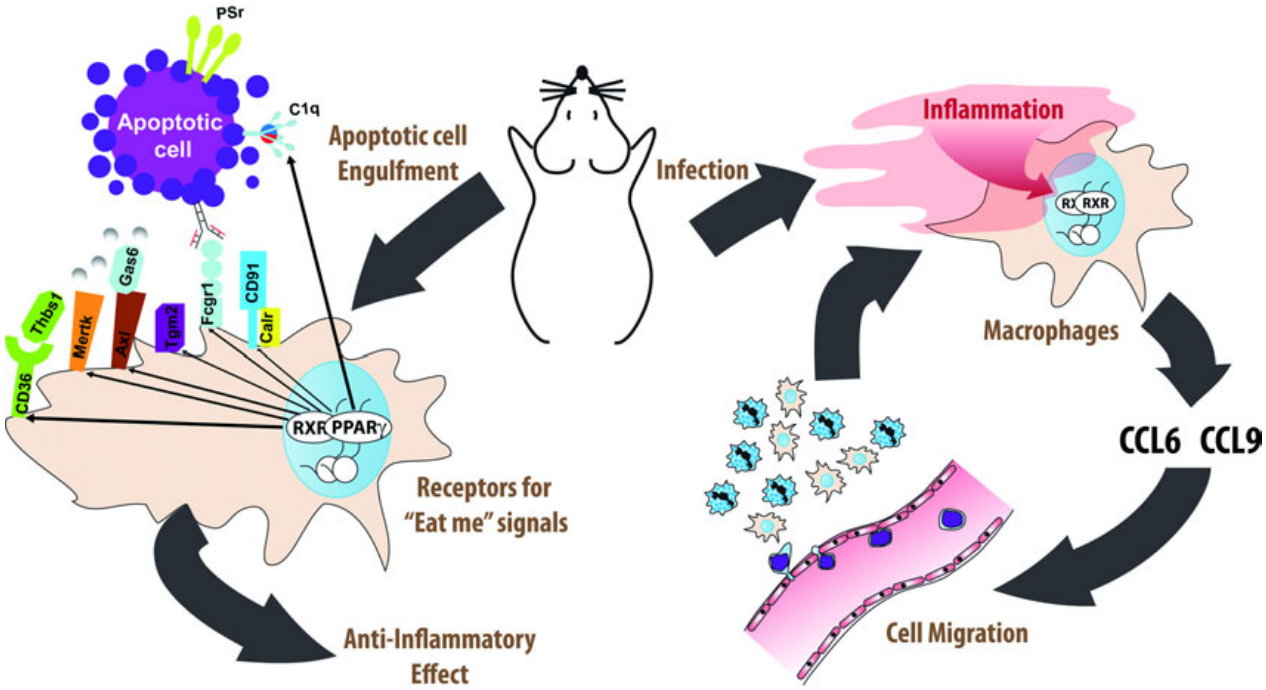
RESEARCH INTEREST

Nuclear hormone receptors (NRs) are important regulators of metabolism and homeostasis, and participate in a wide variety of biological processes including vascular and cardiac function, lipid metabolism, toxin clearance, and inflammation. Work by our group is contributing to the definition of the role of NRs in the immune response, with special emphasis on the function of NRs in tissue inflammation and the differentiation of myeloid cells from stem cell to macrophage. We are interested in the role of RXRs (retinoid X receptors) in chronic inflammatory diseases (autoimmunity, atherosclerosis and diabetes) and the homeostasis of adult stem cells.

We recently found that myeloid-specific RXRα knockout mice exhibit impaired recruitment of leukocytes to sites of inflammation and lower susceptibility to sepsis. These mice moreover develop glomerulonephritis and autoantibodies to nuclear antigens, resembling the nephritis seen in human systemic lupus erythematosus. These findings demonstrate that RXR plays a key role in the regulation of innate immunity and represents a potential target for immunotherapy of sepsis and autoimmunity. These defects eventually lead to the development of insulin resistance and cardiac hypertrophy, and we are currently trying to understand how the lack of RXRs may be involved in the development of these conditions.

Our research into adult stem cells addresses the role of NRs in the mobilization of hematopoietic stem and progenitor cell trafficking to sites of inflammation in mice. We have generated hematopoietic-specific PPARγ and RXRα,β-knockout mice, and our aim is to define the role of these nuclear receptors in tissue repair and regeneration.

2 Regenerative Cardiology



Immunomodulatory functions of RXR: two faces of the same receptor *RXR can form heterodimers with PPARγ* ***(left)*** *and regulate the expression of cell surface receptors that recognize the "eat me" signals from apoptotic cells. Efficient uptake of apoptotic cells induces an anti-inflammatory macrophage phenotype. Absence of this regulatory pathway (for example in mice lacking macrophage PPARγ or RXRα) leads to accumulation of apoptotic debris and evokes autoimmunity. RXR homodimers* ***(right)*** *are essential for the expression of chemokines and thus contribute to proper migration of macrophages to sites of inflammation.*

MAJOR GRANTS

- **Ministerio de Ciencia e Innovación (SAF 2009-07466)**
- **Fundació La Marató TV3 (MTV308)**
- **Fundación Genoma España. MEICA Project (PICPPFGE08)**
- **European Commission FP7. Marie Curie European Reintegration Grant (FP7-PEOPLE-2009-RG)**
- **European Commission FP7. Marie Curie Intra-European Fellowships for Career Development (FP7-PEOPLE-IEF-2008)**
- **Ministerio de Ciencia e Innovación. CDTI (Programa CENIT-2008 1004)**

SELECTED PUBLICATIONS

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 {alpha} controls innate inflammatory responses through the up-regulation of chemokine expression".** *Proc Natl Acad Sci U S A* (2010) 107: 10626-31

Fuentes L, Roszer T, Ricote M. **Inflammatory mediators and insulin resistance in obesity: role of nuclear receptor signaling in macrophages.** *Mediators Inflamm* (2010) 2010: 219583.

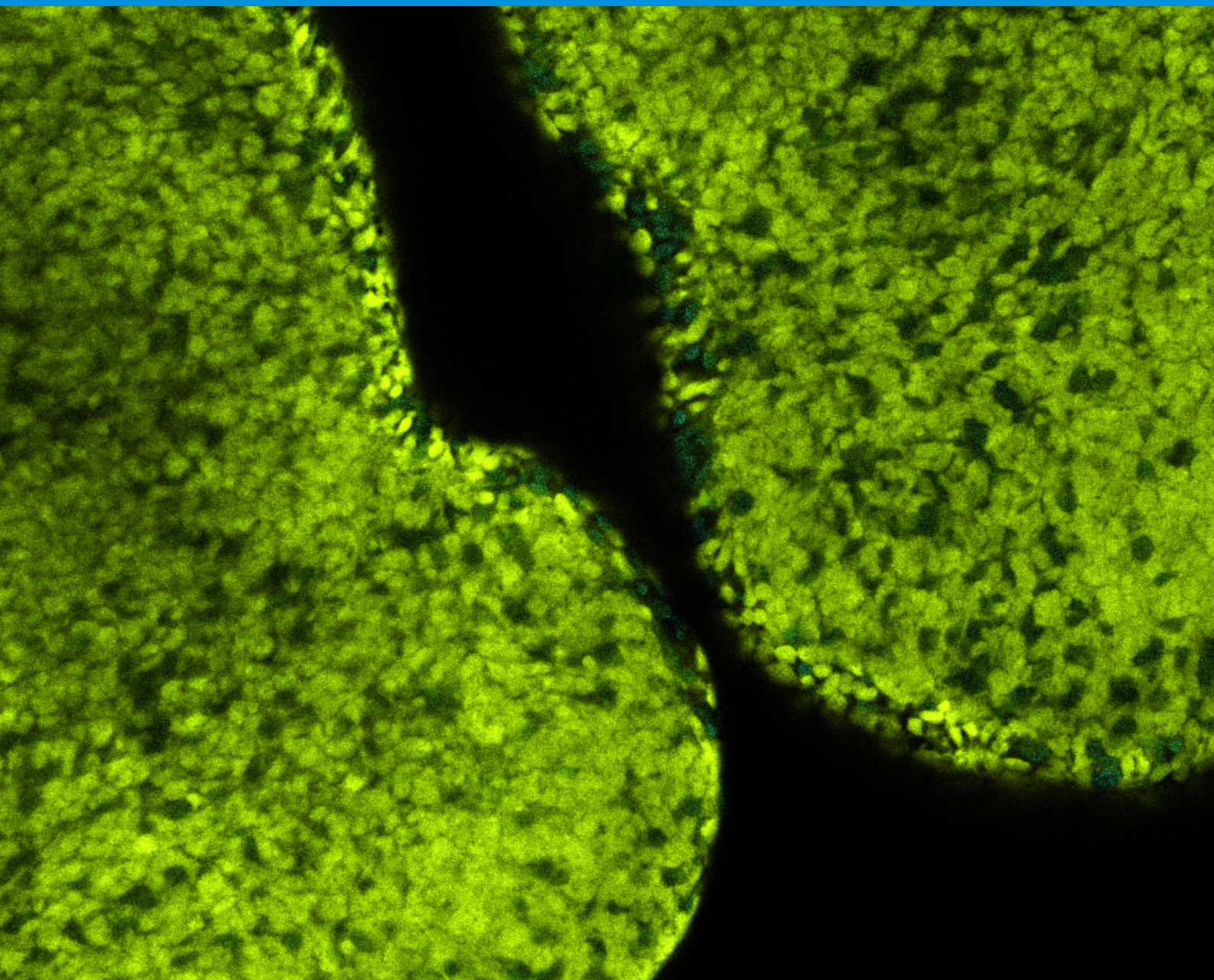
Roszer T, Ricote M. **PPARs in the renal regulation of systemic blood pressure.** *PPAR Research* (2010) 2010: 698730

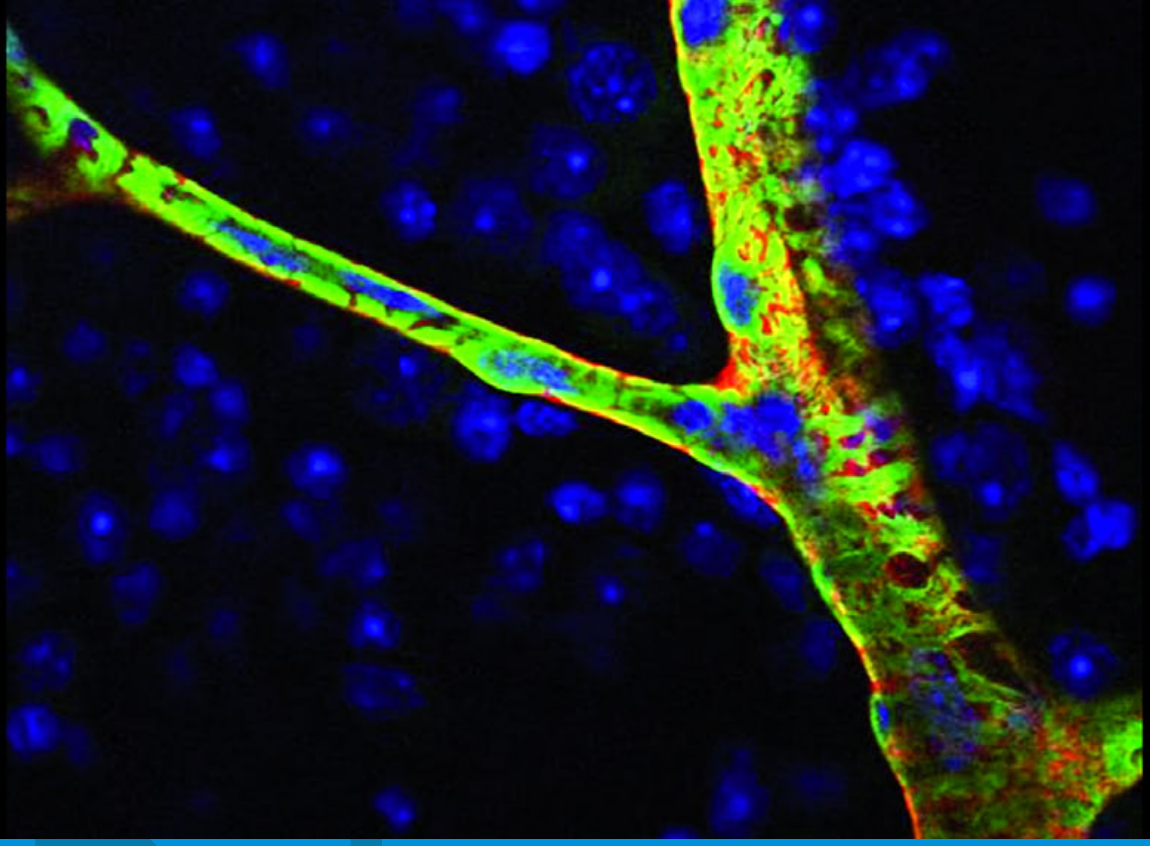
Xavier P, Roszer T, Ricote M. **Lipotoxicity in macrophages: evidence from diseases associated with the metabolic syndrome.** *Biochim Biophys Acta* (2010) 1801: 327-37

Basic Research Departments

3

Vascular Biology and Inflammation





Basic Research Departments

3 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, 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

ADMINISTRATIVE SUPPORT: *Almudena Fernández*
María Jesús de la Calle

Basic Research Departments 3

Vascular Biology and Inflammation

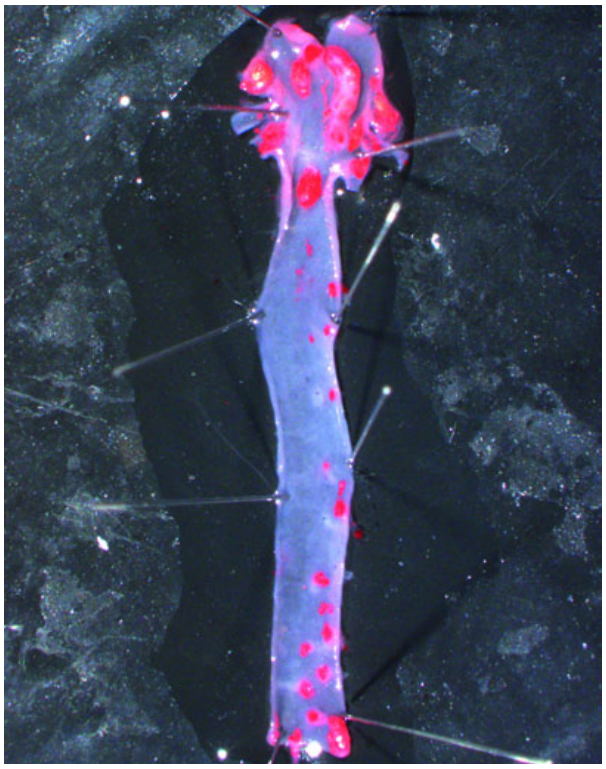
Gene regulation in cardiovascular and inflammatory diseases

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* *Ana Guio* *Gema Benito* *Beatriz Carolina Ornés*

RESEARCH INTEREST

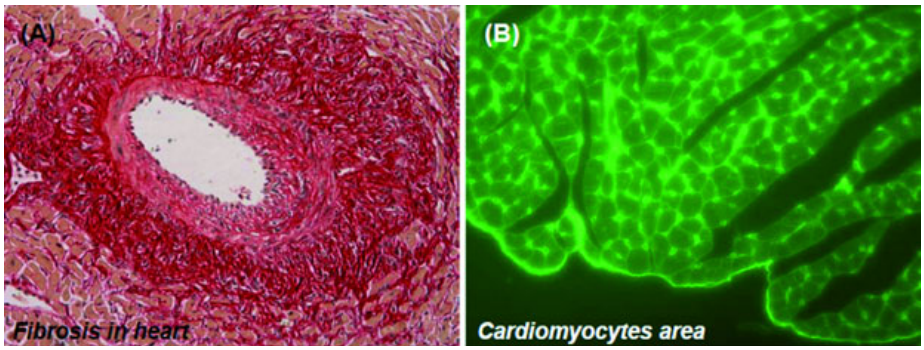
The calcium-calcineurin-NFAT (CN-NFAT) pathway regulates heart valve morphogenesis, pancreatic beta-cell function and the development of the immune, vascular and nervous systems, and is implicated in many related pathological processes. We study the regulation and function of CN-NFAT signaling in lymphocyte activation, angiogenesis and cardiac hypertrophy. Much of our work relates to molecular interactions of the phosphatase calcineurin with NFAT transcription factors and other substrates and regulators. This work has identified sequence motifs important for these interactions and sheds light on the mechanism of immunosuppressive drugs.

Our work on angiogenesis addresses the regulation of NFAT in endothelial cells by VEGF and the profile and actions of prostanoids released by activated endothelium. We use retinopathy of prematurity (ROP) 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 the role of CN in different mouse models of chronic inflammatory diseases, as well as the gene expression program triggered by angiotensin II (Ang-II) in cardiomyocytes and vascular smooth muscle and the role of CN-NFAT signaling in these processes. This work is being conducted through the use of mouse models of vascular remodeling, including inward remodeling (restenosis) and outward remodeling (aneurysm), and is shedding light on the signaling pathways involved in these diseases.

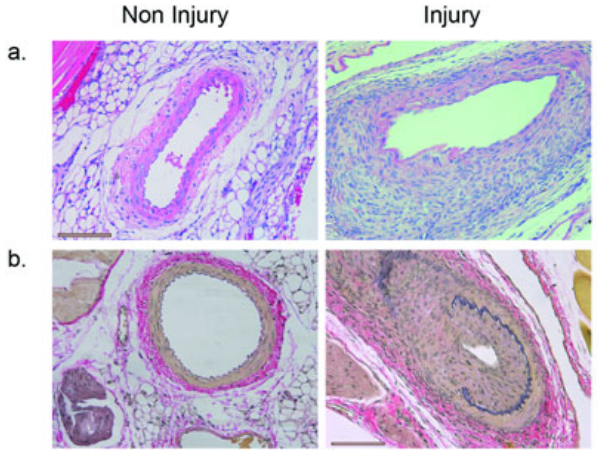


Oil red staining of atheroma plaques in the aorta of a mouse fed a high-cholesterol diet.

3 Vascular Biology and Inflammation



Ang-II causes fibrosis and hypertrophy in the heart. The images show heart sections from a mouse infused with Ang II during 20 days. Left: Picrosirius red staining showing expanded fibrous tissue surrounding heart vessels. Right: An area of hypertrophic cardiac tissue, revealed by staining for cardiomyocytes with fluorescently-labeled wheat germ agglutinin.



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)**
- **Instituto de Salud Carlos III. Red RECAVA (RD06/0014/0005)**
- **Comunidad de Madrid (S-BIO-0194-2006)**
- **Fundación Genoma España**
- **Fundació La Marató TV3 (081731)**

SELECTED PUBLICATIONS

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* (accepted)

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

Rodríguez A, Roy J, Martínez-Martínez S, López-Maderuelo MD, Niño-Moreno P, Ortí L, Pantoja D, Pineda-Lucena A, Cyert M, Redondo JM. **A conserved docking surface on calcineurin mediates interaction with substrates and immunosuppressants.** *Mol Cell* (2009) 33: 616-26 (Highlighted as "must read paper" in Faculty of 1.000)

Martínez-Martínez S, Genescà L, Rodríguez A, Salichs E, Raya A, Were F, López-Maderuelo MD, Redondo JM* and S. de la Luna* **The RCAN carboxyl-end mediates calcineurin docking-dependent inhibition via a site that dictates binding to substrates and regulators.** *Proc Natl Acad Sci USA* (2009) 14: 6117-22
*Co-corresponding author

Salvado MD, Alfranca A, Escolano A, Haeggström J, Redondo JM. **COX-2 limits prostanoid production in activated endotelial cells and is a source of PGH_2 for transcellular metabolism to PGE_2 by tumor cells.** *Arterioscler Thromb Vasc Biol* (2009) 29: 1131-7

Basic Research Departments 3

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* *Miguel Foronda*
Technicians:	*Sara Sánchez* *Dácil M. Pavón* *Teresa Osteso*

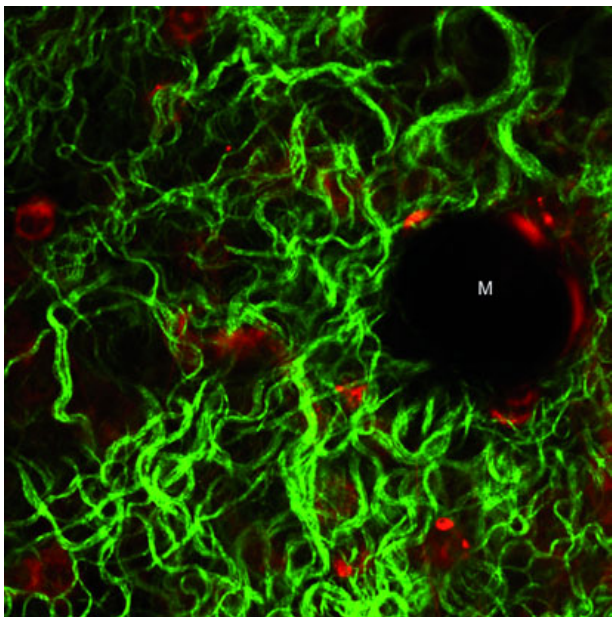
RESEARCH INTEREST

Signals are the language of life, mediating the communication essential for cells' proper behavior. Within cells, intricate networks of proteins transduce signals into the appropriate physiological response, and many diseases are caused by malfunctioning of these signal transduction networks. 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.

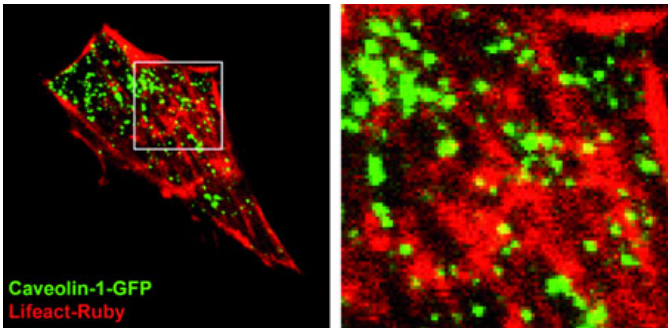
Loss of integrin-mediated adhesion triggers an inward traffic of Cav1-rich membranes, which regulates Rac1 plasma membrane (PM) targeting and hence directs cell migration and controls cell proliferation. We have now found that Rac1 can be palmitoylated, which favors its stabilization in liquid-ordered areas of the PM, inducing actin polymerization which causes the coalescence of ordered microdomains into larger regions, thus promoting PM order. Our recent work has delineated how filamin A regulates actin-linked caveolae dynamics at the PM, and shows that Cav1-membrane inward trafficking depends on actin, microtubules (MT), dynamin2, Abl kinases, the formin mDia1, and phosphorylation of filamin A by PKCα. After de-adhesion, internalized Cav1-rich rosettes are transferred to a 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 Abi1-Arp2/3-mediated branched actin polymerization. Cav1 will form caveolae as stress fibers are formed, but caveolae are flattened by high PM tension induced by excessive mDia1-actin-mediated force. To fully understand the molecular mechanisms by which integrins regulate Cav1 trafficking, we are conducting an RNAi-based high-content image analysis screen in collaboration with the Cellomics Unit.

Our work on EMT and fibrosis during chronic peritoneal inflammation has identified an inducing role for the ERK/NF-kB/Snail1 pathway, while p38 MAPK acts as a brake.

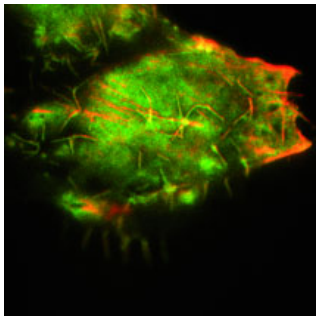


Multiphoton excitation microscopy image showing second harmonic generation (SHG, green) signal and autofluorescence (red) in intact mammary tissue from wild-type mice. The green staining reflects the degree of parallelism in collagen fibers. M=Mammary gland

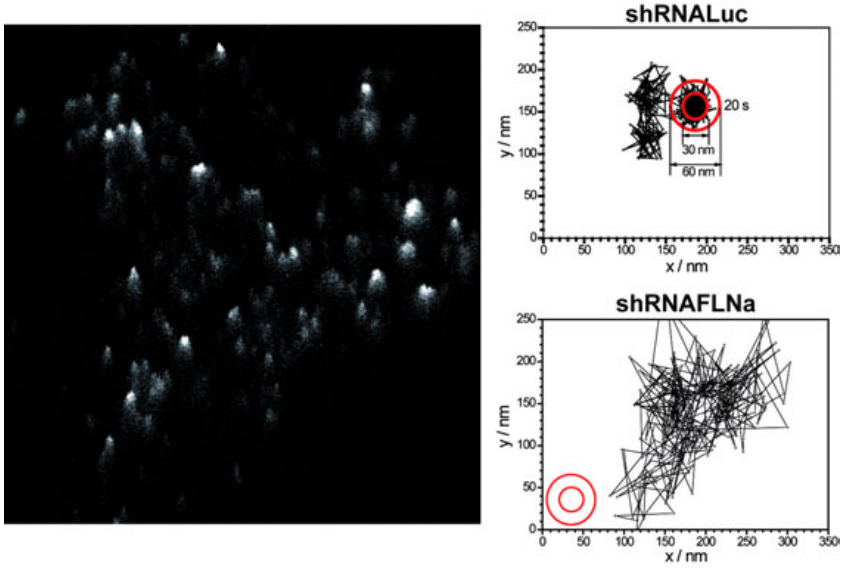
3 Vascular Biology and Inflammation



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) (PI: Asier Echarri)**
- **European Science Foundation. EURYI (*European Young Investigator Award*, 2005-2010)**

SELECTED PUBLICATIONS

Strippoli R, Benedicto I, Pérez-Lozano ML, Foronda M, Sánchez-Perales S, López-Cabrera M and del Pozo MA. **p38 maintains E-cadherin expression by modulating TAK1-NF-kB during epithelial-to mesenchymal transition** (2010) *J Cell Science* 123: 4321-31

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

Cerezo A, Guadamillas MC, Goetz J, Sánchez-Perales S, Klein E, Assoian R and del Pozo MA. **Absence of caveolin-1 increases proliferation and anchorage-independent growth by a Rac-dependent, Erk-independent mechanism.** *Mol and Cell Biol* (2009) 29: 5046-59

Strippoli R, Foronda M, López-Cabrera M and del Pozo MA. **Targeting the ERK/NF-κB/Snail1 pathway as a potential therapeutic strategy to prevent the failure of peritoneal dialysis.** *Nature Rev Cardiol* (CNIC Edition) (2009) 6: 43-8

Basic Research Departments 3

Vascular Biology and Inflammation

Matrix metalloproteinases in angiogenesis and inflammation

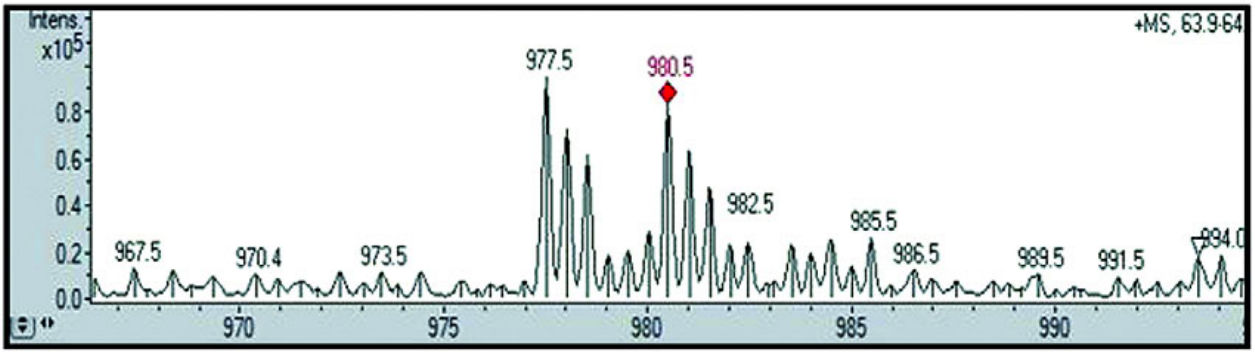
Head of Laboratory:	*Alicia G. Arroyo*
Postdoctoral Researchers:	*Pilar Gonzalo* *Rubén A. Mota*
Predoctoral Researchers:	*María Victoria Hernández de Riquer* *Agnieszka Koziol* *Mara Martín* *Vanessa Moreno*
Masters Student:	*Cristina Clemente*
Technician:	*Ángela Pollán*

RESEARCH INTEREST

Angiogenesis in adults is often coupled to inflammation, and its deregulation might contribute to the development and progression of chronic inflammatory disease. We previously reported the contribution of the matrix metalloproteinase MT1-MMP to inflammation and angiogenesis, in particular to chemokine and nitric oxide-induced angiogenesis, monocyte transmigration, and more recently to myeloid cell fusion. This latter function requires binding of the MT1-MMP cytosolic tail to the adaptor p130Cas, thereby upregulating Rac1 membrane targeting and activity. This finding indicates that the functions of MT1-MMP in inflammation are cell context-dependent, and to explore this in more depth we have conducted proteomic analyses to identify the collection of cellular substrates (degradome) processed by MT1-MMP in endothelial cells and leukocytes. We have also applied this approach to MT4-MMP, a GPI-anchored MMP whose substrates and functions are poorly understood. Preliminary data point to specific and unexpected functions for these proteases in the interplay between inflammation and angiogenesis.

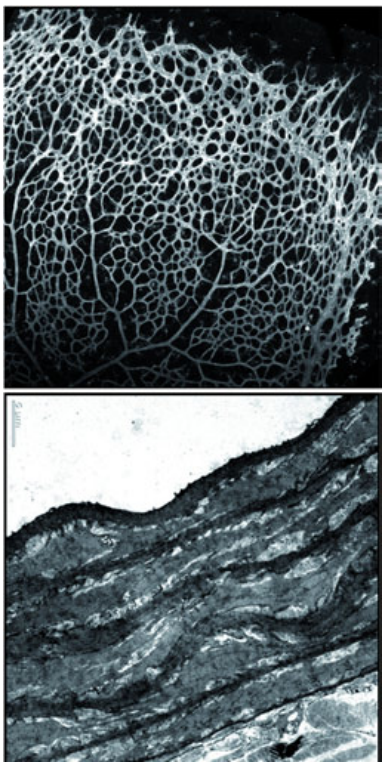
We are using the novel molecular information generated by the proteomic analysis to explore key events in inflammation-driven angiogenesis, such as the induction of endothelial tip cells and the decision between stabilization and regression of the new vasculature, and how these processes are linked to the phenotype of macrophages and other components of the inflammatory infiltrate. These functional studies are conducted in cell-based systems and in genetically modified mouse models of angiogenesis and inflammatory disorders such as atherosclerosis. We are also characterizing the role of more recently identified players in vascular integrity and angiogenesis, mainly MT-MMP substrates such as extracellular matrix metalloproteinase inducer (EMMPRIN).

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

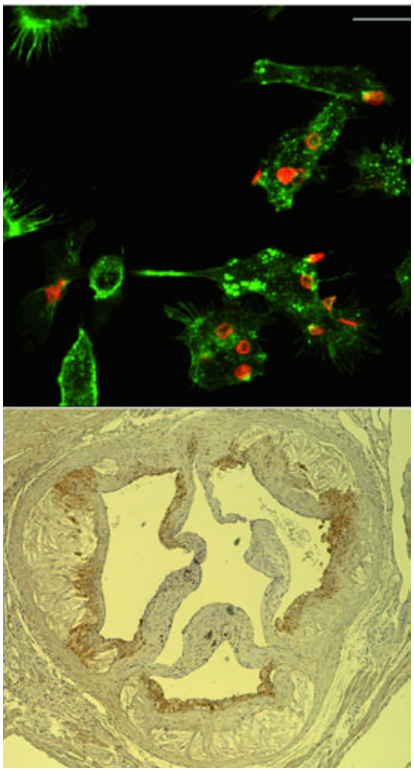


SILAC (stable isotype labeling of aminoacids in culture) *is a quantitative proteomic approach that we are using to identify the degradome of specific proteases in cell types involved in inflammation and angiogenesis. The figure shows a mass spectrum obtained from an actin peptide labeled with heavy or light amino acids.*

3 Vascular Biology and Inflammation



Analysis of angiogenesis and vascular homeostasis. *Top: Whole-mount staining with isolectin B4 reveals active angiogenesis in mouse retinas seven days postpartum. Bottom: Electron micrograph showing the ultrastructure of the mouse aortic wall.*



Macrophages and inflammation. *Top: Mouse peritoneal macrophages (green) can engulf sheep red blood cells (red) by phagocytosis. Bottom: Mac-3 staining reveals the presence of macrophages in atherosclerotic plaques in LDLR $^{-/-}$ mice fed a high fat diet.*

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**

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 and 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 and Ricote M. **Retinoid X receptor alpha controls innate inflammatory responses through the up-regulation of chemokine expression.** *Proc Natl Acad Sci U S A* (2010) 107: 10626-31

Basic Research Departments 3

Vascular Biology and Inflammation

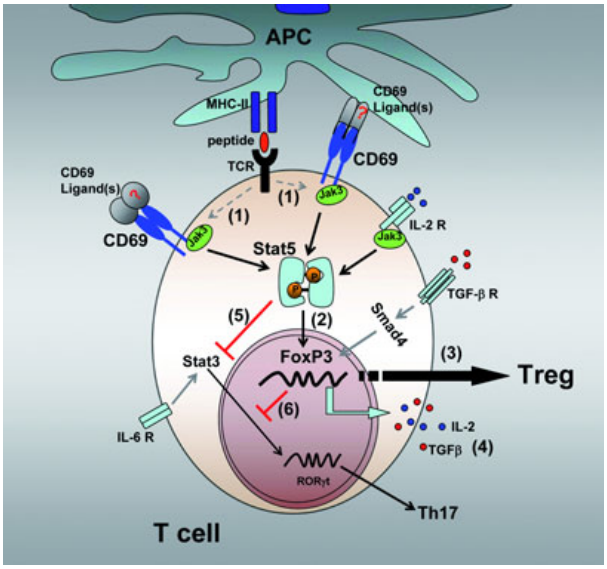
Regulatory molecules of inflammatory processes

Head of Laboratory:	*Pilar Martín*
Postdoctoral Researcher:	*José Rodríguez Cortés*
Predoctoral Researchers:	*Aránzazu Cruz Adalia* *Adela Matesanz Marín*
Visiting Scientist:	*César Augusto Henríquez Camacho*

RESEARCH INTEREST

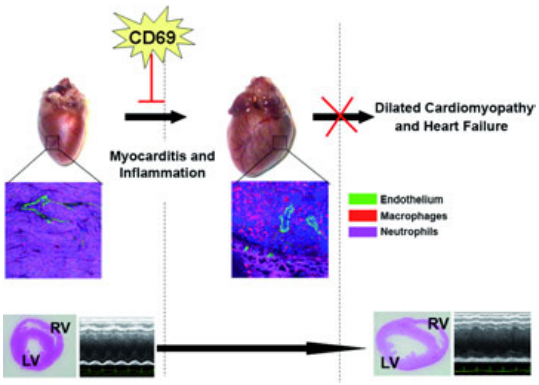
Understanding peripheral mechanisms operating in autoinmmune and chronic inflammatory diseases is critical for the design and development of novel therapies against these immunological disorders. Autoimmune diseases are characterized by a breakdown in the mechanisms of tolerance to self antigens. Autoimmune diseases, which include conditions such as arthritis, asthma, contact dermatitis and myocarditis, affect millions of people worldwide, 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 ascribed to the family of type II C-type lectins. It 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 inflammatory sites. Engagement of CD69 with monoclonal antibodies in the presence of phorbol esters induces Ca^{2+} influx that leads to the activation of ERK, induction of IL-2 and IFN-γ genes, and 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, through the regulation of Th17 effector responses, limits myocardial inflammation and subsequent heart failure. It is likely that a similar process occurs in humans with myocarditis and subsequent dilated cardiomyopathy. These findings reveal the involvement of a novel molecular actor in the immunopathogenesis of myocarditis, which could be a potential therapeutic target.

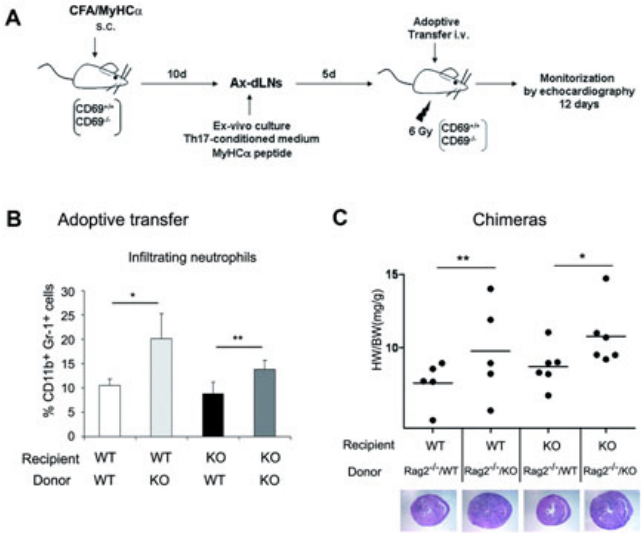


CD69 receptors are expressed on the membrane of T cells following activation (1). The cytoplasmic tail of CD69 associates with Jak3 and Stat 5 proteins, triggering phosphorylation of Stat5 and its translocation to the nucleus (2) where it can activate the transcription factor FoxP3, stimulating the differentiation of regulatory T cells (3). CD69 engagement can also induce expression of IL-2 and TGF-β. These cytokines may act in an autocrine manner to induce the differentiation of regulatory T cells (4). CD69 can inhibit the Th17 differentiation pathway through at least two mechanisms: CD69-activated Stat5 directly inhibits the translocation of Stat3 to the nucleus (5) and indirectly, via FoxP3 activation, antagonizes Stat3-mediated RORγt activation (6). APC, antigen presenting cell; TCR, T cell receptor; Treg, regulatory T cell; P, phosphorylation.

3 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.*



Adoptively transferred CD69$^{-/-}$ Th17 cells can induce severe myocarditis in WT mice. (A) *WT and CD69$^{-/-}$ Th17 cells were produced by sensitizing mice to MyHC-α peptide followed by isolation from axillary-draining lymph nodes (AX-dLNs) and in vitro derivation. The Th17 cells were then injected into either WT or CD69$^{-/-}$ recipient mice.* ***(B)*** *Analysis of inflammation in recipient hearts. Bars represent the proportion of infiltrating neutrophils (CD11b^{+} and Gr-1^{+}) in the myocardium 12 days after Th17 cell transfer.* ***(C)*** *CD69 WT and KO mice were lethally irradiated and reconstituted with a mix of bone marrow cells from RAG2$^{-/-}$ plus CD69$^{-/-}$ or RAG2$^{-/-}$ plus CD69$^{+/+}$ mice. Heart weight/body weight (HW/BW) ratios of individual chimeric mice after the induction of EAM are shown as dots; horizontal bars represent means. Representative myocardial cross sections are shown below the chart. Data correspond to the arithmetic mean and SD (n=6), and p values are indicated (one-way ANOVA and Bonferroni multiple comparisons test).*

MAJOR GRANTS

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

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Cruz-Adalia A, Jiménez-Borreguero LJ, Ramírez-Huesca M, Chico-Calero I, Barreiro O, López-Conesa E, Fresno M, Sánchez-Madrid F, Martín P. **CD69 limits the severity of cardiomyopathy after autoimmune myocarditis.** *Circulation* (2010) 122: 1396-404

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
*Joint 1st authors

Barreiro O*, Martín P*, González-Amaro R and Sánchez-Madrid F. **Molecular cues guiding the inflammatory responses.** *Cardiovasc Res* (2010) 86: 174-82
*Joint 1st authors

Sandoval P, Loureiro J, González-Mateo G, Pérez-Lozano ML, Maldonado-Rodríguez A, Sánchez-Tomero JA, Mendoza L, Santamaría B, Ortiz A, Ruíz-Ortega M, Selgas R, Martín P, Sánchez-Madrid F, Aguilera A and López-Cabrera M. **PPAR-γ agonist Rosiglitazone protects peritoneal membrane from dialysis fluid-induced damage.** *Lab Invest* (2010) 90: 1517-32

Basic Research Departments 3

Vascular Biology and Inflammation

Stress kinases in diabetes, cancer and cardiovascular disease

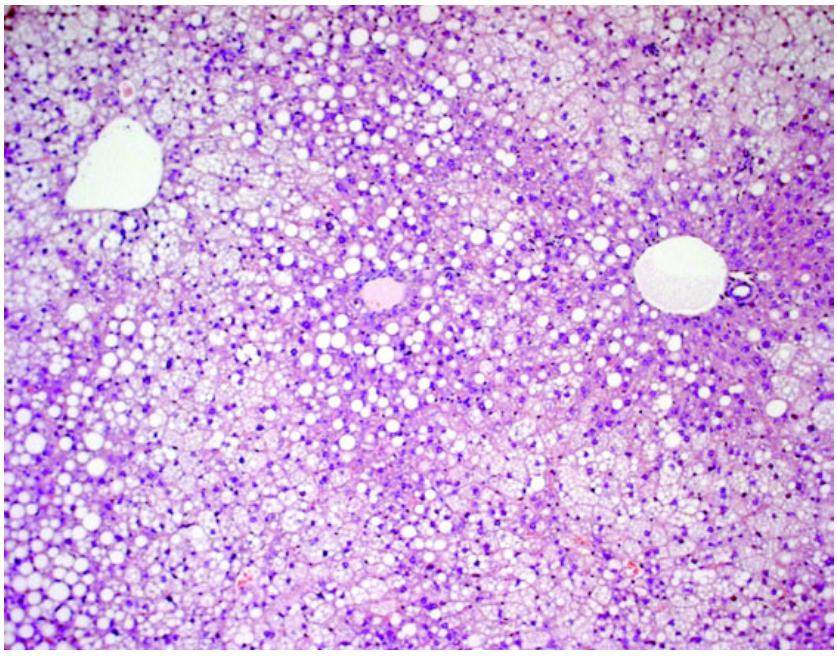
Head of Laboratory:	*Guadalupe Sabio*
Postdoctoral Researcher:	*Nuria Matesanz*
Predoctoral Researchers:	*María Ángeles Verdugo* *Elisa Manieri* *Bárbara González* *Edgar Bernardo*
Technician:	*Luis Leiva*

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.

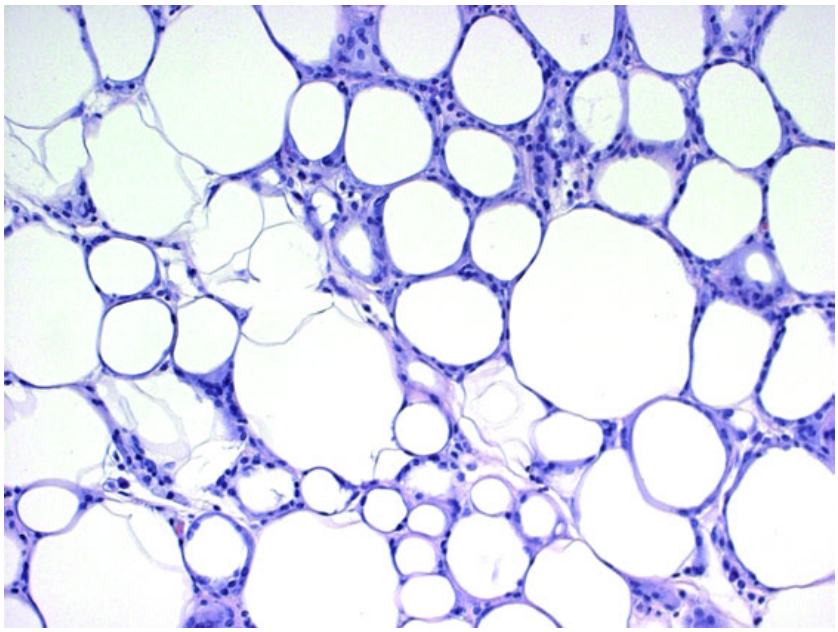
The high cardiovascular risk associated with metabolic syndrome and type 2 diabetes suggests that common mechanisms are involved in the etiology of these conditions, and that disease parameters in both, might be improved by agents acting on the same therapeutic targets. Evidence from basic research suggests 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 genome 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 high-fat diet for 16 weeks.

3 Vascular Biology and Inflammation



H&E-stained section of epididymal fat from C57Bl6/J mice fed a high-fat diet for 16 weeks.

MAJOR GRANTS

- **European Commission. European Research Council Starting Independent Researcher Grant (ERC-StG-260464)**
- **European Foundation for the Study of Diabetes (EFSD 0203)**
- **Papel de la obesidad en el desarrollo del cáncer hepático. Lóreal-Unesco**

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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

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Sabio G, Cavanagh-Kyros J, Ko HJ, Jung DY, Gray S, Jun JY, Barrett T, Mora A, Kim JK, Davis RJ. **Prevention of steatosis by hepatic JNK1.** *Cell Metab* (2009) 10: 491-8

Basic Research Departments 3

Vascular Biology and Inflammation

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

Program Director:	*Francisco Sánchez-Madrid*
Postdoctoral Researchers:	*Olga Barreiro* *Hortensia de la Fuente* *Noa B. Martín Cofreces* *Gloria Martínez del Hoyo* *María Mittelbrunn* *Vera Rocha*
Predoctoral Researchers:	*Francesc Baixauli* *Aránzazu Cruz* *Cristina Gutiérrez* *Giulia Morlino* *Mónica Sala-Valdés* *Norman Núñez* *Emilio Tejera* *Carolina Villarroya*
Technicians:	*Marta Ramírez* *María José López*

RESEARCH INTEREST

Intercellular communication is of critical importance for the innate and adaptive immune responses. Our group is interested in deciphering key communicative events during central processes of the immune response such as antigen presentation for T cell activation (immune synapse) and leukocyte trans-endothelial migration.

Cell-cell synapses are an exquisitely evolved mode of intercellular communication that is essential for neural and immune system functionality. The immune synapse (IS) is a transient, highly-specific and highly-ordered structure formed at the T cell–antigen-presenting cell (APC) interface through the reorganization of transmembrane and membrane-associated molecules. The tubulin cytoskeleton is rapidly directed toward the center of the IS through the translocation of the microtubule-organizing center (MTOC). This MTOC polarization brings the secretory apparatus into close apposition with the APC, thus providing the basis for polarized secretion. We are currently investigating the functional consequences of horizontal transfer of RNA-harboring exosomes from T cells to APCs at the IS, the role of the micro-RNA machinery in T cell activation, and the mechanisms of selective sorting of micro-RNAs and mRNAs into exosomes during cognate interactions. We are also investigating horizontal transfer of genetic information during leukocyte-endothelium interactions.

MTOC translocation is also a mechanism for macromolecule transport and the nucleation of signaling and adapter molecules. We are interested in the regulation of MTOC-dependent mitochondrial polarization to provide a localized bioenergetic source for cytoskeletal rearrangements and exosomal delivery, in particular the role of the microtubule-polymerization promoter EB1. In addition, we are studying the role of the tubulin deacetylase HDAC6, a regulator of MTOC translocation, in inflammatory immune responses.

Another area of interest is the role of immunoregulatory molecules such as tetraspanins CD9 and CD81 and galectins 1, 3 and 9 in autoimmune disease. This is studied in models of two frequent autoimmune diseases: psoriasis, which is a Th1/Th17 inflammatory skin disease, and allergic asthma, which is mainly a Th2 chronic inflammatory disease. We also study the inflammatory response in a model of inflammation based on contact hypersensitivity, and have found in vivo imaging to be fundamental to our understanding in this area.

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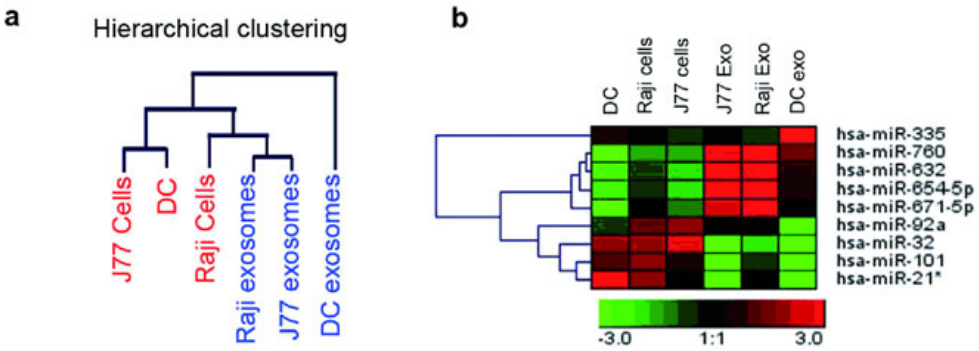
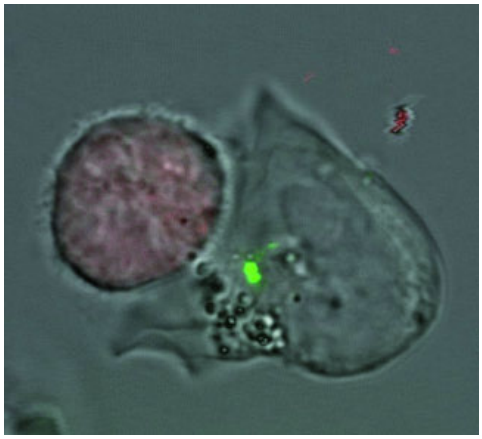
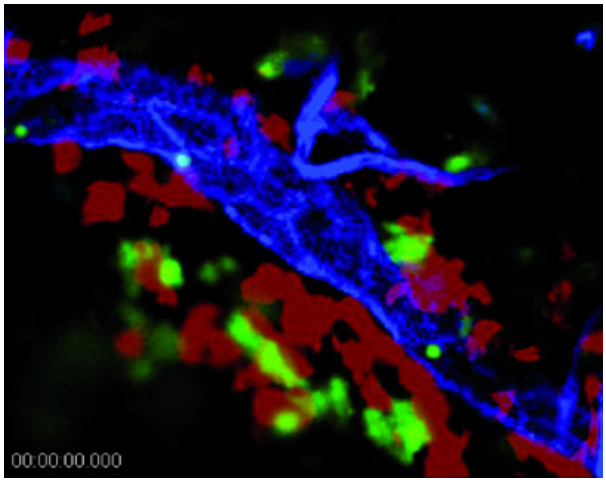


***a,** Microarray analysis of exosomal miRNAs and the miRNAs of their respective donor cells. The panel shows the hierarchical clustering of the vsn-normalized array data in the log2 scale averaged per biological replicate for each origin (exosomes/cells) and cell type (DC: dendritic cells; J77: Jurkat-derived J77 T cell line and Raji: Raji B cell line). **b,** Heatmap of the vsn-normalized data for selected miRNAs.*



Disruption of AKAP450 function impairs MTOC translocation towards the immune synapse. Cell conjugates were formed between J77 cells overexpressing C-terminally GFP-tagged AKAP450 (green) and SEE-pulsed Raji APCs (red). MTOC position (GFP signal) was monitored by confocal fluorescence microscopy.



Intravital microscopy image showing leukocyte-endothelium interactions in an inflamed area (mouse dermis). To allow visualization, we adoptively transferred GFP+ hematopoietic cells to a C57Bl6 recipient mouse, in which the vasculature was traced in blue using an anti-CD31 antibody and perivascular cells were stained red.

MAJOR GRANTS

- 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

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Robles-Valero J, Martín-Cófreces NB, Lamana A, Macdonald S, Volkov Y, Sánchez-Madrid F. **Integrin and CD3/TCR activation are regulated by the scaffold protein AKAP450.** *Blood* (2010) 115: 4174-84

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Martín P, Gómez M, Lamana A, Cruz-Adalia A, Ramírez-Huesca M, 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

Martin P, Gómez M, Lamana A, Marín AM, Cortés JR, Ramírez-Huesca M, Barreiro O, López-Romero P, Gutiérrez-Vázquez 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

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* (accepted)

Basic Research Departments 3

Vascular Biology and Inflammation

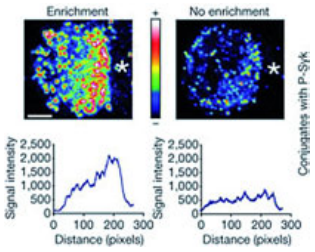
Immunobiology of inflammation

Head of Laboratory:	*David Sancho Madrid*
Predoctoral Researcher:	*Noelia Blanco Menéndez*
Masters Student:	*María Martínez López*
Technician:	*Helena María Izquierdo Fernández*

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.

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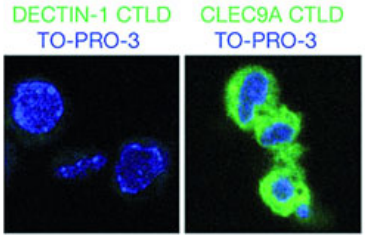

CLEC9A ligand is preformed and intracellular. Fixed and permeabilized mouse embryonic fibroblasts were labeled with the ligand binding domain monomers DECTIN-1 (negative control) or CLEC9A, in green, and counterstained with TO-PRO-3 (nuclear dye) before confocal microscopy. Original magnification, x630.

MAJOR GRANTS

- European Commission. European Research Council Starting Independent Researcher Grant (ERC-StG-260414)

- Ministerio de Ciencia e Innovación (RYC2009-04235)

SELECTED PUBLICATIONS

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

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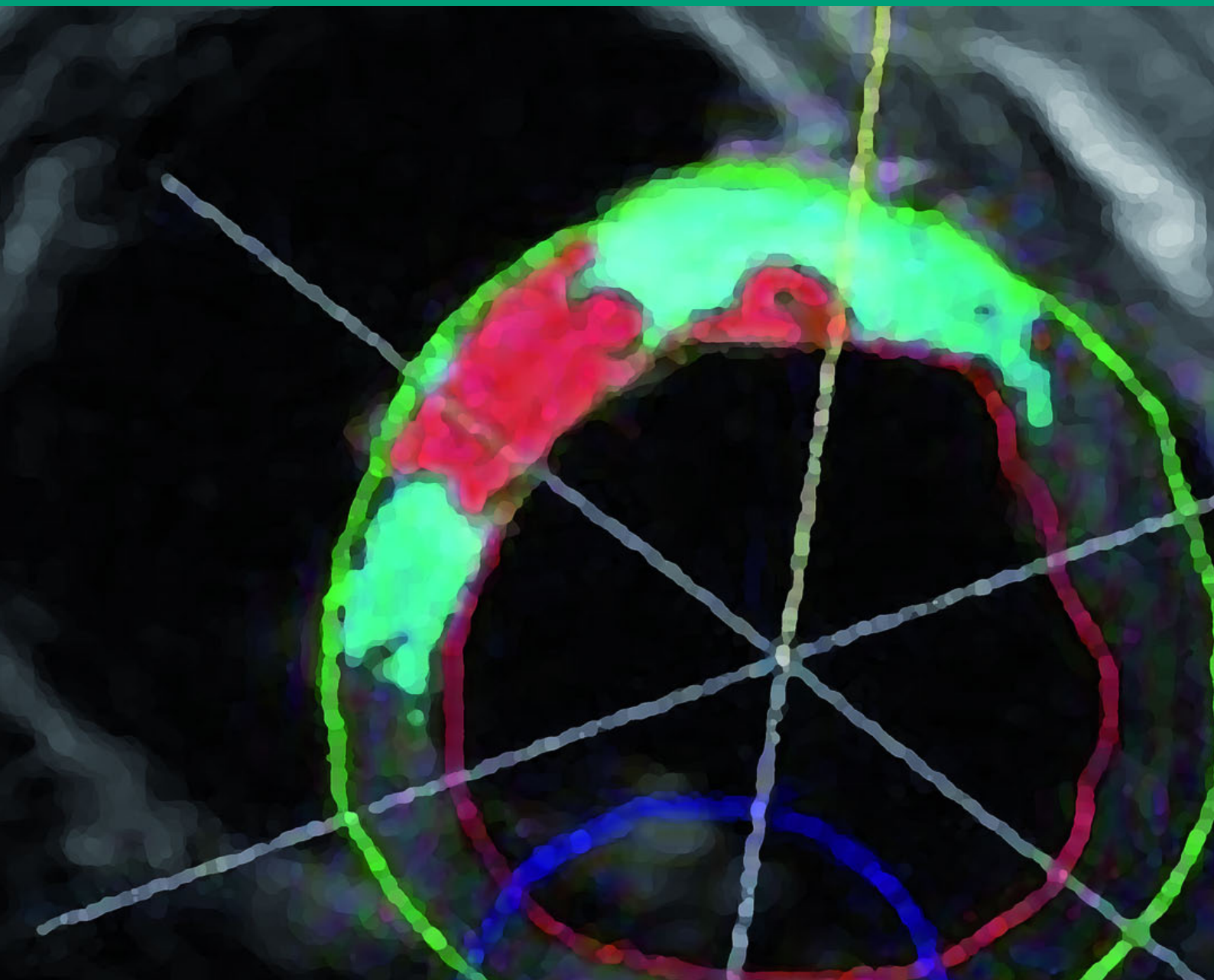
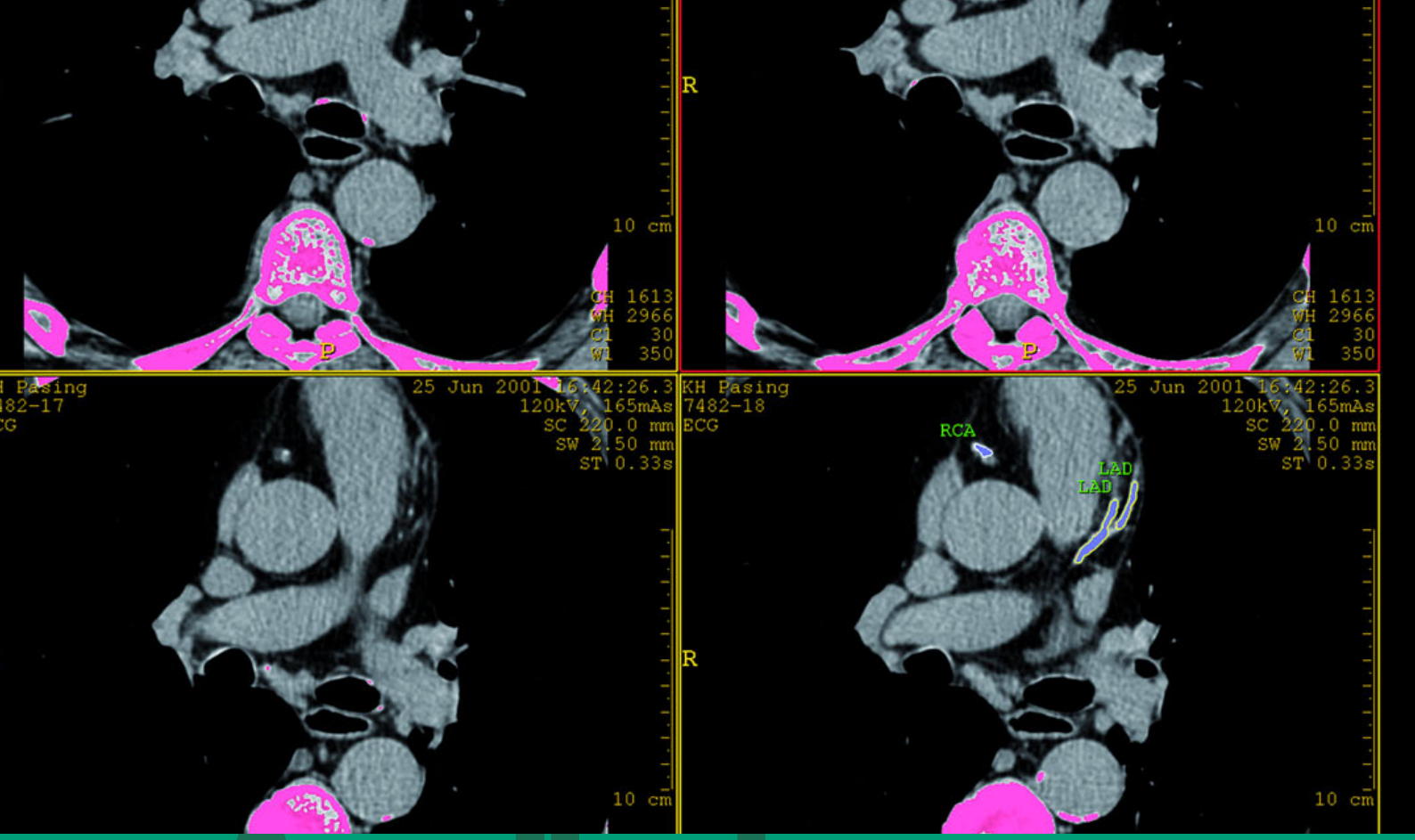
Sancho D, Joffre OP, Keller AM, Rogers NC, Martínez D, Hernanz-Falcón P, Rosewell I, Reis e Sousa C. **Identification of a dendritic cell receptor that couples sensing of necrosis to immunity.** *Nature* (2009) 458: 899-903

Applied Research Departments

Multi-Departamental Clinical Projects





Applied Research Departments

Cardiovascular Translational Research

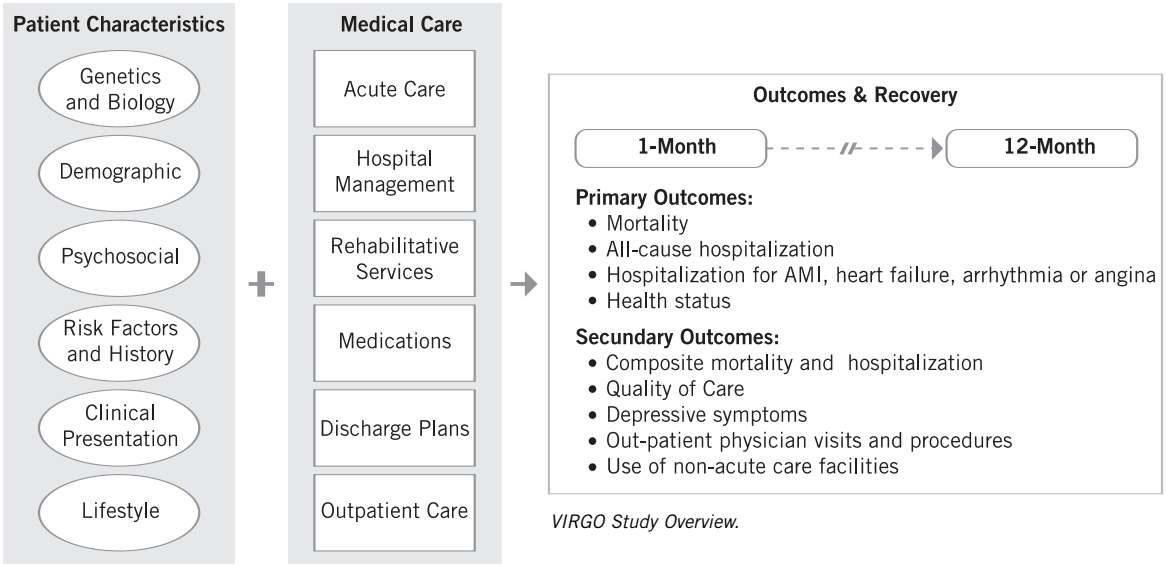
Atherosclerosis is the underlying cause of most cardiovascular disease, the leading cause of death worldwide. Atherosclerotic disease progressively damages vital organs and vascular areas, leading to clinical conditions such as peripheral vascular disease, myocardial infarction and stroke. Aside from the human cost, atherosclerotic disease also incurs a high economic cost. Atherosclerotic lesions evolve subclinically over several decades, and diagnosis is currently only possible once these catastrophic conditions have already appeared. The CNIC's main efforts in applied research are therefore aimed at improving diagnosis through the use of the latest imaging technologies and at testing the efficacy of new treatments.

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 may be 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 is 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 already recruited 395 patients in 24 hospitals in Spain, and we are well on our way to completing recruitment on schedule. IMJOVEN is coordinated by the Department of Translational Research 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.



VIRGO Study Overview.

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 2010, study participants underwent a standardized clinical exam, laboratory assays, and collection of biological samples including serum, plasma, whole blood, urine and DNA.

A medical imaging facility has been established to allow further exploration of the presence of subclinical atherosclerosis in these participants. Over the next three years, 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. In addition, 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); and "DNA methylation and the association of cadmium exposure with chronic kidney disease in a population-based occupational study" (Johns Hopkins NIEHS

PESA, CNIC- Santander

PROGRESSION OF EARLY SUBCLINICAL ATHEROSCLEROSIS, CNIC-SANTANDER

Strategies to identify individuals with subclinical alterations indicating increased risk of cardiovascular disease have been boosted by the recent development of 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 over 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.

Participants are first assessed with basic imaging techniques, including CT imaging to estimate coronary calcium, 3D ultrasound of carotid artery, and 2D ultrasound measurement of abdominal aorta and the rate of ankle-brachial pressure. These techniques are used for the early diagnosis of individuals with subclinical atherosclerosis. Participants are then studied with two advanced imaging techniques: magnetic resonance imaging (MRI) and positron emission tomography (PET). These advanced techniques will help determine participants' atherosclerotic burden and monitor its progression and the presence and progression of inflammation in atherosclerotic plaques.

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 peri-menopause and its relation to cardiovascular risk factors and hormonal changes.

The PESA CNIC-Santander 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.

Polypill/FOCUS

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 fixed dose drug combinations (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 combination for secondary prevention. The CNIC-Ferrer polypill project is led by Valentín Fuster and is coordinated by the Translational Research Department.

During the last year we have conducted several clinical trials to ensure the quality and safety of the polypill. A new study to explore the potential pharmacodynamic interactions with simvastatin was launched in Spain. The kick-off meeting of the FOCUS project, which tests the fixed-dose combination concept for cardiovascular prevention in populations with different socio-economic characteristics, was held in Madrid last June. Patient recruitment will begin during the first half of 2011. An important aim of FOCUS is to understand the factors that determine poor treatment adherence and inappropriate prescribing for secondary cardiovascular prevention. This will allow FOCUS to establish recommendations for better use of medication in patients with ischemic heart disease. After the successful completion of FOCUS, 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).

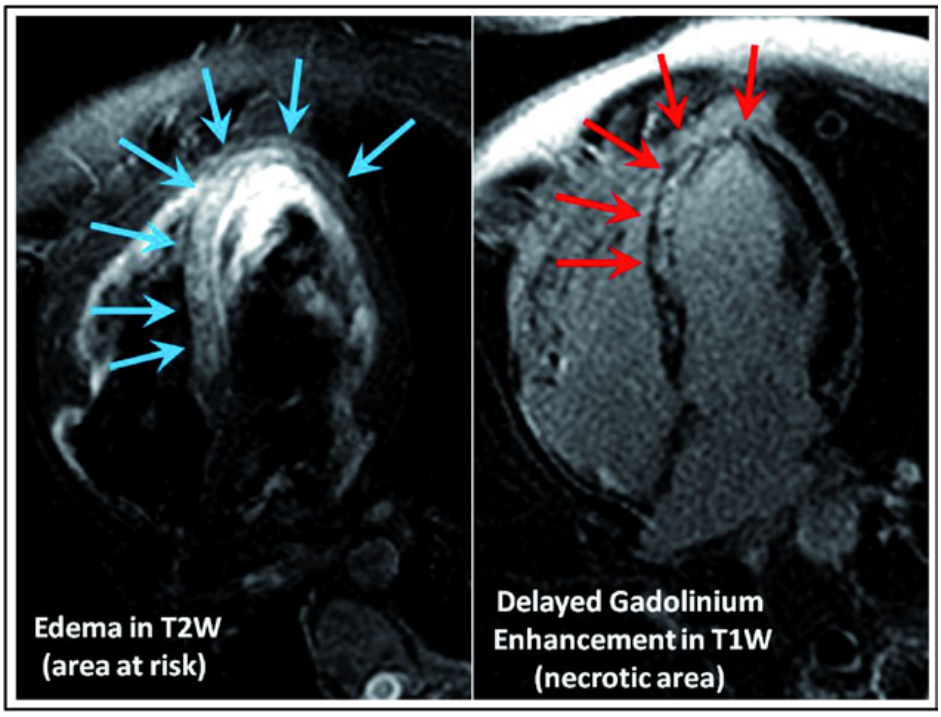
METOCARD-CNIC

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 the 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. In particular, whether early β-blocker administration is able to reduce infarct size is a subject of debate. 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. Patients are currently being recruited in cities across Spain in close collaboration with emergency medical services and hospitals. The main endpoints of this trial will be evaluated by innovative magnetic resonance imaging protocols developed at the CNIC Imaging Facility.



Imaging of human heart after an acute myocardial infarction.

Apical four chamber view of a human heart six days after an acute myocardial infarction. Left panel depicts edema (without contrast infusion). Arrows delineate the at-risk area of the left ventricle. Right panel shows the necrosed area (after Gadolinium contrast injection). Arrows delineate the necrotic area of the left ventricle. Note that the at-risk area is larger than the necrotic area, providing evidence of cardioprotected areas (areas at risk but with no necrosis). (Picture provided by Gonzalo Pizarro, Leticia Fernández-Friera et al., unpublished data.)

Applied Research Departments

Multi-Departamental Clinical Projects

METOCARD-CNIC team:

- Borja Ibáñez, (PI)
- Valentín Fuster, Carlos Macaya, Jesús Jiménez-Borreguero (Co-PIs)

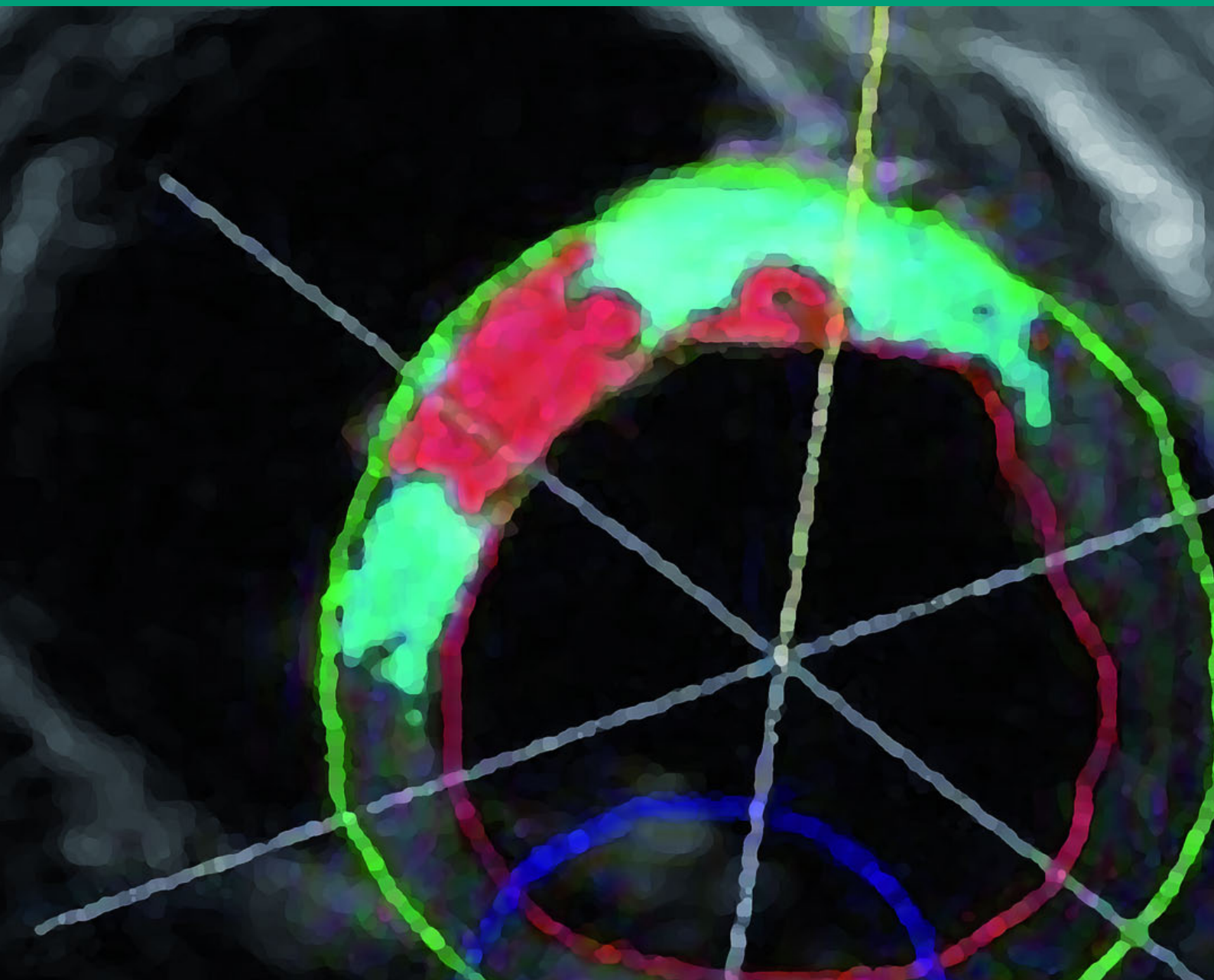
Other relevant investigators:

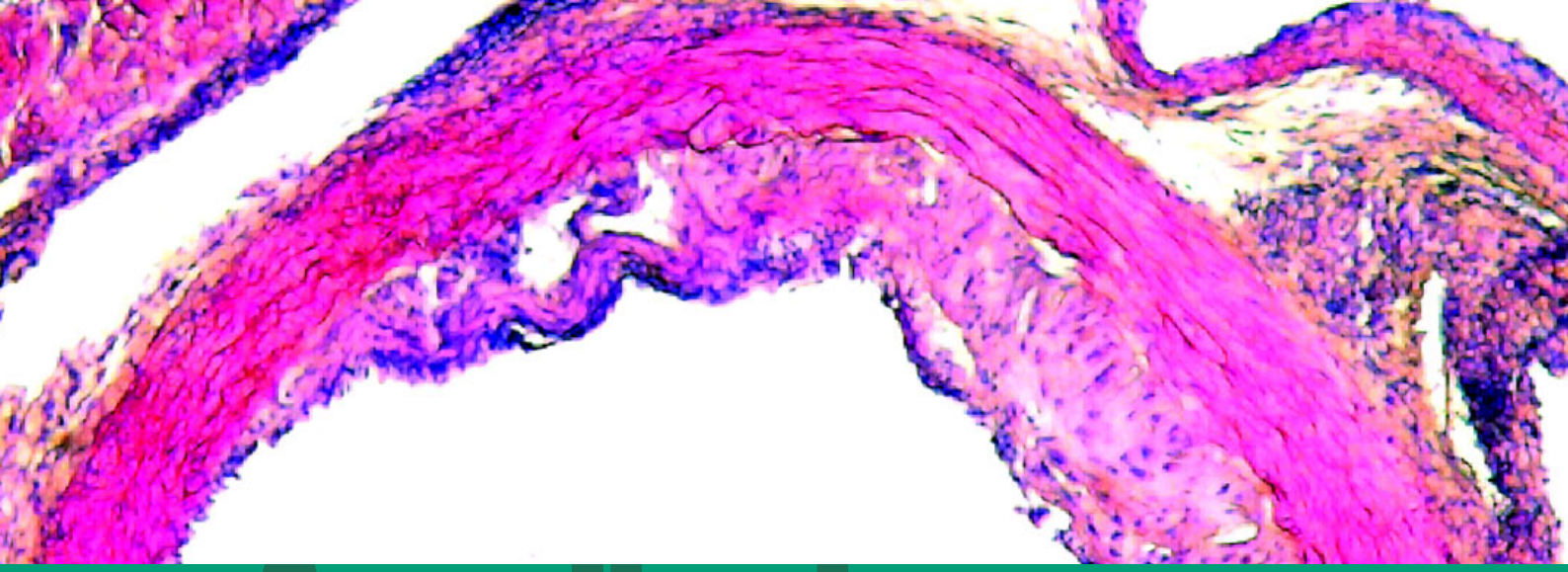
- Gonzalo Pizarro, Leticia Fernández Friera, Luz Álvarez (CNIC)
- A complete list of investigators can be found in www.metocard.es

Applied Research Departments

4

Epidemiology, Atherothrombosis and Imaging





Applied Research Departments

4 Epidemiology, Atherothrombosis and Imaging

The EAI Department pools the expertise of molecular and cell biologists, cardiologists, epidemiologists, nutritionists, statisticians and physicists to develop and apply sophisticated non-invasive procedures for the investigation, diagnosis and treatment of cardiovascular diseases. Several groups work on the development of molecular-resolution imaging technologies and use them to investigate the molecular mechanisms underlying cardiovascular disorders. These imaging technologies can identify and characterize various types of atherosclerotic plaques, providing invaluable information on the underlying molecular mechanisms of disease and leading to tools for accurate diagnosis and targeted drug delivery. Our experimental strategy involves a multifaceted approach that combines in vitro, cellular, animal and human studies and a variety of technologies, including genetic engineering, proteomics, transcriptomics, and the most advanced imaging techniques. The epidemiology and genetics area integrates population studies with the results of basic and clinical research to identify environmental and genetic risk factors underlying the incidence, development and prognosis of cardiovascular disease.

DEPARTMENT DIRECTOR:	*Valentín Fuster*
DEPARTMENT MANAGER:	*Ana Isabel Castillo*
ADMINISTRATIVE SUPPORT:	*Ana Gutiérrez* *Eeva Inari Soininen*
TECHNICIANS:	*Javier Mateos* *Inés Ortega*

Applied Research Departments 4

Epidemiology, Atherothrombosis and Imaging

Cardiovascular imaging

Head of Laboratory:	*Valentín Fuster (CNIC, Mt. Sinai Medical Center, New York)*
Research Scientists:	*Luis Jesús Jiménez Borreguero (CNIC - Hospital de la Princesa Research Agreement)* *Oliver Weber (CNIC, Philips)* *Zahi Fayad (Mt. Sinai Medical Center)* *Juan José Badimón (Mt. Sinai Medical Center)* *Jesús Mateo (CNIC)*
Project Managers:	*Laura García Leal* *Luz Alvarez Vilela*
CardioImage Fellow:	*Gabriela Guzmán (CNIC, Hospital de La Paz, Madrid)*
Predoctoral Researchers:	*Patricia García (CNIC)*
Technicians:	*Carolina Rojas Murcia* *Natalia Serrano Juzgado* *Isabel Pérez García* *Aurora del Barrio Mantecas* *Alberto Ávila Morales* *Ricardo Ponce Sánchez* *Sergio Cárdenas Melero*

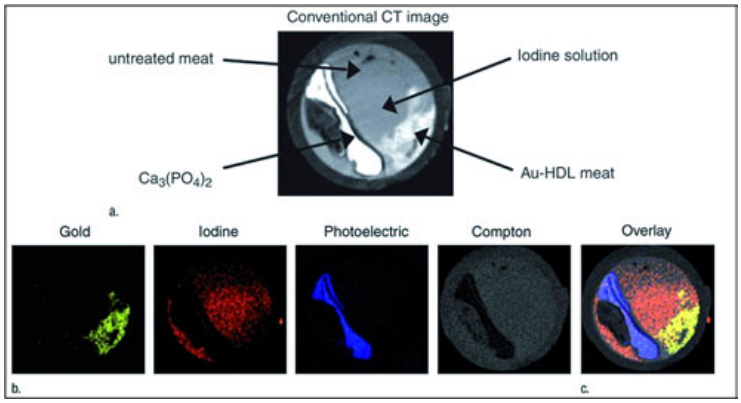
RESEARCH INTEREST

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 diagnosing asymptomatic disease and monitoring treatment outcomes.

Our work covers all of these aspects. Our preclinical work involves the use of positron emission tomography-computed tomography (PET/CT), molecular magnetic resonance imaging and other technologies to characterize plaque composition and development. We also lead the European Commission financed HYPERImage project, devoted to the development and validation of an integrated PET/MR system to substitute PET/CT technology. Last year the new Imaging Facility was established. This major installation, established through the CNIC's strategic alliance with Philips, is equipped with state-of-the-art imaging technology for animal studies at the main CNIC site, and further equipment for studies with patients at the nearby Carlos III Hospital. Aside from capabilities in echocardiography, computed tomography and magnetic resonance imaging, the facility will also be equipped with technology for magnetic particle imaging, a tomographic imaging technique developed by Philips that achieves resolutions finer than one millimeter.

We also participate in the CNIC's clinical studies of imaging technologies, working closely with the Epidemiology group and the Department of Translational Research. Our work in this area involves the use of novel imaging algorithms that can provide significant information for sensitive risk stratification in asymptomatic subjects. Highlights last year included the launch of the PESA-CNIC/Santander trial and the continuation of the AWHS study. These sibling studies examine the association of bioimaging parameters with the presence of genetic, epigenetic, metabolomic, proteomic and environmental factors in two populations with different characteristics. Examinations include include coronary calcium scoring by multidetector comuted tomography scan (MDCT) and carotid and abdominal aorta 3D ultrasound. Participants in PESA showing evidence of atherosclerotic disease are studied in greater depth by MRI and PET/CT. Advanced non-invasive imaging analyses are also the main endpoints of the IMJOVEN and METOCARD-CNIC trials, into the the excess risk in young women with acute myocardial infarction and the cardioprotective effect of pre-reperfusion administration with the β-blocker metoprolol within the first two hours in patients with acute myocardial infarction.

4 Epidemiology, Atherothrombosis and Imaging



Amira 3D reconstruction compiled from a confocal Z-stack of a random mosaic E9.5 heart. The image shows a ventral view of the heart tube, encompassing the outflow tract and the right and left ventricles. The cell distribution in the mosaic reveals the regional tissue deformation occuring during heart morphogenesis

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. **Fine-tuning therapy for acute coronary syndromes.** *N Engl J Med* (2010) 363: 976-7

Fuster V, Farkouh ME. **General Cardiology Perspective: Decision making regarding revascularization of patients with type 2 diabetes mellitus and cardiovascular disease in the bypass angioplasty revascularization investigation 2 diabetes (BARI 2D) trial.** *Circulation* (2010) 121: 2450-2

Fuster V, Bansilal S. **Promoting Cardiovascular and Cerebrovascular Health.** *Stroke* (2010) 41: 1079-83

Muntendam P, McCall C, Sanz J, Falk E, Fuster V; High-Risk Plaque Initiative.
The BioImage Study: novel approaches to risk assessment in the primary prevention of atherosclerotic cardiovascular disease--study design and objectives. *Am Heart J* (2010) 160: 49-57

Sanz J, Moreno PR, Fuster V. **The year in atherothrombosis.** *J Am Coll Cardiol* (2010) 55: 1487-98.

Applied Research Departments 4

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
Yafa Naim Abu Nabah Soriano
José Rivera Torres
Laia Trigueros Motos

Predoctoral Researchers:
Pedro Molina Sánchez
Ana Navarro Puche
Carlos Silvestre Roig

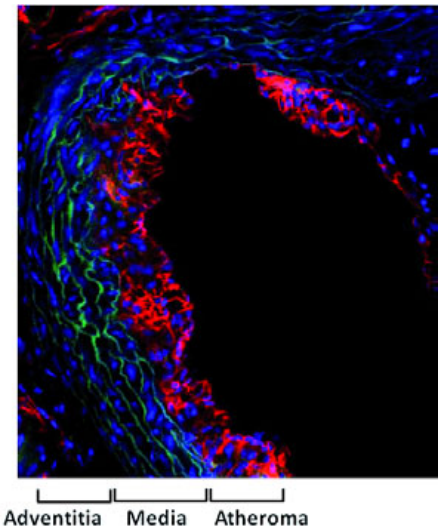
Technicians:
María Jesús Andrés Manzano
Cristina González Gómez

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 towards the growing lesion. Moreover, accumulation of non-cellular material such as modified lipids and extracellular matrix components contributes to atheroma growth. Excessive cellular hyperplasia is also a feature of restenosis, the major limitation to the long-term success of revascularization via stent placement.

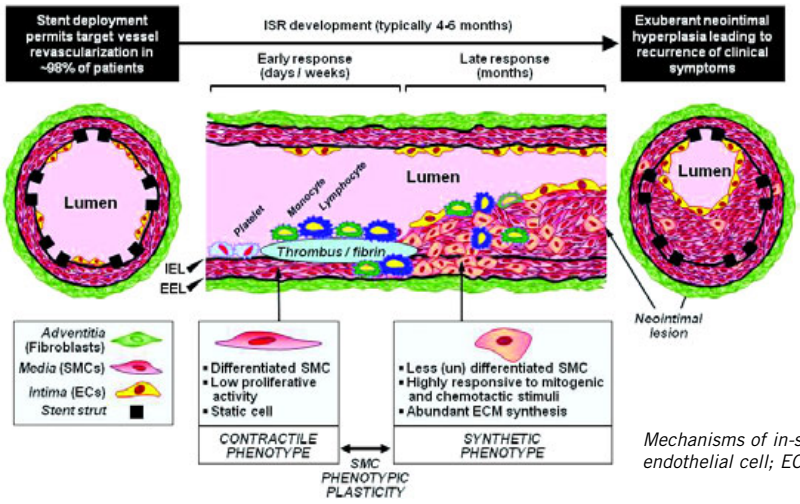
Our research addresses the cellular, molecular and genetic mechanisms that underlie the development of atherosclerosis and restenosis, with particular emphasis on the role of cell cycle regulatory factors, as well as the identification of biomarkers of these diseases. We use a multifaceted approach that 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 screening.

Specific projects in the lab include: 1) Characterization of the molecular and cellular mechanisms that control the development of vascular obstructive lesions in the setting of native atherosclerosis and in-stent restenosis; 2) Studies of the consequences of single nucleotide polymorphisms in cell-cycle regulatory genes for human susceptibility to in-stent restenosis and the underlying molecular mechanisms; and 3) Research into the role of nuclear lamins in the regulation of gene expression, age-associated cardiovascular disease and the immune response.

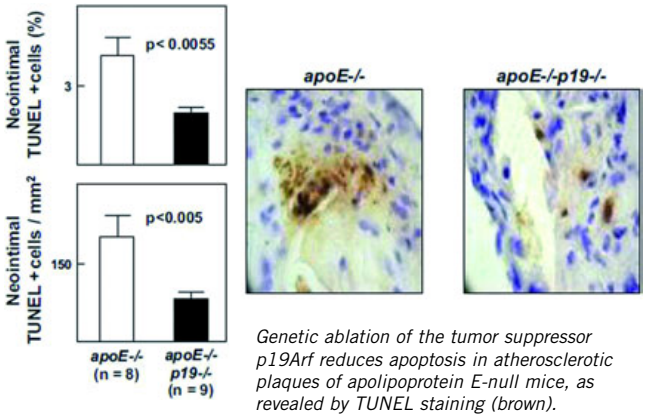


RhoA activity in macrophages within an atherosclerotic plaque of an apolipoprotein E-null mouse, as revealed by phospho-ERM immunstaining (red).

4 Epidemiology, Atherothrombosis and Imaging



Mechanisms of in-stent restenosis (ISR). SMC, smooth muscle cell; EC, endothelial cell; ECM, extarcellular matrix.



Genetic ablation of the tumor suppressor p19Arf reduces apoptosis in atherosclerotic plaques of apolipoprotein E-null mice, as revealed by TUNEL staining (brown).

MAJOR GRANTS

- **Ministerio de Ciencia e Innovación. FIS RETICS (RECAVA: RD06/0014/0021)**
- **Fundación Ramón Areces. Funds held at the CSIC**
- **Ministerio de Ciencia e Innovación (SAF2007-62110). Funds held at the CSIC**

SELECTED PUBLICATIONS

Fuster JJ, Fernández P, González-Navarro H, Silvestre C, Naim Abu Nabah Y, Andrés V. **Control of cell proliferation in atherosclerosis: Insights from animal models and human studies.** *Cardiovasc Res* (2010) 86: 254-64

González-Navarro H, Naim Abu Nabah Y, Vinué A, Andrés-Manzano MJ, Collado M, Serrano M, Andrés V. **p19^{Arf} deficiency reduces macrophage and vascular smooth muscle cell apoptosis and aggravates atherosclerosis.** *J Am Coll Cardiol* (2010) 55: 2258-68

Fuster JJ, González JM, Edo MD, Viana R, Boya P, Cervera J, Verges M, Rivera J, Andrés V. **The tumor suppressor p27^{Kip1} undergoes endo-lysosomal proteolysis through its interaction with sorting nexin 6.** *FASEB J* (2010) 24: 2998-3009

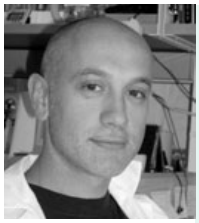
Rodríguez J, Calvo F, González JM, Casar B, Andrés V, Crespo P. **ERK1/2 MAP kinases promote cell cycle entry by rapid, kinase-independent disruption of retinoblastoma-lamin A complexes.** *J Cell Biol* (2010) 191: 967-79

Andrés V, González JM. **Role of A-type lamins in signaling, transcription and chromatin organization.** *J Cell Biol* (2009) 187: 945-57

Applied Research Departments 4

Epidemiology, Atherothrombosis and Imaging

Imaging cardiovascular inflammation and the immune response



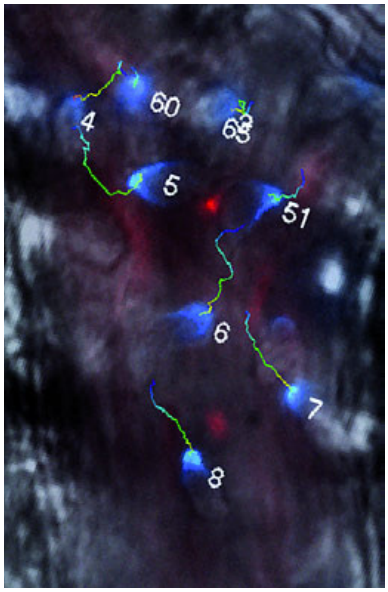
Head of Laboratory:	*Andrés Hidalgo Alonso*
Postdoctoral Researchers:	*María Nacher Espuig*
Predoctoral Researchers:	*María Casanova Acebes*
Technician:	*Christophe Pitaval*

RESEARCH INTEREST

Our laboratory is interested in various aspects of the inflammatory response. We are developing techniques based on multichannel fluorescence intravital microscopy to visualize the molecular and cellular phenomena that occur within the inflamed vasculature. We are also interested in understanding the mechanisms by which leukocyte production and release during inflammation modulates homeostatic processes.

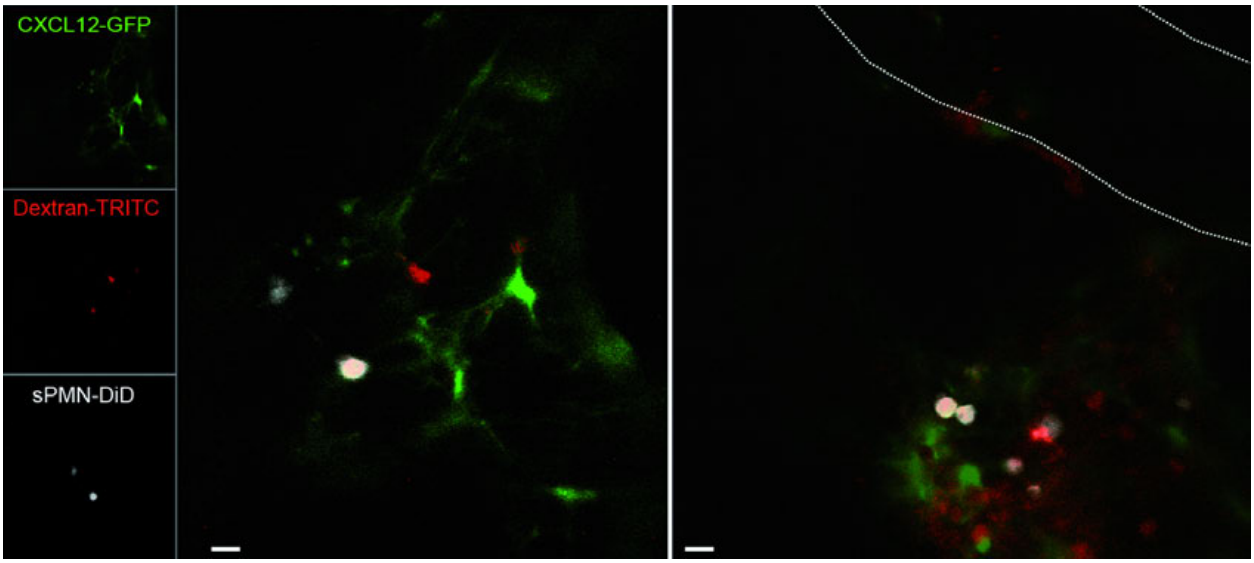
Imaging inflammation: Leukocytes and platelets are recruited to inflamed vessels via adhesion receptors, chemokines and cytokines. During this process, leukocytes redistribute surface receptors to discrete domains, each of which can mediate interactions with circulating platelets and erythrocytes. These interactions can lead to an excessive activation of the leukocyte, which in turn releases toxic mediators that damage the surrounding endothelium. We want to understand the biology of these interactions, including how they lead to the formation of polarized leukocyte domains, the receptors that mediate them and their consequences in inflammatory disease. We are particularly interested in understanding the potential contribution of these interactions to vascular injury under atherogenic conditions.

Control of leukocyte production and release: We are also interested in dissecting the links between inflammation and alterations in the bone marrow niches, the home of hematopoietic stem cells and their differentiated progeny. We are addressing this through the use of gene-targeted mouse models with alterations in the immune and hematopoietic compartments. Our goal is to define the signals that these biological systems use to communicate with each other and to understand how this is regulated and altered during disease.



Imaging leukocyte behavior during inflammation.
Neutrophils adhered to inflamed vessels move or "crawl" on the endothelium, a process mediated by activated β2-integrins. Tracking the paths of these cells during one minute (colored lines) in the venules of mice provides an index of leukocyte activation status under different experimental or genetic conditions. Bar = 10 μm.

4 Epidemiology, Atherothrombosis and Imaging



Interactions of phagocytes with the hematopoietic niche.
In vivo imaging of the bone marrow of mice treated with senescent neutrophils (white). Macrophages are labeled with TRITC-Dextran (red) and niche cells—characterized by the production of high levels of CXCL12—are genetically tagged with GFP (green). We are interested in the interplay among these cells and its consequences during inflammatory injury and repair.

MAJOR GRANTS

- **MINISTERIO DE CIENCIA E INNOVACION (SAF2009-11037)**
- **MINISTERIO DE CIENCIA E INNOVACION (RYC-2007-00697)**
- **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

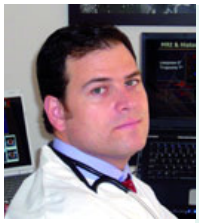
Hidalgo A, Chang J, Jang J, Peired AJ, Chiang EY and Frenette PS. **Heterotypic interactions enabled by polarized neutrophil microdomains mediate thrombo-inflammatory injury.** *Nature Med* (2009) 15: 384-91

Hidalgo A and Frenette PS. **When integrins fail to integrate.** *Nature Med* (2009) 15: 249-50

Applied Research Departments 4

Epidemiology, Atherothrombosis and Imaging

Imaging in experimental cardiology



Head of Laboratory:	*Borja Ibáñez Cabeza*
Postdoctoral Researchers:	*David Sanz-Rosa* *David Vivas Balcones* *(CNIC - Hospital Clínico San Carlos, Madrid)* *Leticia Fernández Friera* *(CNIC - Mt. Sinai Medical Center, New York, Hospital Marques de Valdecilla-IFIMAV, Santander)* *Gonzalo Pizarro Sánchez* *(CNIC - Hospital Quirón Madrid)*
Predoctoral Researcher:	*Jaime García-Prieto*
Technician:	*José Luis Martín Rivillo*

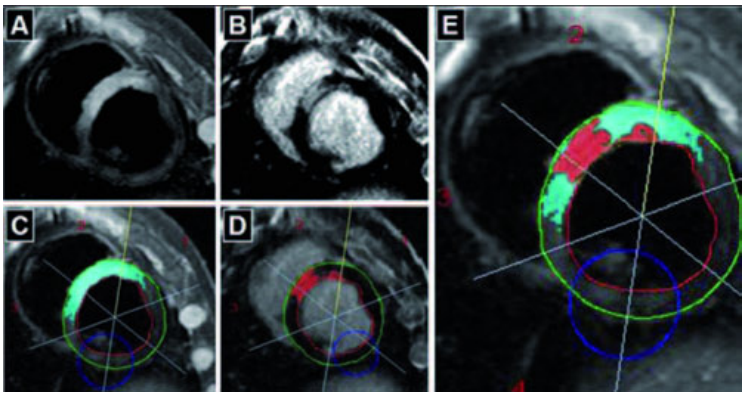
RESEARCH INTEREST

Our laboratory focuses on the development of experimental models of cardiovascular diseases in order to obtain knowledge on the mechanisms underlying the origin and progression of these diseases and to test the efficacy of novel interventions. Our studies span the molecular origins of disease and their manifestations at the macro anatomical and physiological levels, and our group comprises 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 multi-disciplinary programs in close collaboration with hospitals and clinical researchers.

One of our main interests is cardioprotection during myocardial infarction (MI). We have established different models of MI in rodents and large animals, and we are using these to study the mechanisms underlying of the beneficial effects of various cardioprotective strategies (mainly those related to modulation of the adrenergic system).

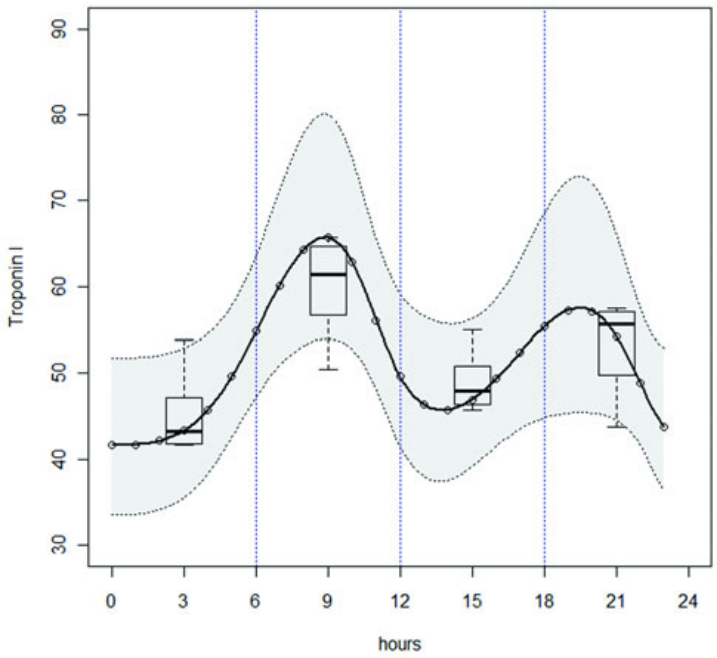
We also investigate the relationship between circadian oscillations and spontaneous cardioprotection. Our aim here is to exploit natural changes in the levels of salvage kinases, which have been shown to significantly affect infarct size.

Working closely with the Department of Translational Research, we are leading a clinical trial (METOCARD-CNIC), which uses magnetic resonance imaging to to evaluate the effectiveness of a cardioprotective strategy based on beta adrenergic modulation in patients with a previous myocardial infarction. We also participate in European Commission funded HYPERImage project for the development of new imaging technologies.



Analysis of MI size by magnetic resonance imaging. *With sequences potentiated in T2 (A, C) and T1 (B, D after administration of gadolinium) it is possible to quantify the at-risk and infarcted areas. The ability to use non-invasive in vivo imaging techniques to determine the extension of necrosis into the at-risk area makes it possible to reduce the number of experimental animals.*

4 Epidemiology, Atherothrombosis and Imaging



Time-of-the-day of the onset of acute MI influences infarct size. *The spline regression curve shows clear circadian oscillations in infarct size that were independent of clinical variables. Patients suffering an MI during the dark-to-light transition have larger infarct sizes than those in whom ischemia begins at other times of day.*

MAJOR GRANTS

- **Ministerio de Sanidad y Politica Social FICI (EC10-042)**
- **Ministerio de Ciencia e innovación. FIS (PI10/02268)**

SELECTED PUBLICATIONS

Cimmino G, Ibáñez B, Giannarelli C, Prat-González S, Hutter R, Garcia M, Sanz J, Fuster V, Badimon JJ. **Carvedilol administration in acute ayocardial infarction results in stronger inhibition of early markers of left ventricular remodeling than metoprolol.** *Int J Cardiol* (accepted)

Ibáñez B, Fuster V. **Ischaemic conditioning for myocardial salvage after AMI.** *Lancet* (2010) 375: 1691; author reply 1692

Speidl WS, Cimmino G, Ibáñez B, Elmariah S, Hutter R, Garcia MJ, Fuster V, Goldman ME, Badimon JJ. **Recombinant apolipoprotein A-I Milano rapidly reverses aortic valve stenosis and decreases leaflet inflammation in an experimental rabbit model.** *Eur Heart J* (2010) 31: 2049-57

Badimón JJ, Ibáñez B. **Increasing High-Density Lipoprotein as a Therapeutic Target in Atherothrombotic Disease.** *Rev Esp Cardiol* (2010) 63: 323-33

Ibáñez B, Cimmino G, Prat-González S, Vilahur G, Hutter R, García MJ, Fuster V, Sanz J, Badimon L and Badimon JJ. **The cardioprotection granted by metoprolol is restricted to its administration prior to coronary reperfusion.** *Int J Cardiol* (accepted)

Applied Research Departments 4

Epidemiology, Atherothrombosis and Imaging

Vascular wall remodeling and cardiovascular disease

Head of Laboratory: *Carlos Zaragoza Sánchez*

Postdoctoral Researcher: *Beatriz Herranz Sánchez*

Predoctoral Researchers: *Begoña Lavin Plaza*
Carlos Tarín

Technician: *Mónica Gómez Parrizas*

RESEARCH INTEREST

Our research is focused on the actions of vasoactive factors and proteolytic enzymes during the early steps of vascular wall remodeling, a fundamental process which plays a key role in the development and progression of atherosclerosis, aneurysm, myocardial infarction, and arterial hypertension, four of the most prevalent diseases worldwide. We study animal models of these diseases generated in the laboratory, and our ultimate goal is to translate the results of our research into validated clinical tools for diagnosis and treatment.

The following projects are currently running in our laboratory.

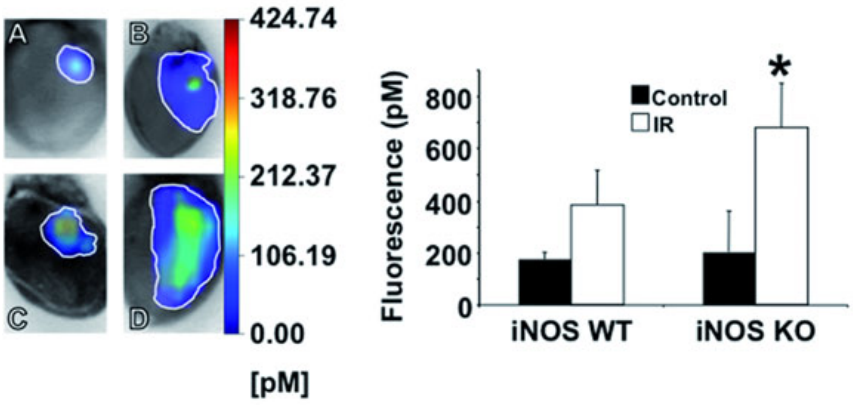
1) Identification of molecular determinants involved in the development, progression, and rupture of abdominal aortic aneurysms (AAA) and the development of new molecular imaging tools for noninvasive detection.

2) Determination of the contribution of proteolytic enzymes to the migration and homing of endothelial progenitor cells (EPCs) during vascular wall repair, and the development of new non-invasive tools for molecular tracking by highfrequency molecular ultrasound.

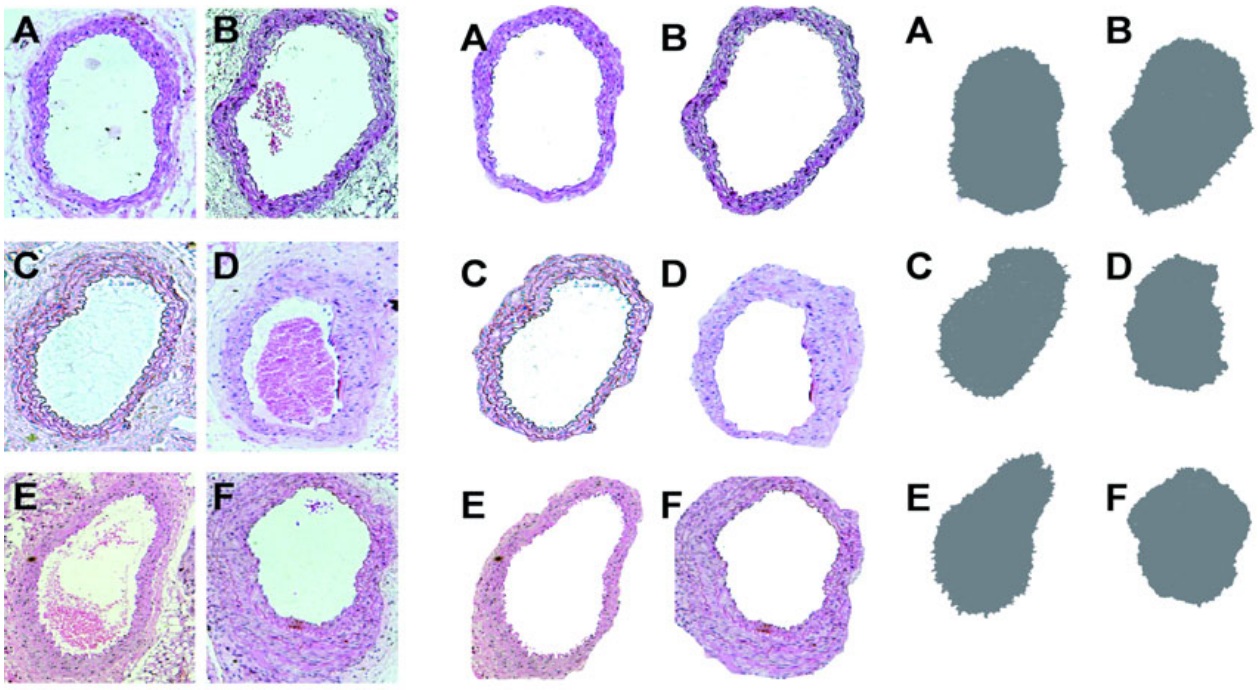
3) Identification of molecular determinants responsible for cardioprotection during late ischemic preconditioning, with the aim of devising noninvasive strategies for in vivo molecular imaging detection of myocardial infarction biomarkers by nanoparticle technology coupled with magnetic resonance.

4). Participation in the European Comission funded FP7 HYPERImage project. Generation of a hybrid PET/MR system for concurrent clinical and preclinical detection: WP4, preclinical validation of the system towards cardiology: atherosclerosis and myocardial infarction; WP6, management of knowledge

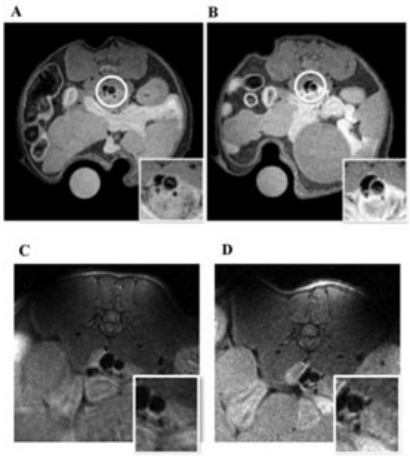
Noninvasive molecular imaging detection of collagen by magnetic resonance in abdominal aortic aneurysms. A, *Sagittal image acquired by magnetic resonance at time 0 in a mouse injected with nanoparticles conjugated to peptide EP-3553.* ***B,*** *Image acquired at 24 h. Insets show magnified views of the abdominal aorta.* ***C and D,*** *Similar images from mice injected with nanoparticles conjugated to a scramble peptide.*



4 Epidemiology, Atherothrombosis and Imaging



Lack of iNOS increases myocardial damage during ischemia/reperfusion. *iNOS wild type (A, B) and iNOS knockout (KO) mice were injected with the cathepsin-B probe Prosense-680 and either mock operated (A,C; control) or subjected to left coronary artery ischemia/reperfusion (B,D; IR). The images show representative fluorescence molecular tomography detection of cathepsin-B in hearts isolated postmortem. The chart shows results from x mice(* p<0.05 iNOS WT IR vs iNOS KO IR).*



eNOS-deficient mice show significantly greater neointimal thickening than wild type mice in response to in vivo aortic endothelial denudation. *Left panels show serial sections of aortas extracted with increasing time after wire-mediated endothelial denudation. The middle panels show the smooth muscle cells in isolation. The right panels show the lumen area.*

MAJOR GRANTS

- Ministerio de Ciencia e Innovación (SAF2008-04629).

SELECTED PUBLICATIONS

Zaragoza C, Ibañez B, Jiménez-Borreguero LJ, Schulz V, Fayad Z, Fuster V. **Future perspectives in cardiovascularimaging: Simultaneous PET/MRI technology in biomedical research.** *Nat Rev Cardiol* (CNIC Edition) (2010) 7: 7-10

Saura M, Tarin C, Zaragoza C. **Recent Insights into the implication of nitric oxide in bone development.** *Sci World J* (2010) 10: 624-32

Lizarbe TR, Tarin C, Gomez M, Lavin B, Aracil E, Orte LM, Zaragoza C. **Nitric oxide induces the progression of abdominal aortic aneurysms through the matrix metalloproteinase inducer EMMPRIN.** *Am J Pathol* (2009) 175: 1421-30

Martinez-Miguel P, Raoch V, Zaragoza C, Valdivieso JM, Rodriguez-Puyol M, Rodriguez-Puyol D , Lopez-Ongil S. **Endothelin-converting enzyme-1 increases in atherosclerotic mice: potential role of oxidized low density lipoproteins.** *J Lipid Res* (2009) 50: 364-75

Tarin C, Gomez M, Calvo E, Lopez JA, Zaragoza C. **Endothelial nitric oxide deficiency reduces MMP-13-mediated cleavage of ICAM-1 in vascular endothelium: a role in atherosclerosis.** *Arterioscler Thromb Vasc Biol* (2009) 29: 27-32

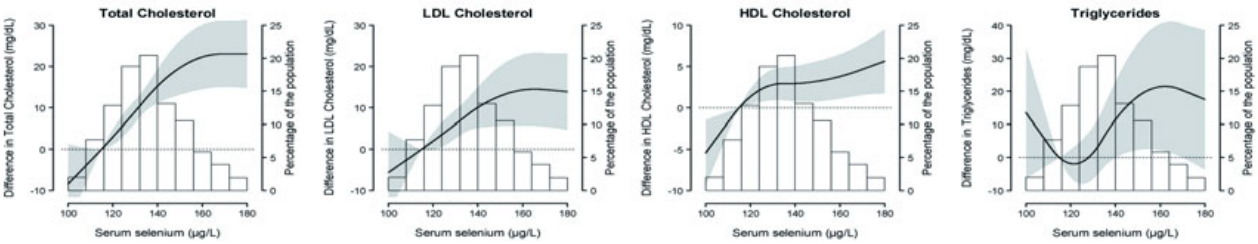
CNIC-JHU COLLABORATIVE PROGRAM: Epidemiology and population genetics

Program Director:	*Eliseo Guallar*
Senior Researcher:	*José Mª Ordovás (CNIC - Tufts University Research Agreement)*
Research Scientists:	*Manuel Franco* *José Luis Peñalvo* *Martín Laclaustra*
Post-residency Researcher:	*María Téllez*
Biostatistician:	*Pedro López*
Support Scientist:	*Marta Ledesma* *Laura García*
Technicians:	*Alicia Usón* *Belén Moreno* *Raquel Langarita* *Esther Rovira* *Damaris Tamayo*

RESEARCH INTEREST

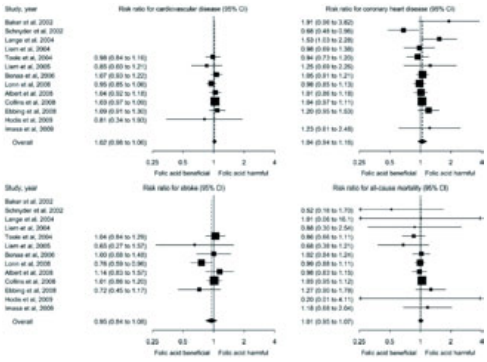
The group conducts high-quality and high-impact population research studies into the environmental, individual and genetic risk factors that are causally related to cardiovascular disease. The group works closely with Department of Translational Cardiovascular Research in the design of clinical studies and the analysis of population data from advanced imaging methodologies. We are deeply involved in the Aragon Workers Health Study (AWHS). Enrollment was completed in 2010, with 5589 workers recruited, a response rate of 95%. Follow-up is continuing as scheduled, and in 2011 we will commence measurement of subclinical atherosclerosis in the cohort. The group also plays a major role in the planning of the PESA (Progression of Subclinical Atherosclerosis) study, that started in 2010 and has already recruited over 500 participants, and in the IMJOVEN study, which has recruited over 300 young women who have suffered a myocardial infarction. 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.

Members of the group pursue highly innovative research lines that cover the major risk factors for cardiovascular disease, including diet (Ordovás, Guallar, Franco, Laclaustra, Peñalvo), genetics and epigenetics (Ordovás, Téllez), metabolic factors (Ordovás, Laclaustra, Peñalvo), the environment (Guallar, Téllez), and psychosocial factors (Franco). 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.

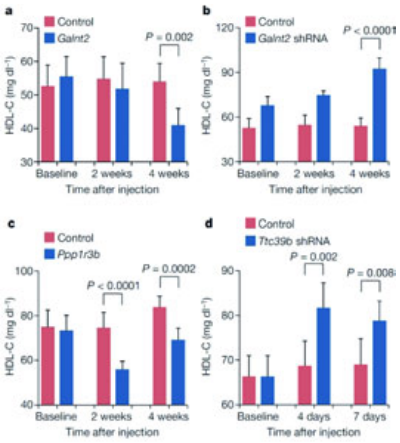


Adjusted differences (95% CI) in serum lipids by serum selenium concentrations in the US population (NHANES 2003-2004)
Models were adjusted for sex, age, race, education, body mass index, smoking, cotinine, postmenopausal status, cholesterol, total fat, saturated fatty acids, selenium intake, and use of vitamin and mineral supplements. Lipid concentrations at the 10th percentile (115 microg/L) of the serum selenium distribution were used as reference. The histograms show the distribution of selenium concentrations in the study population.

4 Epidemiology, Atherothrombosis and Imaging



Risk ratios and pooled estimates for cardiovascular disease, coronary heart disease, stroke, and all-cause mortality in randomized controlled trials of folic acid supplementation
The area of each square is proportional to the study weight in the analysis. Pooled estimates (diamonds) *and 95% CIs* (horizontal lines) *were obtained from inverse-variance weighted random-effects models.*



Effects of altered Galnt2, Ppp1r3b or Ttc39b expression in mouse liver on plasma lipid levels
Charts show plasma HDL-C levels at baseline and at the indicated times after injection with viral vectors. a, Overexpression, and b, knockdown of Galnt2; n = 56 mice per group. c, Overexpression of Ppp1r3b; n = 57 mice per group. d, Knockdown of Ttc39b. n = 56 mice per group. Error bars show standard deviations. Since independent experiments were performed at different times or sites, baseline HDL-C levels varied.

MAJOR GRANTS

- **ALIBIRD (P2009/AGR-1469). PI: E. Guallar**
- **Centro Nacional de Investigaciones Cardiovasculares (FPIT CNIC-08). PI: E. Guallar**
- **Ministerio de Ciencia e Innovación (SAF2008-01995). PI: J.L. Peñalvo**
- **Ministerio de Ciencia e Innovación (RYC-2010-07554). PI: M. Franco**
- **European Commission FP7. Marie Curie European Reintegration Grant (GA-249302). PI, M. Franco**
- **Ministerio de Ciencia e Innovación. FIS (CP08/00112). PI: M. Laclaustra**
- **Ministerio de Ciencia e Innovación. FIS (CM08/0037). PI: M. Téllez**

SELECTED PUBLICATIONS

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Fuster V, Lois F, Franco M. **Early identification of atherosclerotic disease by noninvasive imaging.** *Nat Rev Cardiol* (2010) 7: 327-33

Nurmi T, Mursu J, Peñalvo JL, Poulsen HE, Voutilainen S. **Dietary intake and urinary excretion of lignans in Finnish men.** *Br J Nutr* (2010) 103: 677-85

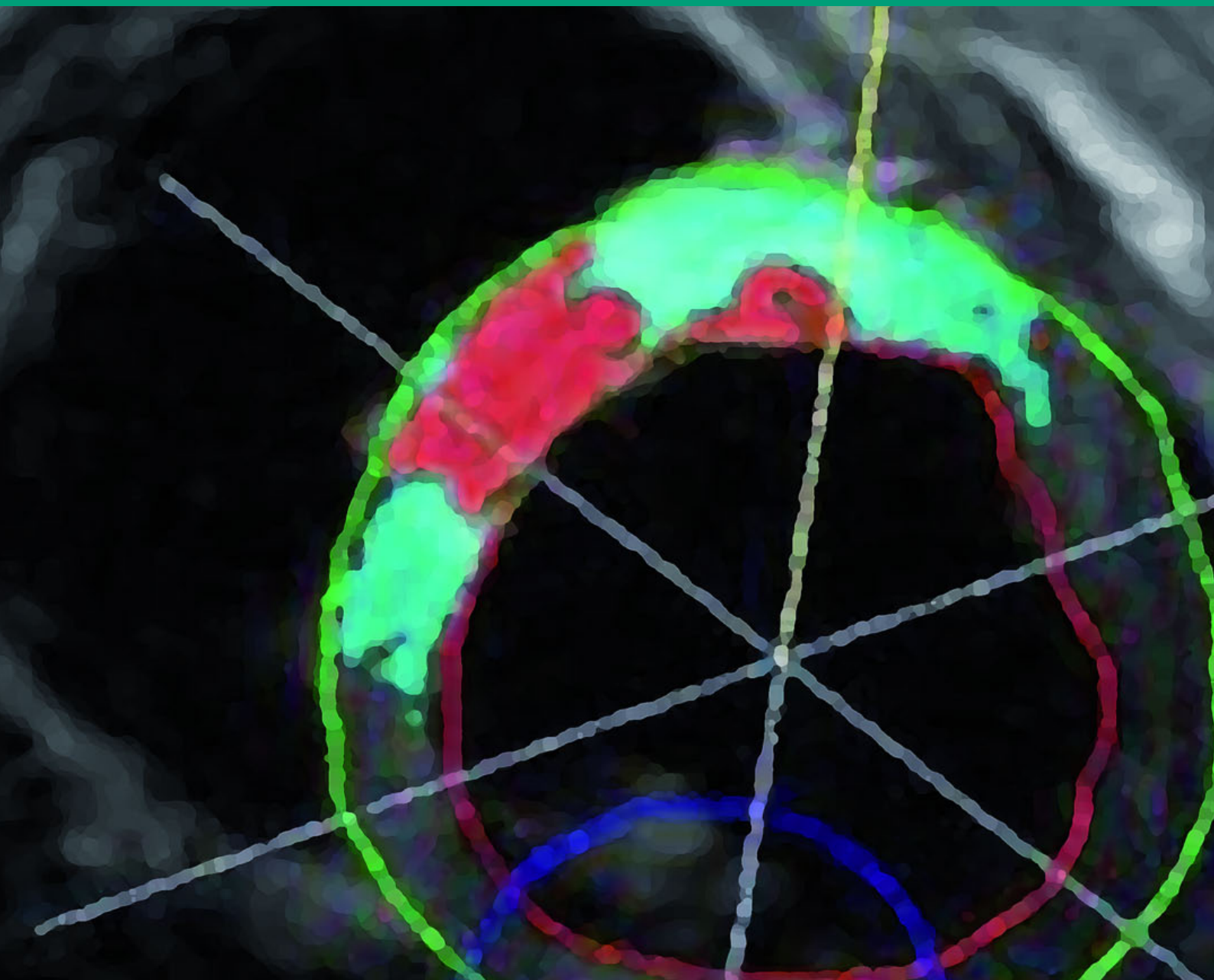
Laclaustra M, Stranges S, Navas-Acien A, Ordovas JM, Guallar E. **Serum selenium and serum lipids in US adults: National Health and Nutrition Examination Survey (NHANES) 2003-2004.** *Atherosclerosis* (2010) 210: 643-8

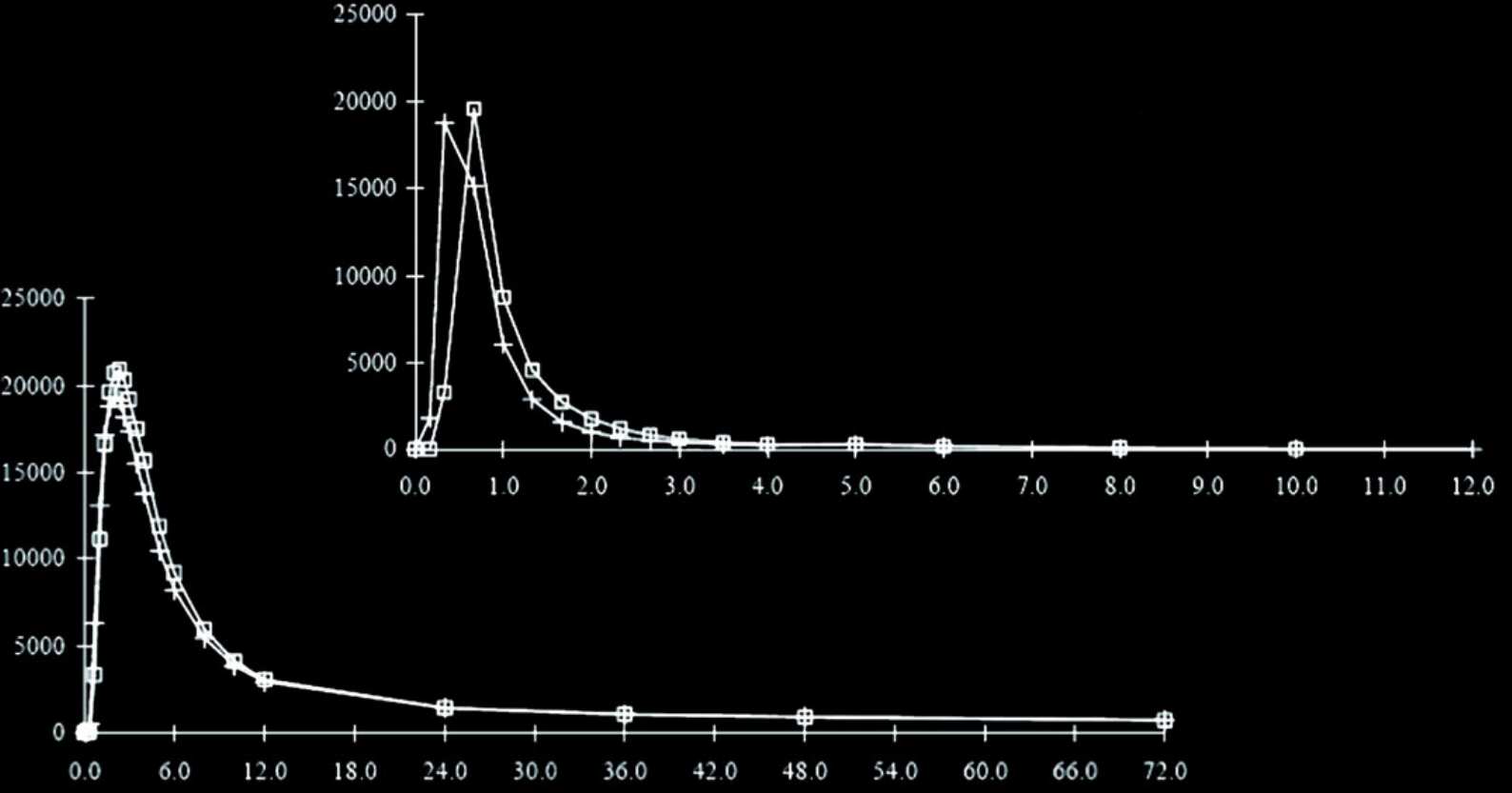
Tellez-Plaza M, Navas-Acien A, Crainiceanu CM, Sharrett AR, Guallar E. **Cadmium and peripheral arterial disease: gender differences in the 1999-2004 US National Health and Nutrition Examination Survey.** *Am J Epidemiol* (2010) 172: 671-81

Applied Research Departments

5

Cardiovascular Translational Research





Applied Research Departments

5 Cardiovascular Translational Research

The TCR department is the nexus between the CNIC and the hospital system, facilitating collaboration between clinical research groups and the CNIC's scientists, encouraging the application and clinical testing of new technologies, and training clinical researchers.

Applied Research Departments 5

Cardiovascular Translational Research

Fixed-dose combination therapy for cardiovascular prevention: The CNIC Polypill Project

Head of Laboratory: *Ginés Sanz*

Project Manager: *Luz Álvarez*

Administrative Support: *Laura González Betlinski*

RESEARCH INTEREST

During 2010 we carried out the clinical phase of the project. Most of the planned studies have been completed, and demonstrate the safety and tolerability of the polypill. Participants are now being recruited for the study of pharmacodynamic interaction with simvastatin (350 patients) and we expect to have results by the end of 2011.

The polypill registration dossier has been presented in several countries in Central and South America, and marketing of the drug has been already approved in Guatemala.

The kick-off meeting for the FOCUS study was held in Madrid last June and the inclusion of patients will being in May 2011. FOCUS is a European Union (FP7) funded project which will test the efficacy of the polypill in a population of more than 1300 patients in five countries: Argentina, Brazil, Italy, Paraguay and Spain. The study, led by Dr. Valentin Fuster, is coordinated by the Department of Translational Cardiovascular Research.

MAJOR GRANTS

- **Ministerio de Ciencia e Innovación. FIS (PI07/0773)**
- **European Commission FP7 (241559 FOCUS)**

SELECTED PUBLICATIONS

Bueno H, Betriu A, Heras M, Alonso JJ, Cequier A, García EJ, López-Sendón JL, Macaya C, Hernández-Antolín R; TRIANA Investigators. **Primary angioplasty vs. fibrinolysis in very old patients with acute myocardial infarction: TRIANA (TRatamiento del Infarto Agudo de miocardio eN Ancianos) randomized trial and pooled analysis with previous studies.** *Eur Heart J* (accepted)

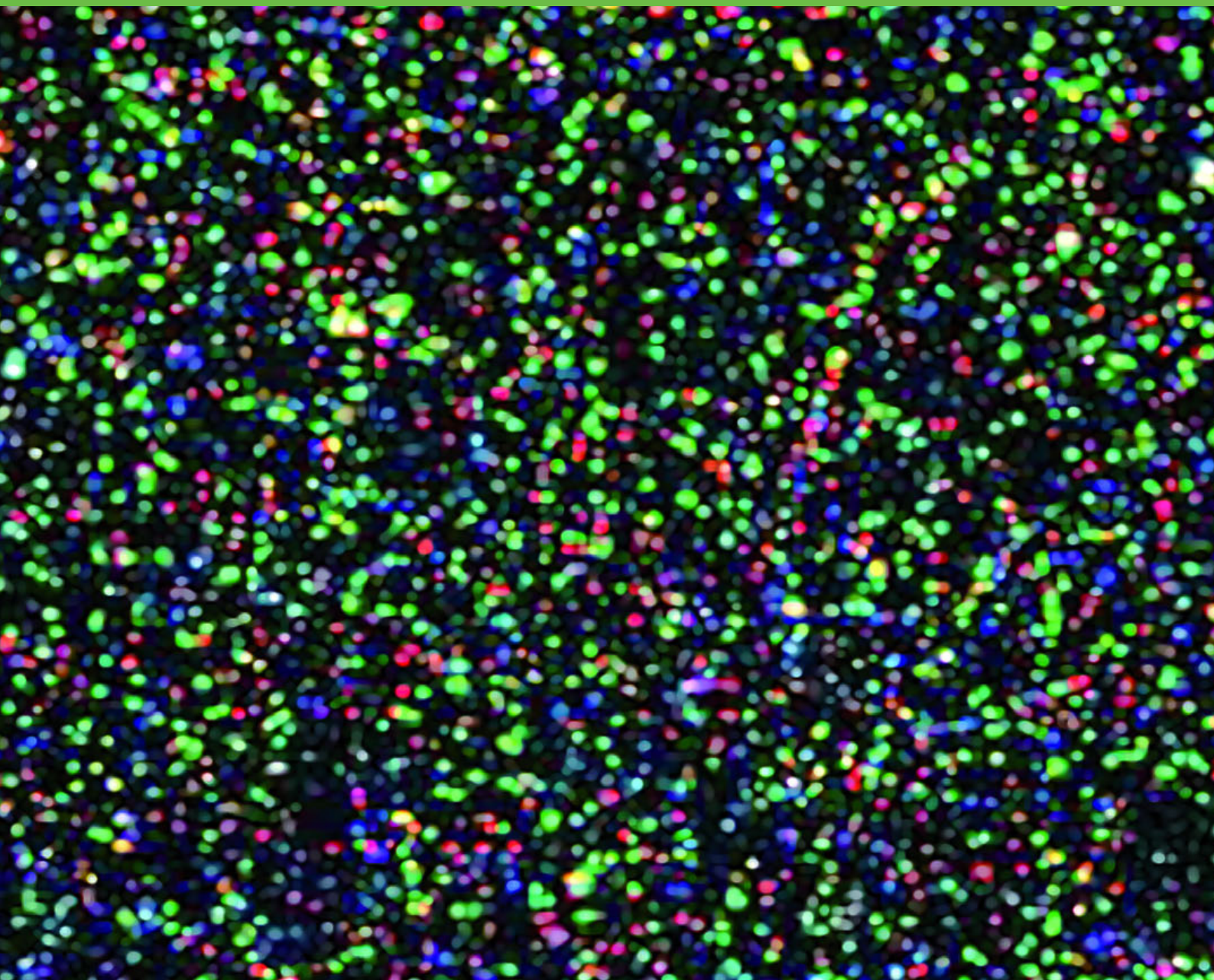
Garcia-Alvarez A, Sitges M, Pinazo MJ, Regueiro-Cueva A, Posada E, Poyatos S, Ortiz-Pérez JT, Heras M, Azqueta M, Gascon J, Sanz G. **Chagas cardiomyopathy: the potential of diastolic dysfunction and brain natriuretic peptide in the early identification of cardiac damage.** *PLoS Negl Trop Dis* (2010) 4: e826

García-García C, Sanz G, Valle V, Molina L, Sala J, Subirana I, Martí H, Marrugat J, Bruguera J, Masià R, Elosua R. **Trends in in-hospital mortality and six-month outcomes in patients with a first acute myocardial infarction. Change over the last decade.** *Rev Esp Cardiol* (2010) 63: 1136-44

Rigol M, Solanes N, Farré J, Roura S, Roqué M, Berruezo A, Bellera N, Novensà L, Tamborero D, Prat-Vidal C, Huzman MA, Batlle M, Hoefsloot M, Sitges M, Ramírez J, Dantas AP, Merino A, Sanz G, Brugada J, Bayés-Genís A, Heras M. **Effects of adipose tissue-derived stem cell therapy after myocardial infarction: impact of the route of administration.** *J Card Fail* (2010) 16: 357-66

Jansà M, Hernández C, Vidal M, Nuñez M, Bertran MJ, Sanz S, Castell C, Sanz G. **Multidimensional analysis of treatment adherence in patients with multiple chronic conditions. A cross-sectional study in a tertiary hospital.** *Patient Educ Couns* (2010) 81: 161-8

6 Technical Units



Microscopy and Dynamic Imaging

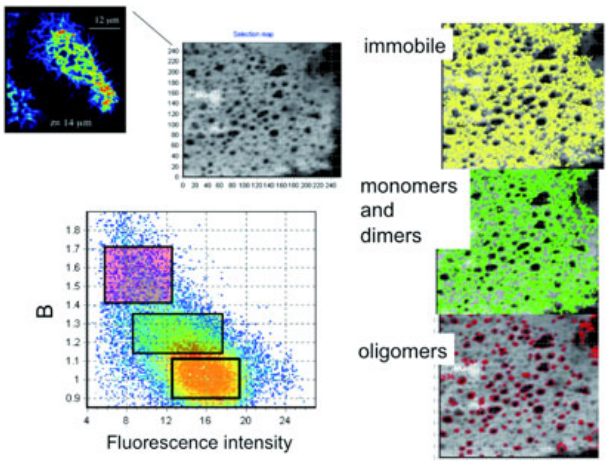
Head of Unit:	*Valeria R. Caiolfa*
Support Scientists:	*Moreno Zamai* *Christian Hellriegel* *Antonio Manuel Santos Beneit* *Elvira Arza*
Postdoctoral Researchers:	*Antonio Manuel Santos Beneit* *Elvira Arza*

RESEARCH INTEREST

The Microscopy and Dynamic Imaging Unit, established in 2008, provides state-of-the-art expertise and training in optical microscopy to scientists at the CNIC and beyond. Resources are maintained for spectroscopy, microscopy, biochemistry, cell culture and data analysis. Areas of expertise include multi-dimensional (multi-D) imaging, immunolabeling, time-lapse, multi-color TIRFM, and 3D cross sectioning. We also provide capabilities in the tracking of single molecules, intracellular vesicles and cells, and in fluctuation analysis techniques such as FCS (fluorescence correlation spectroscopy), RICS (raster image correlation spectroscopy) and N&B (number and brightness analysis). These approaches are used to quantify diffusion of single proteins, monomer-dimer-oligomer equilibrium, stoichiometry of protein-ligand binding, etc.

In addition to providing customized training and data analysis, the Unit is also involved in several collaborative projects that require the development of new technologies and new analytical protocols. Two-photon FRET-FLIM is currently being applied in collaborative projects with the Vascular Biology and Inflammation Department and external groups. We have also started a new project for mapping stem cell differentiation in vivo, in collaboration with the Cardiovascular Developmental Biology Department.

More than 130 internal researchers routinely use the facility, and in 2010 services were opened to external users. During the year, the Unit organized four internal courses for beginners and one advanced international workshop with more than 40 participants from the CNIC and other institutes.



Example of N&B analysis by which we can determine the oligomerization of labeled proteins in living cells. The figure shows the basal membrane of HEK293 cells transfected with a GPI-anchored membrane receptor that was fused to mEGFP as fluorescent tag. N&B counts the fluorophore molecules that diffuse together in the cell by measuring their brightness (B). The higher is the B value, the higher is the oligomerization state of the protein. B values around 1 are indicative of immobile molecules. In the figure, the different forms of the receptor are localized in the image according to their B values (yellow= immobile; green = monomers and dimmers; red = higher oligomers).

SELECTED PUBLICATIONS

Scielzo C, Bertilaccio MT, Simonetti G, Dagklis A, ten Hacken E, Fazi C, Muzio M, Caiolfa V, 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

Transgenesis

Head of Unit: *Luis-Miguel Criado Rguez.*

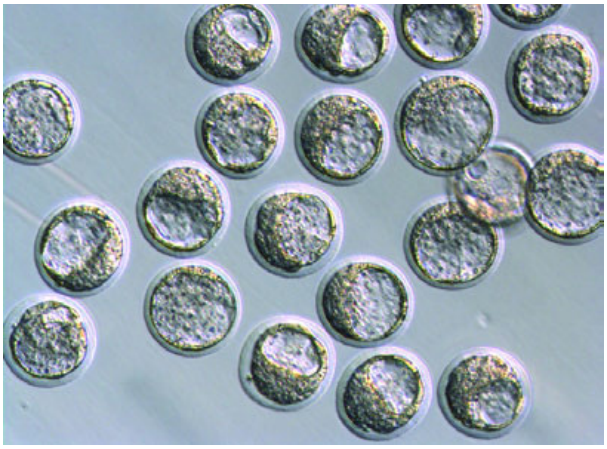
Support Scientist: *José María Fernández Toro*

Technician: *David Esteban Martínez*

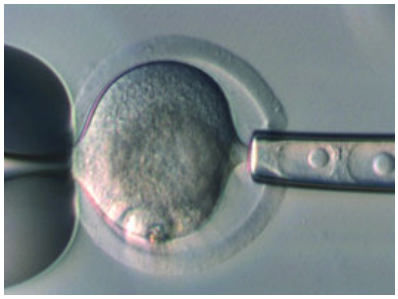
RESEARCH INTEREST

The Transgenesis Unit provides a range of services for the production of genetically-modified mice -so called 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.

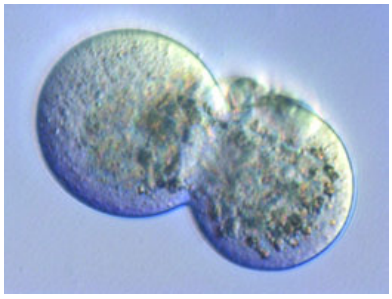
Transgenic mice are produced in the Unit by the established methodologies of microinjection of DNA in solution into zygote pronuclei (pronuclear microinjection) or of recombinant lentiviruses beneath the zygote zona pellucida (subzonal or perivitelline microinjection). Chimeric mice for the generation 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 strains by embryo transfer, cryopreservation of mouse strains (frozen embryos or sperm), in vitro fertilization (IVF), and intracytoplasmic sperm injection (ICSI).



***C57BL/6JOlaHsd** murine blastocysts used for microinjection with murine stem cells (mES-Cells) for the production of chimeric mice.*



***Mechanical enucleation of a mouse zygote.** The two pronuclei can be seen inside the enucleation needle (right).*



***Production of heteroplasmic mouse embryo.** Electrofusion of a NC (NZB mtDNA-C57BL/6JOlaHsd gDNA) cytoplast with a C57BL/6JOlaHsd zygote. The zonae pellucidae have been removed.*

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* (2009) 73: 1088-95

Genomics

Head of Unit: *Ana Dopazo*

Support Scientists: *Sergio Callejas*
Alberto Benguría
Fátima Sánchez Cabo

Technician: *Rebeca Álvarez*

RESEARCH INTEREST

The Genomics Unit is dedicated to providing high-quality genomic technology to the scientific community at the CNIC and beyond. The Unit is equipped with 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.

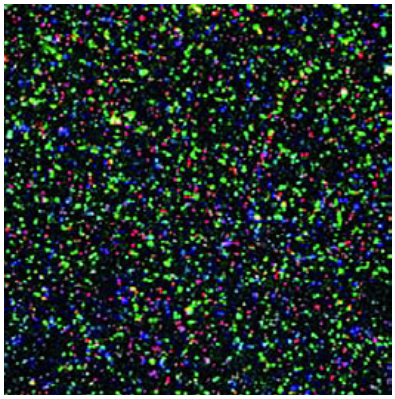
The Unit's capabilities expanded in 2010 to incorporate the new technology of next-generation DNA sequencing, with the acquisition of an Illumina Genome Analyzer platform. This enables CNIC scientists to undertake ultra-deep sequencing projects that, because of their size, would not otherwise be possible. The Unit has also begun to offer high-throughput microRNA sequencing services in addition to the already existing microRNA microarray profiling service, thus allowing researchers to obtain a complete picture of microRNA expression in biological samples.

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. The Unit also provides user advice and training on topics related to its activity.

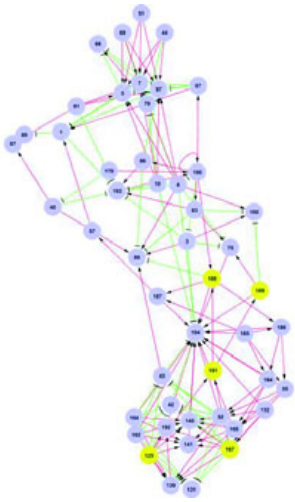
The Genomics Unit actively participates in the ongoing CNIC IM-JOVEN clinical study. IM-JOVEN is part of a large, multicenter case-controlled study aimed at identifying the clinical, genetic and demographic characteristics that determine the occurrence of myocardial infarction in young women.

Hierarchical clustering of microarray data

6 Technical Units



DNA clusters for analysis by next-generation sequencing



IPA gene network

SELECTED PUBLICATIONS

Cubelos B, Sebastian-Serrano A, Beccari L, Calcagnotto ME, Cisneros E, Kim S, Dopazo A, Alvarez-Dolado M, Redondo JM, Bovolenta P, Walsh CA and Nieto M. **Cux1 and Cux2 regulate dendritic branching, spine morphology, and synapses of the upper layer neurons of the cortex.** *Neuron* (2010) 66: 523-35

Gomez-Cabello D, Callejas S, Benguria A, Moreno A, Alonso J and Palmero I. **Regulation of the microRNA processor DGCR8 by the tumor suppressor ING1.** *Cancer Res* (2010) 70: 1866-74

Lamas JR, Rodriguez-Rodriguez L, Vigo AG, Alvarez-Lafuente R, Lopez-Romero P, Marco F, Camafeita E, Dopazo A, Callejas S, Villafuertes E, Hoyas JA, Tornero-Esteban MP, Urcelay E and Fernandez-Gutierrez B. **Large-scale gene expression in bone marrow mesenchymal stem cells: a putative role for COL10A1 in osteoarthritis.** *Ann Rheum Dis* (2010) 69: 1880-5

Lopez-Huertas MR, Callejas S, Abia D, Mateos E, Dopazo A, Alcami J and Coiras M. **Modifications in host cell cytoskeleton structure and function mediated by intracellular HIV-1 Tat protein are greatly dependent on the second coding exon.** *Nucleic Acids Res* (2010) 38: 3287-307

Luna-Zurita L, Prados B, Grego-Bessa J, Luxan G, Del Monte G, Benguria A, Adams RH, Perez-Pomares JM and de la Pompa JL. **Integration of a Notch-dependent mesenchymal gene program and Bmp2-driven cell invasiveness regulates murine cardiac valve**

Pluripotent Cell Technology

Head of Technical Service: *Giovanna Giovinazzo*

Support Scientist: *Francisco Gutierrez*

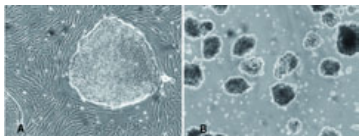
Technician: *Mara Ángeles Sanguino*

RESEARCH INTEREST

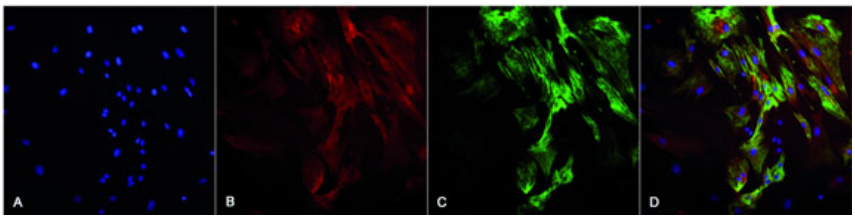
The Pluripotent Cell Technology (PCT) facility provides centralised support with the culture and manipulation of mouse and human pluripotent stem cells. The comprehensive range of support services offered also includes expert advice and training and the development and implementation of new technologies.

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 take 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 blastocyst injection. If required, we also advise on appropriate targeting and screening strategies. The technology developed in the unit has achieved efficient transmission of targeted mESCs to the germline, allowing us to generate numerous lines of genetically modified mice.

Recent collaborations with CNIC research groups have involved us in the derivation of mutant homozygotic mESC lines and the differentiation of mESCs to cardiomyocytes. Throughout the last year we also focused on the design and fine-tuning of protocols for the routine culture of human pluripotent stem cells. This pioneering technology will underpin the use and application of cutting-edge human pluripotent cell technologies by CNIC researchers.



A, *Human embryonic stem cells at day 5 in culture.* ***B,*** *Human embryoid bodies at day 1 of differentiation.*



Immunofluorescence staining of cardiac cells differentiated from human embryonic stem cells. ***A,*** *DAPI.* ***B,*** *Troponin I.* ***C,*** *Actinin.* ***D.*** *Merge.*

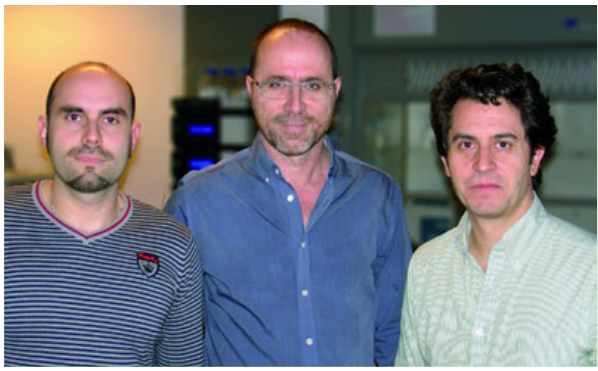
SELECTED PUBLICATIONS

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* (accepted)

Proteomics

Head of Unit: *Juan Antonio López*

Support Scientists: *Enrique Calvo*
Emilio Camafeita



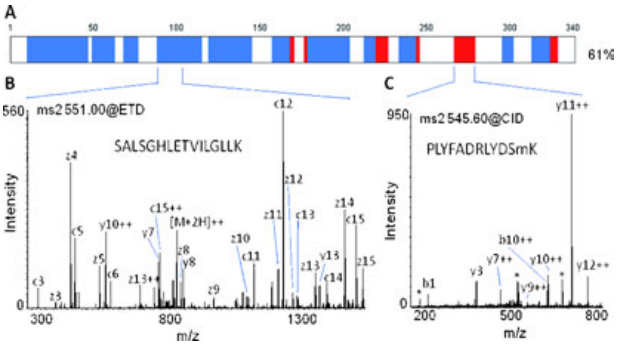
RESEARCH INTEREST

The Proteomics Unit has broad experience in proteomics approaches to the separation, quantification, identification and characterization of proteins in biological systems, and maintains a program of continuous technological development and improvement to meet the demanding requirements of the research community. Over the last year substantial progress was made in the initial steps of sample preparation, especially relating to the selection of specific subproteomes (for example based on protein activity) and fishing for proteins that interact with selected baits for interactome analysis.

We have made continuous improvements in the separation and quantitative analysis of differential protein expression by gel-based separation (2D-DIGE) and "gel-free" technologies based on nanoHPLC coupled to mass spectrometry. Proteins and peptides and their post-translational modifications are identified and characterized by MALDI-TOF/TOF and ESI mass spectrometers, the latter comprising a hybrid triple quadrupole (QqQ) and a linear ion trap coupled to an Orbitrap high resolution mass analyzer. Particular progress has been made in post-acquisition analysis and data visualization of the spectra through the use of combined validation technologies included in the Scaffold (Proteome Software) program.

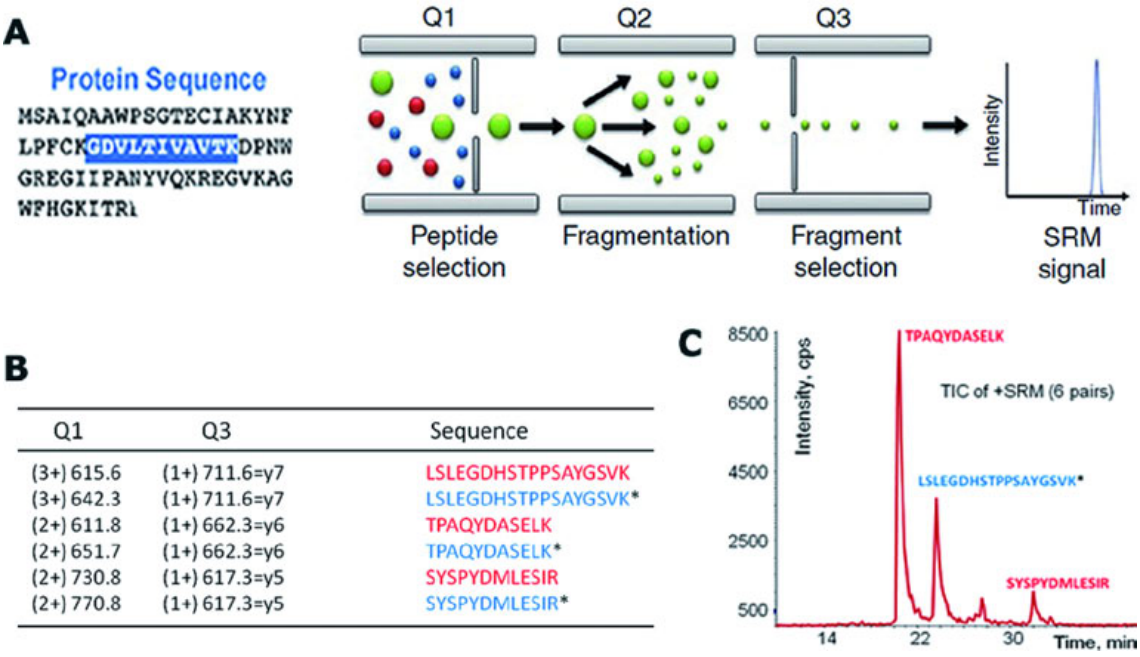
These approaches make use of shotgun and targeted proteomic analyses. While current approaches to global proteome profiling use high-throughput tandem mass spectrometry methods, these have limited sensitivity and are often unable to reliably detect and quantify low-abundance proteins in complex biological specimens such as a biopsy or cell extract. As an alternative 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.

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 the detection of differential protein expression, chemical and posttranslational modifications, and protein-protein interactions in diverse biological systems.



Annexin A2 sequence analysis by combined digestion and activation on the LTQ Orbitrap ETD spectrometer. A, *Annexin A2 sequence coverage using trypsin (blue boxes) and Lys-C (red) enzymes followed by LC-MS/MS analysis.* ***B,*** *Fragmentation spectra from an annexin-A2–derived tryptic peptide fragmented by electron transfer dissociation (ETD).* ***C,*** *Fragmentation spectra from an annexin peptide derived from Lys-C digestion fragmented by collision induced dissociation (CID).* (From *Fernandez-Garcia* et al., *2010.)*

6 Technical Units



B

Q1	Q3	Sequence
(3+) 615.6	(1+) 711.6=y7	LSLEGDHSTPPSAYGSVK
(3+) 642.3	(1+) 711.6=y7	LSLEGDHSTPPSAYGSVK*
(2+) 611.8	(1+) 662.3=y6	TPAQYDASELK
(2+) 651.7	(1+) 662.3=y6	TPAQYDASELK*
(2+) 730.8	(1+) 617.3=y5	SYSPYDMLESIR
(2+) 770.8	(1+) 617.3=y5	SYSPYDMLESIR*

Selected reaction monitoring (SRM). A, *Setup of a triple quadrupole instrument such as the Applied 4000 QTrap. In the SRM mode, samples separated by nanoHPLC are injected into the mass spectrometer, but only selected precursor ions from the protein(s) of interest are allowed to enter q2 and undergo fragmentation. Q3 is then tuned to detect only selected fragment (product) ions.* ***B,*** *Selected masses from annexin A2 set at Q1 and Q3 for the analysis of phosphorylation at Thr19, Thr105 and Ser236. Masses corresponding to both the nonphosphorylated peptides (red) and phosphorylated peptides (blue) were monitored along the chromatogram in Q1, and a characteristic fragment ion was set in Q3 for each peptide.* ***C,*** *Total ion chromatogram (TIC) of the six precursor/product pairs selected. The sequences found are indicated next to their corresponding retention time. (*Modified from *Fernandez-Garcia* et al., *2010.)*

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Duch A, Palou G, Jonsson ZO, Palou R, Calvo E, Wohlschlegel J, Quintana DG. **A DBF4 mutant that contributes to bypass the RAD53 mediated block of origins of replication in response to genotoxic stress.** *J Biol Chem* (accepted)

Garrido-Gomez T, Dominguez F, Lopez 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 Metab* (accepted)

Tuñon J, Martin-Ventura JL, Blanco-Colio LM, Lorenzo O, Lopez JA, Egido J. **Proteomic strategies in the search of new biomarkers in atherothrombosis.** *J Am Coll Cardiol* (2010) 55: 2009-16

Fernandez-Garcia B, Casado P, Prado MA, Ugarte-Gil LJ, Artime N, Cabal-Hierro L, Calvo E, Lopez JA, Ramos S, Lazo PS. **Proteomic analysis of annexin A2 phosphorylation induced by microtubule interfering agents and KSP inhibitors.** *J Proteome Res* (2010) 9: 4649-60

Lamas JR, Rodríguez-Rodríguez L, Vigo AG, Alvarez-Lafuente R, López-Romero P, Marco F, Camafeita E, Dopazo A, Callejas S, Villafuertes E, Hoyas JA, Tornero-Esteban MP, Urcelay E, Fernández-Gutiérrez B. **Large-scale gene expression in bone marrow mesenchymal stem cells: a putative role for COL10A1 in osteoarthritis.** *Ann Rheum Dis* (2010) 69: 1880-85

Cellomics

Head of Unit: *María Montoya*

Support Scientists: *José Manuel Ligos*
Hind Azegrouz

Predoctoral Researchers: *Begoña Díez*
Carmen Muñoz

Technicians: *Raquel Nieto*
Mariano Vitón
Mª Montserrat Arroyo

RESEARCH INTEREST

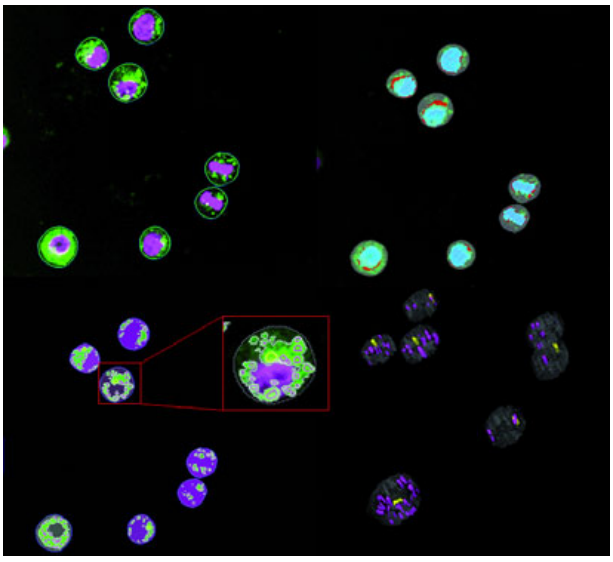
The fundamental unit of biological processes is the cell, and modern research is ever more dependent on the capacity to conduct sophisticated cell-based characterizations. The Cellomics Unit is dedictated to the two principal cell analytical techniques: flow cytometry and high content screening (HCS).

The Unit houses state-of-the-art flow cytometry and HCS equipment, and provides the necessary technical expertise in the use of this equipment to support the CNIC's research objectives. The Unit's staff assist researchers in experimental design and data interpretation for flow cytometry experiments, and design and perform high content screens, including miniaturization, automation, analysis and result validation. The Unit also provides training to CNIC research staff in flow cytometry and HCS technologies.

The Unit is equipped with

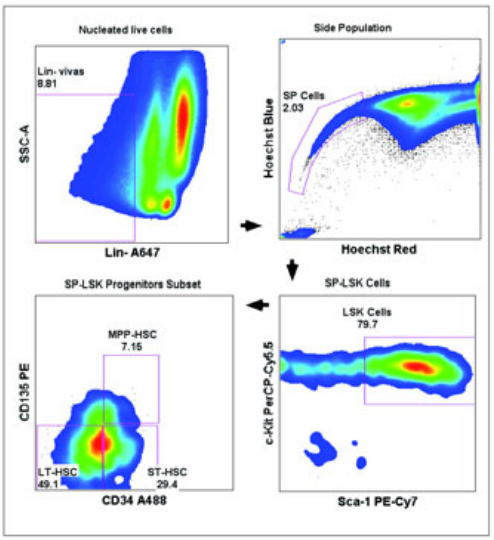
- Three latest generation digital analytical flow cytometers: two Becton Dickinson FACSCanto II machines and one Cyan (Beckman Coulter).
- Two high speed flow sorters: A MoFlo (Beckman Coulter) and a custom made FACSAria II (Becton Dickinson).
- A liquid handling workstation connected to a cell culture incubator with 110 plate throughput (Freedom EVO, Tecan).
- An automated confocal microscope for microplate reading (Opera, Perkin Elmer).
- A full range of dedicated cytometry and image analysis software packages (Modfit, FlowJo, Acapella, Definiens, MatLab).

The Unit is involved in research into the regulation of membrane trafficking during cell migration. We are interested in the role of Rab8, a GTPase that regulates intracellular membrane trafficking to the plasma membrane, in cell migration and cytoskeletal rearrangements. A high content screen has been developed to identify novel molecules involved in the regulation of Rab8 activivty.

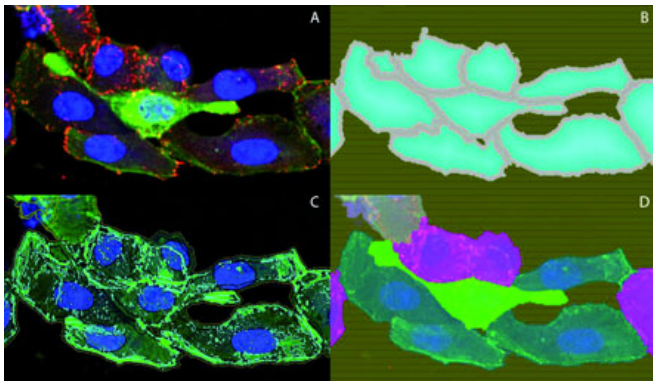


High content analysis of Cav internalization. *Hela cells expressing Cav1-GFP (green) are stained with Hoechst (pink). Confocal microscopy images were acquired using an automated microplate reader (Opera QEHS, PE) and then analyzed using a custom-made tool implemented in the* ***Definiens Developer*** *XD environment. Images show segmentation of nuclei (pale blue) and perinuclear space (red), and vesicles classified (green/pink/yellow) according to various parameters, both in 2D and 3D.*

6 Technical Units



FACS-based multiparametric detection of a hematopoietic stem cell subset. *Long Term (LT-HSC: SP+, Lin-,Sca1+, c-Kit+, CD34-, CD135-), Short Term (ST-HSC: SP+, Lin-, Sca1+, c-Kit+, CD34+, CD135-) and MultiPotential (MPP: SP+, Lin-, Sca1+, c-Kit+, CD34+, CD135+). Hematopoietic progenitors were detected in mouse bone marrow efflux through a combination of Hoechst 33342 staining (side population) and five-fluorescence inmunostaining.*



High content screen development for genes that regulate Rab8 GTPase-induced cytoskeletal rearrangements. *Hela cells expressing Rab8Q67L-GFP were fluorescently stained for nuclei and cytoplasm with Hoechst and CellMask (blue), polymerized actin with phalloidin (green) and focal adhesions with anti-vinculin (red). Confocal microscopy images were acquired using an automated microplate reader (Opera QEHS, PE); an overleay of fluorescence images is shown in* ***A.*** *Images were analyzed using a custom made tool implemented in the* Definiens Developer *XD environment.* ***B*** *shows segmentation of cell membrane (white) and interior areas (blue).* ***C*** *shows segmentation of polymerized actin structures (green), and* ***D*** *represents classification categories (green/blue/pink).*

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

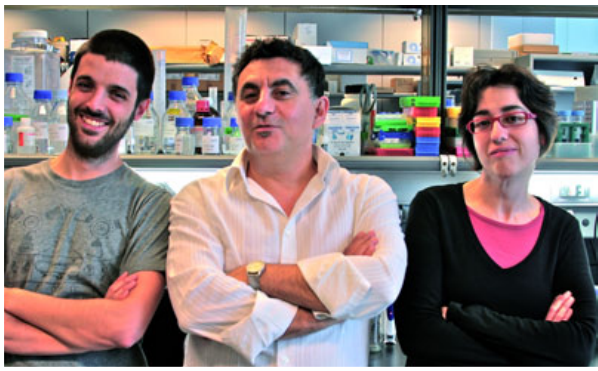
Trucco E, Azegrouz H, Dhillon B. **Modeling the tortuosity of retinal vessels: does calibre play a role?** *IEEE Trans Biomed Eng* (2010) 57: 2239-47

Viral Vectors

Head of Technical Service: *Juan Carlos Ramírez*

Support Scientist: *Raúl Torres*

Technician: *Aída García*

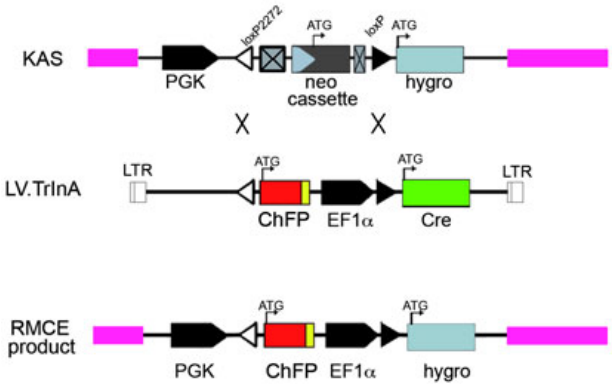


RESEARCH INTEREST

The Viral Vectors Unit provides CNIC researchers with replication deficient non-integrative adenovirus, HIV-derived lentivirus, and retroviral vectors. Virus stocks are produced, titrated and quality controlled by the rescue of replication-competent viruses and qPCR-quantification of particle/transduction units.

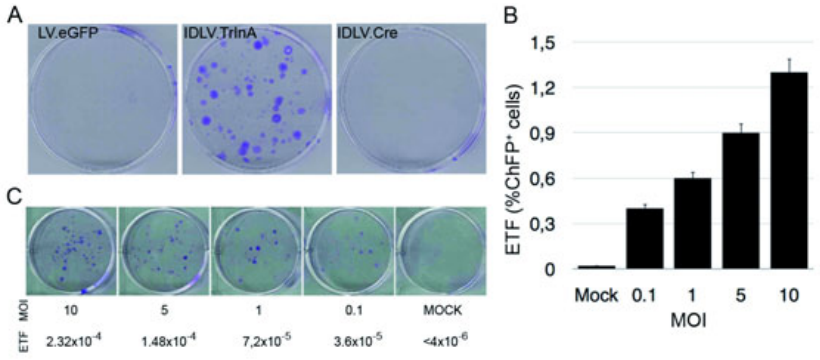
Over the last year our vector library grew to include close to a hundred viral backbones, matching the diverse expression requirements at the Center. Promoters driving constitutive (EF1alpha, CMV, PGK, Ubq, CAG), heart-specific (MHC) or tetracycline-inducible expression are assembled together with Picornaviridae IRES or 2A peptides for polycistronic mRNA synthesis of genes of interest in combination with selectable markers (PuroR, hygroR, neoR) or fluorescent reporter genes (green, cerulean, tomato, cherry, orange, td-tomato).

The Unit's research and development program has recently focused on two main areas. Our recent work on the role of the chemokine SDF-1gamma demonstrated that this cardiac-specific isoform targets the nucleolus; we are now collaborating with the Pluripotent Cell Technology facility to generate isoform-specific knockout mice. Secondly, we are working on a novel strategy to promote homologous recombination in genome-targeted cells by combining recombinant-mediated cassette exchange (RMCE) and integration-defective lentivirus (IDLV) technologies (*Kas-Trina* system). Cells targeted by a zinc-finger nuclease in the AAVS1 site with the *Kas* cassette are transduced with IDLV (*Trina*), and the self-limiting expression of the *cre* recombinase from the virus promotes RMCE at high frequency (up to 10%) and in a MOI-dependent manner. RMCE is revealed by promoter trapping of selectable markers and fluorescent proteins. We are currently testing the feasibility, reliability and simplicity of this system in human primary cells and established cell lines.



Cre recombinase-mediated insertion and cassette exchange strategy. *Structure of the genetic landing pad (KAS) and the incoming construct (LV.TrInA), showing the most important elements: promoters PGK, EF-1α and SV40 in the neo cassette (black arrows), reporter genes (coloured boxes), recombinase cre (green box), translation start site (cornered arrows), and polyA signals (crossed boxes). Triangles represent sites for loxP (black) and lox2272 (white). Homology arms are pink and lentiviral LTRs are open boxes flanking the KAS and TrInA cassette. The expected RMCE product is depicted below.*

6 Technical Units



Randomly integrated chromosomal copies of the genetic landing pad KAS are targeted by IDLV expressing the TrInA cassette. *HEK293A cells resistant to G418 (293AKAS) were pooled and transduced with a low MOI (2 transduction units/cell) of IDLV-TrInA.* ***A,*** *Transduced cells were selected with hygromycin and the colonies counted. The figure shows stained plates after selection of cells that were MOCK-transduced (LV.eGFP, left) or transduced with IDLV-TrInA (center) or IDLV-Cre (right).* ***B,*** *Quantification of RMCE frequency in 293AKAS cells by FACS analysis of ChFP+ cells upon transduction with IDLV-TrInA at different MOIs.* ***C,*** *Quantification of RMCE frequency in 293AKAS cells by culturing under selection conditions with hygromycin after transduction at different MOIs with IDLV-TrInA as in B. ETF = Effective Targeting Frecquency, expressed as the ratio between ChFP+ cells (B) or hygro^R cells (C) and the total number of integrants (G418^R).*

MAJOR GRANTS

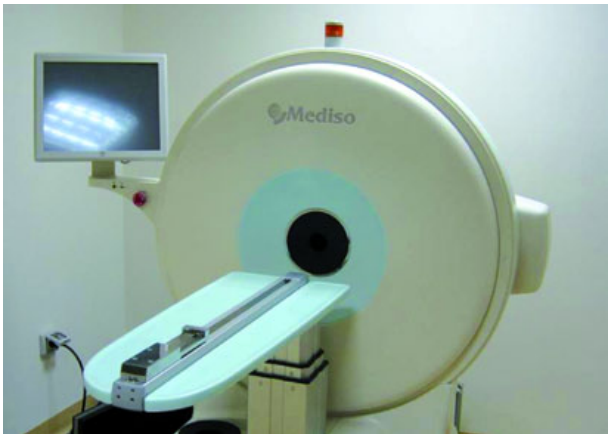
- **Ministerio de Ciencia e Innovación (IPT-010000-2010-040)**

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.
- The *Veterinary Medicine and Experimental Surgery Core* (ESC) provides highly specialized expertise in animal medical problems, disease follow-up, surgical procedures, minimally invasive intervention, and life support.
- 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.



Nano PET/CT apparatus for imaging studies in rodents

SELECTED PUBLICATIONS

Kent ML, Feist SW, Harper C, Hoogstraten-Miller S, Law JM, Sanchez-Morgado JM, Tanguay RL, Sanders GE, Spitsbergen JM, Whipps CM. **Recommendations for control of pathogens and infectious diseases in fish research facilities.** *Comp Biochem Physiol C Toxicol Pharmacol* (2009) 149: 240-8

Poynter S, Phipps JD, Naranjo-Pino A, Sanchez-Morgado JM. **Difficulties in the molecular diagnosis of helicobacter rodent infections.** *Vet Microbiol* (2009) 134: 272-8

Sanchez-Morgado JM, Gallagher A, Johnson LK. **Mycobacterium gordonae infection in a colony of african tropical clawed frogs (Xenopus tropicalis).** *Lab Anim* (2009) 43: 300-3

Appendix

Publications 2010

Training Programs and Courses

Seminars, Events and Awards

Funding

Staff Figures

Appendix

Publications 2010

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), 2009. 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 DEVELOPMENTAL BIOLOGY

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
IF: 4.351

Brand NJ, Lara-Pezzi E, Rosenthal N and Barton PJ.
Analysis of cardiac myocyte biology in transgenic mice: a protocol for preparation of neonatal mouse cardiac myocyte cultures.
Methods Mol Biol (2010) 633: 113-24
IF: n/a

Carramolino L, Fuentes J, Garcia-Andres C, Azcoitia V, Riethmacher D and Torres M.
Platelets play an essential role in separating the blood and lymphatic vasculatures during embryonic angiogenesis.
Circ Res (2010) 106: 1197-201
IF: 9.214

Fernandez-Tresguerres B, Canon S, Rayon T, Pernaute B, Crespo M, Torroja C and Manzanares M.
Evolution of the mammalian embryonic pluripotency gene regulatory network.
Proc Natl Acad Sci U S A (2010) 107: 19955-19960
IF: 9.432

Flores I and Blasco MA.
The role of telomeres and telomerase in stem cell aging.
FEBS Lett (2010) 584: 3826-3830
IF: 3.541

Garcia-Andres C and Torres M.
Comparative expression pattern analysis of the highly conserved chemokines SDF1 and CXCL14 during amniote embryonic development.
Dev Dyn (2010) 239: 2769-77
IF: 2.833

Gonzalez-Rosa JM, Padron-Barthe L, Torres M and Mercader N.
[Lineage tracing of epicardial cells during development and regeneration].
Rev Esp Cardiol (2010) 63 Suppl 2: 36-48
IF: 2.746

Grego-Bessa J, Pérez-Pomares JM and de la Pompa JL.
Signaling Pathways in Valve Formation: The Origin of Congenital Defects.
Heart Development and Regeneration (2010) 1: 389-413
IF: n/a

Grimes AC, Duran AC, Sans-Coma V, Hami D, Santoro MM and Torres M.
Phylogeny informs ontogeny: a proposed common theme in the arterial pole of the vertebrate heart.
Evol Dev (2010) 12: 552-67
IF: 3.179

Jimenez-Guri E, Udina F, Colas JF, Sharpe J, Padron-Barthe L, Torres M and Pujades C.
Clonal analysis in mice underlines the importance of rhombomeric boundaries in cell movement restriction during hindbrain segmentation.
PLoS One (2010) 5: e10112
IF: 4.351

Kouwenhoven EN, van Heeringen SJ, Tena JJ, Oti M, Dutilh BE, Alonso ME, de la Calle-Mustienes E, Smeenk L, Rinne T, Parsaulian L, Bolat E, Jurgelenaite R, Huynen MA, Hoischen A, Veltman JA, Brunner HG, Roscioli T, Oates E, Wilson M, Manzanares M, Gomez-Skarmeta JL, Stunnenberg HG, Lohrum M, van Bokhoven H and Zhou H.
Genome-wide profiling of p63 DNA-binding sites identifies an element that regulates gene expression during limb development in the 7q21 SHFM1 locus.
PLoS Genet (2010) 6: e1001065
IF: 9.532

Lara-Pezzi E and Rosenthal N.
Genetic Enhancement of Cardiac Regeneration.
Heart Development and Regeneration (2010) 2: 981-997
IF: n/a

Luna-Zurita L, Prados B, Grego-Bessa J, Luxan G, Del Monte G, Benguria A, Adams RH, Perez-Pomares JM and de la Pompa JL.
Integration of a Notch-dependent mesenchymal gene program and Bmp2-driven cell invasiveness regulates murine cardiac valve formation.
J Clin Invest (2010) 120: 3493-507
IF: 15.387

Macgrogan D, Nus M and de la Pompa JL.
Notch signaling in cardiac development and disease.
Curr Top Dev Biol (2010) 92: 333-65
IF: 5.753

Appendix

Publications 2010

Pernaute B, Canon S, Crespo M, Fernandez-Tresguerres B, Rayon T and Manzanares M.
Comparison of extraembryonic expression of Eomes and Cdx2 in pregastrulation chick and mouse embryo unveils regulatory changes along evolution.
Dev Dyn (2010) 239: 620-9
IF: 2.833

Pittman AM, Naranjo S, Jalava SE, Twiss P, Ma Y, Olver B, Lloyd A, Vijayakrishnan Y, Qureshi M, Broderick P, van Wezel T, Morreau H, Tuupanen S, Aaltonen LA, Alonso ME, Manzanares M, Gavilan A, Visakorpi T, Gomez-Skarmeta JL and Houlston RS.
Allelic Variation at the 8q23.3 Colorectal Cancer Risk Locus Functions as a Cis-Acting Regulator of EIF3H.
PLoS Genet (2010) 6: e1001126
IF: 9.532

Ragvin A, Moro E, Fredman D, Navratilova P, Drivenes O, Engstrom PG, Alonso ME, Mustienes Ede L, Skarmeta JL, Tavares MJ, Casares F, Manzanares M, van Heyningen V, Molven A, Njolstad PR, Argenton F, Lenhard B and Becker TS.
Long-range gene regulation links genomic type 2 diabetes and obesity risk regions to HHEX, SOX4, and IRX3.
Proc Natl Acad Sci U S A (2010) 107: 775-80
IF: 9.432

Rossello CA and Torres M.
Gene transfer by electroporation into hemogenic endothelium in the avian embryo.
Dev Dyn (2010) 239: 1748-54
IF: 2.833

Spruce T, Pernaute B, Di-Gregorio A, Cobb BS, Merkenschlager M, Manzanares M and Rodriguez TA.
An early developmental role for miRNAs in the maintenance of extraembryonic stem cells in the mouse embryo.
Dev Cell (2010) 19: 207-19
IF: 13.363

EPIDEMIOLOGY, ATHEROTHROMBOSIS AND IMAGING

Araujo F, Mateo de Castro J and Encinas Barrios C.
[Ezetimibe: caution in the face of doubt].
Farm Hosp (2010) 34: 212-4
IF: n/a

Badimon JJ and Ibanez B.
Increasing high-density lipoprotein as a therapeutic target in atherothrombotic disease.
Rev Esp Cardiol (2010) 63: 323-33
IF: 2.746

Carson AP, Fox CS, McGuire DK, Levitan EB, Laclaustra M, Mann DM and Muntner P.
Low Hemoglobin A1c and Risk of All-Cause Mortality Among US Adults Without Diabetes.
Circ Cardiovasc Qual Outcomes (2010) 3: 661-7
IF: n/a

Cruz-Adalia A, Jimenez-Borreguero LJ, Ramirez-Huesca M, Chico-Calero I, Barreiro O, Lopez-Conesa E, Fresno M, Sanchez-Madrid F and Martin P.
CD69 Limits the Severity of Cardiomyopathy After Autoimmune Myocarditis.
Circulation (2010) 122: 1396-404
IF: 14.816

Elmariah S, Delaney JA, O'Brien KD, Budoff MJ, Vogel-Claussen J, Fuster V, Kronmal RA and Halperin JL.
Bisphosphonate Use and Prevalence of Valvular and Vascular Calcification in Women MESA (The Multi-Ethnic Study of Atherosclerosis).
J Am Coll Cardiol (2010) 56: 1752-9
IF: 12.535

Fadrowski JJ, Navas-Acien A, Tellez-Plaza M, Guallar E, Weaver VM and Furth SL.
Blood lead level and kidney function in US adolescents: The Third National Health and Nutrition Examination Survey.
Arch Intern Med (2010) 170: 75-82
IF: 9.813

Franco M, Sanz B, Otero L, Dominguez-Vila A and Caballero B.
Prevention of childhood obesity in Spain: a focus on policies outside the health sector. SESPAS report 2010.
Gac Sanit (2010) 24: 49-55
IF: 1.172

Fuster JJ and Andres V.
A role for miR-33 in p53 regulation: New perspectives for hematopoietic stem cell research.
Cell Cycle (2010) 9: 3397-8
IF: 4.087

Fuster JJ, Fernandez P, Gonzalez-Navarro H, Silvestre C, Nabah YN and Andres V.
Control of cell proliferation in Atherosclerosis: insights from animal models and human studies.
Cardiovasc Res (2010) 86: 254-64
IF: 5.801

Fuster JJ, Gonzalez JM, Edo MD, Viana R, Boya P, Cervera J, Verges M, Rivera J and Andres V.
Tumor suppressor p27Kip1 undergoes endolysosomal degradation through its interaction with sorting nexin 6.
FASEB J (2010) 24: 2998-3009
IF: 6.401

Fuster V.
Fine-tuning therapy for acute coronary syndromes.
N Engl J Med (2010) 363: 976-7
IF: 47.050

Fuster V.
[Science, health and education: a priority and a model.].
Arch Cardiol Mex (2010) 80: 261-271
IF: n/a

Fuster V, Jimenez-Borreguero JL, Sanz G and Ibanez B.
HIV and cardiovascular disease: urgent need for sub-clinical detection.
Nat Rev Cardiol (CNIC Edition) (2010) 7: 17-21
IF: n/a

Fuster V, Lois F and Franco M.
Early identification of atherosclerotic disease by noninvasive imaging.
Nat Rev Cardiol (2010) 7: 327-33
IF: n/a

Fuster V and Sweeny JM.
Clopidogrel and the reduced-function CYP2C19 genetic variant: a limited piece of the overall therapeutic puzzle.
JAMA (2010) 304: 1839-40
IF: 28.899

Appendix

Publications 2010

Fuster V and Vahl TP.
The role of noninvasive imaging in promoting cardiovascular health.
J Nucl Cardiol (2010) 17: 781-90
IF: 2.777

Galende E, Karakikes I, Edelmann L, Desnick RJ, Kerenyi T, Khoueiry G, Lafferty J, McGinn JT, Brodman M, Fuster V, Hajjar RJ and Polgar K.
Amniotic fluid cells are more efficiently reprogrammed to pluripotency than adult cells.
Cell Reprogram (2010) 12: 117-25
IF: 2.692

Garcia-Alvarez A, Fernandez-Friera L, Fuster V and Sanz J.
Role of new imaging techniques in the emergency department diagnosis of acute coronary syndrome.
Emergencias (2010) 22: 125-129
IF: n/a

Gomez-Abellan P, Gomez-Santos C, Madrid JA, Milagro FI, Campion J, Martinez JA, Ordovas JM and Garaulet M.
Circadian expression of adiponectin and its receptors in human adipose tissue.
Endocrinology (2010) 151: 115-22
IF: 4.752

Gonzalez-Navarro H, Abu Nabah YN, Vinue A, Andres-Manzano MJ, Collado M, Serrano M and Andres V.
p19(ARF) deficiency reduces macrophage and vascular smooth muscle cell apoptosis and aggravates Atherosclerosis.
J Am Coll Cardiol (2010) 55: 2258-68
IF: 12.535

Guallar E, Miller ER, 3rd, Ordovas JM and Stranges S.
Vitamin D supplementation in the age of lost innocence.
Ann Intern Med (2010) 152: 327-9
IF: 16.225

Ibanez B and Fuster V.
Ischaemic conditioning for myocardial salvage after AMI.
Lancet (2010) 375: 1691; author reply 1692
IF: 30.758

Jones S, Solomon A, Sanz-Rosa D, Moore C, Holbrook L, Cartwright EJ, Neyses L and Emerson M.
The plasma membrane calcium ATPase (PMCA) modulates calcium homeostasis, intracellular signalling events and function in platelets.
J Thromb Haemost (2010) 8: 2766-74
IF: 6.069

Laclaustra M.
Adipose tissue, the metabolic syndrome and Atherosclerosis.
Nat Rev Cardiol (CNIC Edition) (2010) 7: 29-34
IF: n/a

Laclaustra M.
Fisiopatología y síndrome metabólico: el papel del tejido adiposo.
La opinión de los expertos (2010) 51: 4-8
IF: n/a

Laclaustra M, Ordovas JM and Civeira F.
Fisiopatología del sindrome metabólico. De la resistencia a la insulina y las adipocians a la disfunción endotelial.
El síndrome metabólico (2010) 23-40
IF: n/a

Laclaustra M, Stranges S, Navas-Acien A, Ordovas JM and Guallar E.
Serum selenium and serum lipids in US adults: National Health and Nutrition Examination Survey (NHANES) 2003-2004.
Atherosclerosis (2010) 210: 643-8
IF: 4.522

Lamas JR, Rodriguez-Rodriguez L, Varade J, Lopez-Romero P, Tornero-Esteban P, Abasolo L, Urcelay E and Fernandez-Gutierrez B.
Influence of IL6R rs8192284 Polymorphism Status in Disease Activity in Rheumatoid Arthritis.
J Rheumatol (2010) 37: 1579-81
IF: 3.854

Lamas JR, Rodriguez-Rodriguez L, Vigo AG, Alvarez-Lafuente R, Lopez-Romero P, Marco F, Camafeita E, Dopazo A, Callejas S, Villafuertes E, Hoyas JA, Tornero-Esteban MP, Urcelay E and Fernandez-Gutierrez B.
Large-scale gene expression in bone marrow mesenchymal stem cells: a putative role for COL10A1 in osteoarthritis.
Ann Rheum Dis (2010) 69: 1880-5
IF: 8.111

Liao SL, Elmariah S, van der Zee S, Sealove BA and Fuster V.
Does medical therapy for thoracic aortic aneurysms really work? Are beta-blockers truly indicated? CON.
Cardiol Clin (2010) 28: 261-9
IF: 1.247

Lobatto ME, Fayad ZA, Silvera S, Vucic E, Calcagno C, Mani V, Dickson S, Nicolay K, Banciu M, Schiffelers RM, Metselaar J, van Bloois L, Wu HS, Fallon J, Rudd J, Fuster V, Fisher EA, Storm G and Mulder WJ.
Multimodal clinical imaging to longitudinally assess a nanomedical anti-inflammatory treatment in experimental Atherosclerosis.
Mol Pharm (2010) 7: 2020-9
IF: 4.531

Lobo E, Dufouil C, Marcos G, Quetglas B, Saz P, Guallar E and Lobo A.
Is There an Association Between Low-to-Moderate Alcohol Consumption and Risk of Cognitive Decline?
Am J Epidemiol (2010) 172: 708-16
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Lamas JR, Rodriguez-Rodriguez L, Vigo AG, Alvarez-Lafuente R, Lopez-Romero P, Marco F, Camafeita E, Dopazo A, Callejas S, Villafuertes E, Hoyas JA, Tornero-Esteban MP, Urcelay E and Fernandez-Gutierrez B.
Large-scale gene expression in bone marrow mesenchymal stem cells: a putative role for COL10A1 in osteoarthritis.
Ann Rheum Dis (2010) 69: 1880-5
IF: 8.111

Lopez-Huertas MR, Callejas S, Abia D, Mateos E, Dopazo A, Alcami J and Coiras M.
Modifications in host cell cytoskeleton structure and function mediated by intracellular HIV-1 Tat protein are greatly dependent on the second coding exon.
Nucleic Acids Res (2010) 38: 3287-307
IF: 7.479

Lopez-Romero P, Gonzalez MA, Callejas S, Dopazo A and Irizarry RA.
Processing of Agilent microRNA array data.
BMC Res Notes (2010) 3: 18
IF: n/a

Luna-Zurita L, Prados B, Grego-Bessa J, Luxan G, Del Monte G, Benguria A, Adams RH, Perez-Pomares JM and de la Pompa JL.
Integration of a Notch-dependent mesenchymal gene program and Bmp2-driven cell invasiveness regulates murine cardiac valve formation.
J Clin Invest (2010) 120: 3493-507
IF: 15.387

Martinez-Pinna R, Barbas C, Blanco-Colio LM, Tunon J, Ramos-Mozo P, Lopez JA, Meilhac O, Michel JB, Egido J and Martin-Ventura JL.
Proteomic and metabolomic profiles in atherothrombotic vascular disease.
Curr Atheroscler Rep (2010) 12: 202-8
IF: n/a

Mlecnik B, Sanchez-Cabo F, Charoentong P, Bindea G, Pages F, Berger A, Galon J and Trajanoski Z.
Data integration and exploration for the identification of molecular mechanisms in tumor-immune cells interaction.
BMC Genomics (2010) 11 Suppl 1: S7
IF: 3.759

Pallares P, Garcia-Fernandez RA, Criado LM, Letelier CA, Fernandez-Toro JM, Esteban D, Flores JM and Gonzalez-Bulnes A.
Substantiation of ovarian effects of leptin by challenging a mouse model of obesity/type 2 diabetes.
Theriogenology (2010) 73: 1088-95
IF: 2.073

Puig-Kroger A, Aguilera-Montilla N, Martinez-Nunez R, Dominguez-Soto A, Sanchez-Cabo F, Martin-Gayo E, Zaballos A, Toribio ML, Groner Y, Ito Y, Dopazo A, Corcuera MT, Alonso Martin MJ, Vega MA and Corbi AL.
The novel RUNX3/p33 isoform is induced upon monocyte-derived dendritic cell maturation and downregulates IL-8 expression.
Immunobiology (2010) 215: 812-20
IF: 3.586

Trucco E, Azegrouz H and Dhillon B.
Modeling the tortuosity of retinal vessels: does calibre play a role?
IEEE Trans Biomed Eng (2010) 57: 2239-47
IF: 2.154

Tunon J, Martin-Ventura JL, Blanco-Colio LM, Lorenzo O, Lopez JA and Egido J.
Proteomic strategies in the search of new biomarkers in atherothrombosis.
J Am Coll Cardiol (2010) 55: 2009-16
IF: 12.535

Zanella F, Renner O, Garcia B, Callejas S, Dopazo A, Peregrina S, Carnero A and Link W.
Human TRIB2 is a repressor of FOXO that contributes to the malignant phenotype of melanoma cells.
Oncogene (2010) 29: 2973-82
IF: 7.135

Appendix

Publications 2010

	TOTAL (*)	TOTAL IF Publications (*)	CUMULATIVE IF	AVERAGE IF
TOTAL	139	111	866.548	7.807
CARDIOVASCULAR DEVELOPMENTAL BIOLOGY	19	16	108.312	6.770
EPIDEMIOLOGY, ATHEROTHROMBOSIS AND IMAGING	58	43	413.865	9.625
REGENERATIVE CARDIOLOGY	25	21	139.681	6.651
TRANSLATIONAL CARDIOVASCULAR RESEARCH	7	5	13.899	2.780
VASCULAR BIOLOGY AND INFLAMMATION	19	16	148.950	9.309
TECHNICAL UNITS	20	17	127.095	7.476

(*) 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 2010

Name	Secondary School	Comunidad Autónoma
Andrés Sanz, Julio Alberto	I.E.S. El Portillo	Aragón
García Vargas, Laura	I.E.S. Nicolás Salmerón y Alonso	Andalucía
López Machado, Marta	Santa María del Pilar	Aragón
Muñoz González, Héctor	I.E.S. Satafi	Madrid
Pérez Hernández, Ana Isabel	I.E.S. Javier García Téllez	Extremadura
Pérez Ramírez, Álvaro	I.E.S Fernando III El Santo	Andalucía
Sánchez Martínez, Domingo Antonio	C.E.S. Ciudad del Sol	Murcia
Santos Urios, María	I.E.S. La Nía	Valencia

CICERONE Program

The CICERONE Program is open to advanced undergraduate students studying towards 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.

Appendix

Training Programs and Courses

Fellowships in 2010

Name	Degree	University
Albacete, Lucas	Biochemistry	Autónoma de Madrid
Alonso, Álvaro	Biology	Autónoma de Madrid
Arenas, Enrique Javier	Biotechnology	Politécnica de Valencia
Bernal Mera, Aurora	Biology	Autónoma de Madrid
Bilal Álvarez, Usama	Medicine	Oviedo
Borkowska, Natalia	Biotechnology	Warsaw /Polonia
Cano Romero, Francisco	Biochemistry	Granada
Castillo, Verónica	Pharmacy	Granada
Clemente Toribio, Cristina	Biology	Autónoma de Madrid
Cosín Roger, Jesús	Biotechnology	Politécnica de Valencia
Cruz Uréndez, Francisco Miguel	Biochemistry	Granada
Cumienny, Rafal	Biotechnology	JagiellonianUniversity / Poland
Dhungel, Bijay	Molecular Biology	Acharia Nagarjuna (India) / University of Eastern Finland
Gómez, Jesús María	Biotechnology	Francisco de Vitoria
Hamzcyk, Magda	Biotechnology	Jagiellonian University/Poland
Lázaro, Ana	Biology	Autónoma de Madrid
Luengo Lago, Alba	Chemical Engineering	Columbia University
Maroto, Lucía	Food Technology	Autónoma de Madrid
Martín Higueda, Cristina	Biology	La Laguna
Martínez López, María	Biochemistry	Granada
Merás Colunga, Pablo	Medicine	Oviedo
Mota, Alba	Biochemistry	Autónoma de Madrid
Navarro, Miguel	Medicine	Complutense de Madrid
Oller Pedrosa, Jorge	Biochemistry	Autónoma de Madrid
Ruiz Rivero, Juncal	Medicine	Complutense de Madrid
Sánchez, Damián	Medicine	Autónoma de Madrid
Surkont, Jaroslaw	Biotechnology	Jagiellonian University / Poland
Torres, María del Mar	Biochemistry	Autónoma de Madrid
Villaroy Beltri, Carolina	Biochemistry	Autónoma de Madrid
Wade, Kaitlin	Biology and Mathematics	University of Bristol

Appendix

Training Programs and Courses

CICERONE Workshop: "What you need to know about cardiovascular research"

This group of lectures provides a general introduction to cardiovascular research in Spain, and also gives participants the chance to ask questions to key researchers and opinion leaders in the field. The 2010 edition of the CICERONE workshop took place in Valencia as part of the Cardiovascular Diseases Meeting of the Sociedad Española de Cardiología.

Date: 21 October 2010
Attendees: 40

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: 19-20 July 2010
Attendees: 112

Appendix

Training Programs and Courses

Recent Graduates

CARDIOVASCULAR POSTGRADUATE 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 has 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-18 February 2011
Venue: CNIC
Students: 10

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 2010

Name	Master	Degree-University
Bernal Mera, Aurora	Molecular Biomedicine	Autónoma de Madrid
Clemente Toribio, Cristina	Cellular and Molecular Biology	Autónoma de Madrid
Cruz Uréndez, Francisco Miguel	Molecular Biomedicine	Granada
María Gómez, Jesús	Molecular Biomedicine	Francisco de Vitoria
Martínez López, María	Molecular Biomedicine	Granada
Lázaro Carrillo, Ana	Molecular Biomedicine	Autónoma de Madrid
Torres Capelli, María del Mar	Cellular and Molecular Biology	Granada
Villarroya Beltri, Carolina	Molecular Biomedicine	Autónoma de Madrid

Appendix

Training Programs and Courses

PREDOCTORAL (PhD) Program

The PREDOCTORAL Program provides a common framework for all researchers at the CNIC who are working towards 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 2010

Name	Title of thesis	University	CNIC Department	Thesis Advisor(s)
Cruz Adalia, Aranzazu	Leukocyte activation molecule CD69 as a regulator of inflammatory processes	Faculty of Medicine. Universidad Autónoma de Madrid	Vascular Biology and Inflammation	Francisco Sánchez-Madrid and Pilar Martín
García Maceira, Patricia	Regulation of the HIF path: implications for tumor autogenesis	Faculty of Biology Sciences. Universidad Complutense de Madrid	Epidemiology, Atherothrombosis and Imaging	Jesús Mateo de Castro
López Frontal, Raquel	Role of the tumor suppressor P19ARF in the inflammatory response	Faculty of Sciences. Universidad Autónoma de Madrid	Regenerative Cardiology	Sonsoles Hortelano
Luna Zurita, Luis	Integration of signals during heart valves development: role of the Notch pathway	Faculty of Sciences. Universidad Autónoma de Madrid	Cardiovascular Developmental Biology	José Luis de la Pompa
Olmos Buchelt, Yolanda	Characterization of the complex that regulates the protection system against mitochondrial oxidative stress	Faculty of Biology. Universidad Complutense de Madrid	Regenerative Cardiology	María Monsalve Pérez
Sala Valdés, Mónica	Links between the tetraspanin web and the actin cytoskeleton and their role in migration and immune synapse formation	Faculty of Biology. Universidad Autónoma de Madrid	Vascular Biology and Inflammation	María Yáñez-Mó and Francisco Sánchez-Madrid
Tarín Cerezo, Carlos A.	Remodeling processes mediated by vasoactive nitric oxide and its relevance to cardiovascular disease through the activation of proteolytic enzymes	Faculty of Medicine. Universidad Alcalá de Henares	Epidemiology, Atherothrombosis and Imaging	Carlos Zaragoza Sánchez

Appendix

Training Programs and Courses

Graduate students carrying their PhD theses at the CNIC during 2010

Name	Funding Agency	University	CNIC Departmen	Joined previously through another Training Programme
Aix Sacido, Esther	FPU (Spanish Ministry of Education and Science)	Universidad Autónoma de Madrid	Cardiovascular Developmental Biology	BMM9 2009 - 2010 / MASTER Program 2009
Alameda Serrano, Daniel	FPI (Spanish Ministry of Education and Science)	Universidad Autónoma de Madrid	Regenerative Cardiology	CICERONE Program 2007
Bednareck, Dorota	FPI (Spanish Ministry of Education and Science)	Universidad Autónoma de Madrid	Cardiovascular Developmental Biology	No
Bergacín Liberman, Gabriel	CNIC contract	Universidad Autónoma de Madrid	Regenerative Cardiology	No
Blanco Menéndez, Noelia	CNIC contract	Universidad Autónoma de Madrid	Vascular Biology and Inflammation	No
Casanova Acebes, María	FPI (Spanish Ministry of Education and Science)	Universidad Autónoma de Madrid	Epidemiology, Atherothrombosis and Imaging	No
Cedenilla Horcajuelo, Marta	FPU (Spanish Ministry of Education and Science)	Universidad Autónoma de Madrid	Regenerative Cardiology	CICERONE Program 2008 / Cardiovascular Postgraduate Program 2008 - 2009 / MASTER Program 2008
D'Amato, Gaetano	Marie Curie Initial Training Network (NotchIT)	Universidad Autónoma de Madrid	Cardiovascular Developmental Biology	No
Del Monte Nieto, Gonzalo	Spanish Ministry of Science and Innovation	Universidad Autónoma de Madrid	Cardiovascular Developmental Biology	No
Díez Cabezas, Begoña	FPU (Spanish Ministry of Education and Science)	Universidad Autónoma de Madrid	Vascular Biology and Inflammation	No
Escolano Artigas, Amelia	FPI (Spanish Ministry of Education and Science)	Universidad Autónoma de Madrid	Vascular Biology and Inflammation	No
Escudero Gonzalez, Beatriz	FIS (Spanish Ministry of Health)	Universidad Autónoma de Madrid	Regenerative Cardiology	No

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Training Programs and Courses

Fernández-Tresguerres Torrecillas, Beatriz	FPI (Spanish Ministry of Education and Science)	Universidad Autónoma de Madrid	Cardiovascular Developmental Biology	No
Foronda Álvaro, Miguel	FPU (Spanish Ministry of Education and Science)	Universidad Autónoma de Madrid	Vascular Biology and Inflammation	CICERONE Program 2007 and 2008 / MASTER Program 2008
Gárate Mutiloa, Zita	Vasque Government	Universidad Autónoma de Madrid	Regenerative Cardiology	Cardiovascular Postgraduate Program 2009-2010
García-Prieto Cuesta, Jaime	CNIC contract	Universidad Autónoma de Madrid	Epidemiology, Atherothrombosis and Imaging	No
Giraldo Prado, Patricia	CNIC contract	Universidad Autónoma de Madrid	Regenerative Cardiology	No
Gómez Cabañas, Laura	FPU (Spanish Ministry of Education and Science)	Universidad Autónoma de Madrid	Regenerative Cardiology	CICERONE Program 2008 / MASTER Program 2008
González Rosa, Juan Manuel	FPU (Spanish Ministry of Education and Science)	Universidad Autónoma de Madrid	Cardiovascular Developmental Biology	CICERONE Program 2008 / MASTER Program 2008
Guadamilla Mora, Marta C.	FPI (Spanish Ministry of Education and Science)	Universidad Complutense de Madrid	Vascular Biology and Inflammation	No
Gutiérrez Vázquez, Cristina	CAM (Madrid Autonomic Region)	Universidad Autónoma de Madrid	Vascular Biology and Inflammation	CICERONE Program 2007 / Cardiovascular Postgraduate Program 2008 -2009
Hernández de Riquer, Mª Victoria	FPI (Spanish Ministry of Education and Science)	Universidad Complutense de Madrid	Vascular Biology and Inflammation	No
Herrera Merchan, Antonio	Human Frontier Science Foundation	Universidad Autónoma de Madrid	Regenerative Cardiology	No
Izarra Pérez, Alberto	FPI (Spanish Ministry of Education and Science)	Universidad Autónoma de Madrid	Regenerative Cardiology	No
Koziol, Agnieszka	FPU (Spanish Ministry of Education and Science)	Universidad Autónoma de Madrid	Vascular Biology and Inflammation	No

Appendix

Training Programs and Courses

Lara Astiaso, David	FPU (Spanish Ministry of Education and Science)	Universidad Autónoma de Madrid	Regenerative Cardiology	Cardiovascular Postgraduate Program 2008 -2009 / MASTER Program 2008
Latorre Pellicer, Ana	Diputación General de Aragón	Universidad de Zaragoza	Regenerative Cardiology	No
Lavín Plaza, Begoña	FPI (Spanish Ministry of Education and Science)	Universidad Autónoma de Madrid	Epidemiology, Atherothrombosis and Imaging	No
López Fontal, Raquel	FIS (Spanish Ministry of Health)	Universidad Autónoma de Madrid	Regenerative Cardiology	No
Lozano Vidal, Noelia	FPU (Spanish Ministry of Education and Science)	Universidad Autónoma de Madrid	Vascular Biology and Inflammation	Cardiovascular Postgraduate Program 2009 -2010 / MASTER Program 2009
Luna Zurita, Luis	CNIC contract	Universidad Autónoma de Madrid	Cardiovascular Developmental Biology	No
Luxán García, Guillermo	FPI (Spanish Ministry of Education and Science)	Universidad Autónoma de Madrid	Cardiovascular Developmental Biology	No
Marco Lázaro, Ricardo	CNIC contract	Universidad de Zaragoza	Regenerative Cardiology	No
Martín Alonso, Mara	FPI (Spanish Ministry of Education and Science)	Universidad Autónoma de Madrid	Vascular Biology and Inflammation	CICERONE Program 2008 / Cardiovascular Postgraduate Program 2009 -2010
Mateos San Martín, Daniel	CAM (Madrid Autonomic Region)	Universidad Autónoma de Madrid	Cardiovascular Developmental Biology	CNIC
Matesanz Marín, Adela	FPI (Spanish Ministry of Education and Science)	Universidad Autónoma de Madrid	Vascular Biology and Inflammation	No
Méndez Barbero, Nerea	FPU (Spanish Ministry of Education and Science)	Universidad Autónoma de Madrid	Vascular Biology and Inflammation	Cardiovascular Postgraduate Program 2008 -2009 / MASTER Program 2008
Mendoza Daroca, Pilar	Human Frontier Science Foundation	Universidad Autónoma de Madrid	Regenerative Cardiology	No

Appendix

Training Programs and Courses

Molina Sánchez, Pedro	FPU (Spanish Ministry of Education and Science)	Universidad de Valencia	Epidemiology, Atherothrombosis and Imaging	No
Montes Ruiz, Antonio José	Human Frontier Science Foundation	Universidad Autónoma de Madrid	Regenerative Cardiology	CICERONE Program 2007 / Cardiovascular Postgraduate Program 2008 -2009
Moreno Rodríguez, Vanessa	CAM (Madrid Autonomic Region)	Universidad Autónoma de Madrid	Vascular Biology and Inflammation	No
Munch, Juliane	Notch IT, Marie Curie	Universidad Autónoma de Madrid	Cardiovascular Developmental Biology	Cardiovascular Postgraduate Program 2009 -2010
Muñoz Agudo, Carmen	FPI (Spanish Ministry of Education and Science)	Universidad Autónoma de Madrid	Vascular Biology and Inflammation	No
Muriel López, Olivia	FIS (Spanish Ministry of Health)	Universidad Autónoma de Madrid	Vascular Biology and Inflammation	CICERONE Program 2006
Núñez Andrade, Norman	FPI (Spanish Ministry of Education and Science)	Universidad Autónoma de Madrid	Vascular Biology and Inflammation	No
Olmos Buchelt, Yolanda	SAF (Spanish Ministry of Science and Innovation)	Universidad Complutense de Madrid	Regenerative Cardiology	No
Rayón Alonso, Teresa	FPU (Spanish Ministry of Education and Science)	Universidad Autónoma de Madrid	Cardiovascular Developmental Biology	No
Rodríguez, Juan Camilo Estrada	Red TERCEL (La Paz Hospital)	Universidad Autónoma de Madrid	Regenerative Cardiology	No
Roselló Díez, Alberto	FPI (Spanish Ministry of Education and Science)	Universidad Autónoma de Madrid	Cardiovascular Developmental Biology	No
Sala Valdés, Mónica	FIS (Spanish Ministry of Health)	Universidad Autónoma de Madrid	Vascular Biology and Inflammation	No
Sánchez Ramos, Cristina	FPI (Spanish Ministry of Education and Science)	Universidad Autónoma de Madrid	Regenerative Cardiology	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

Appendix

Training Programs and Courses

Tejera Puente, Emilio	FIS (Spanish Ministry of Health)	Universidad Autónoma de Madrid	Vascular Biology and Inflammation	No
Tomé Pizarro, María	CNIC contract	Universidad Autónoma de Madrid	Regenerative Cardiology	CICERONE Program 2008 / Cardiovascular Postgraduate Program 2008 - 2009
Uribe Sokolov, Verónica	FPU (Spanish Ministry of Education and Science)	Universidad Autónoma de Madrid	Cardiovascular Developmental Biology	CICERONE Program 2007 and 2008 / MASTER Program 2008
Urso, Katia	FIS (Spanish Ministry of Health)	Universidad Autónoma de Madrid	Vascular Biology and Inflammation	No
Valiente Alandí, Iñigo	FPU (Spanish Ministry of Education and Science)	Universidad Autónoma de Madrid	Regenerative Cardiology	CICERONE Program 2008 / Cardiovascular Postgraduate Program 2008-2009 / MASTER Program 2008
Wild, Brigitte	Spanish Ministry of Science and Innovation contract	Universidad Autónoma de Madrid	Regenerative Cardiology	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 objective 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 2010

Name	Instution
Arias Guedón, Teresa	Centro de Investigación Aplicada - Navarra
Mateo de Castro, Jesús	Centro Nacional de Investigaciones Cardiovasculares - Madrid

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 2010

Name	Hospital	CNIC Department
Dobarro Pérez, David	Hospital Universitario de La Paz - Madrid	Cardiovascular Developmental Biology
Domínguez Vila, Adrián	Hospital Materno-infantil de Las Palmas de Gran Canaria - Canarias	Epidemiology, Atherothrombosis and Cardiovascular Imaging
Henríquez Camacho, César Augusto	Hospital Clínico San Carlos - Madrid	Vascular Biology and Inflammation
Kallmeyer Mayor, Andrea	Hospital Clínico San Carlos - Madrid	Epidemiology, Atherothrombosis and Cardiovascular Imaging

CARDIOVASCULAR PATHOPHYSIOLOGY Course: "From symptoms to genes"

The course in CARDIOVASCULAR PATHOPHYSIOLOGY 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: 19 and 20 November 2010
Venue: CNIC Lecture Hall
Attendees: 101

Appendix

Seminars, Events and Awards

January

04 **Elisa Yañiz,**
Mount Sinai School of Medicine, Cardiovascular Research Center, New York, USA

25 **Roger Hajjar,**
Mount Sinai School of Medicine, New York, USA

February

01 **Javier Nieto,**
Universidad de Wisconsin, USA

08 **Ramón Muñoz Chapuli,**
Universidad de Málaga, Spain

15 **Takayuki Asahara,**
Stem Cell Translational Research, Institute of Biomedical Research and Innovation, Kobe, Japan

25 **Keisuke Kaji,**
Institute for Stem Cell Research, MRC Centre for Regenerative Medicine, University of Edinburgh, UK

March

01 **Horng Chen,**
Mayo Clinic, Rochester, USA

04 **Matthieu Pesa,**
University of Burgundy, Dijon, France

15 **Tadaomi Takenawa,**
Graduate School of Medicine, Kobe University, Japan

22 **Ralf Adams,**
Max-Planck-Institut für molekulare Biomedizin, Münster, Germany

April

12 **Alessandro Giacomello,**
Cenci-Bolognetti Foundation, Pasteur Institute, University La Sapienza, Rome, Italy

16 **Eloi Montanez,**
Max Planck Institute of Biochemistry, Martinsried, Germany

20 **Antonio García de Herreros,**
IMIM-Hospital del Mar, Universidad Pompeu Fabra, Barcelona, Spain

27 **Cédric Patthey,**
Umea Center for Molecular Medicine, Sweden

30 **Joan Isren,**
Mount Sinai School of Medicine (MSSM), New York, USA

May

03 **Rainer Pepperkok,**
EMBL, Heidelberg, Germany

05 **Sir Magdi Yacoub,**
Imperial College London, UK

06 *Meeting between the Preventive Cardiology Section and Rehabilitation of the SEC and the CNIC*

07 **Simón Méndez-Ferrer,**
Mount Sinai School of Medicine (MSSM), New York, USA

Appendix

Seminars, Events and Awards

17 **Pier Paolo Di Fiore,**
Istituto FIRC di Oncologia Molecolare, Milan, Italy

20 **Ugo Cavallaro,**
IFOM - FIRC Institute of Molecular Oncology, Milan, Italy

24 **Deepak Srivastava,**
Gladstone Institute of Cardiovascular Disease, San Francisco, USA

25 **William A. Muller,**
Northwestern University, Feinberg School of Medicine, Chicago, USA

25 **Thierry Pedrazzini,**
University of Lausanne Medical School, Switzerland

27 **Alexandra Aicher,**
Nottingham Trent University, Interdisciplinary Biomedical Research Centre School of Science and Technology, UK

May 31 - Jun 4 *CNIC Workshop "Principle of Fluorescence Techniques 2010"*

31 **Christine Mummery,**
Leiden University Medical Center, The Netherlands

June

02 **Paul Kubes,**
University of Calgary, Alberta, Canada

07 **Ihor Lemishka,**
Mount Sinai School of Medicine, New York, USA

11 *Experimental Design CNIC Conference 2010*

14 **Robert A. Hegele,**
Robarts Research Institute, The University of Western Ontario, Canada

16 **Guadalupe Sabio Buzo,**
Centro Nacional de Biotecnología - CSIC, Madrid, Spain

18 **Katia Georgopoulos,**
Massachusetts General Hospital, Harvard Medical School, Charlestown, USA

21 **Angelika Schnieke,**
Livestock Biotechnology, Freising-Weihenstephan, Germany

22 **Christopher Antos,**
Center for Regenerative Therapies, Dresden, Germany

28 **Silvia Priori,**
Università di Pavia, Cardiologia Molecolare, Fondazione Salvatore Maugeri, Pavia, Italy

29 **Jörg Männer,**
University of Goettingen, Germany

July

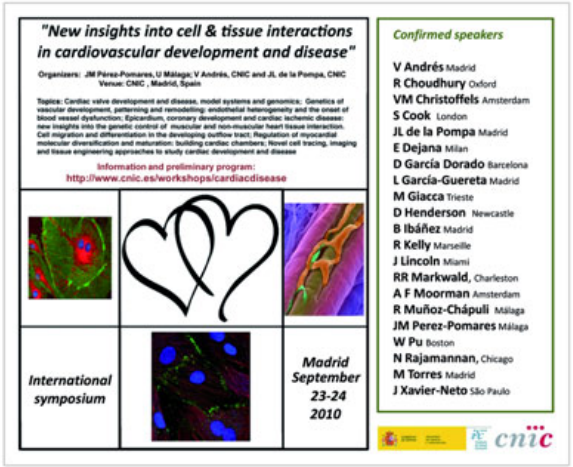
05 **Gerald R. Crabtree,**
Howard Hughes Medical Institute, Stanford University School of Medicine, USA

06 **Vincent M. Christoffels,**
University of Amsterdam, The Netherlands

12 **Nadia Rosenthal,**
EMBL Monterotondo, Italy

14 **Lars Knoch,**
Sigma-Aldrich, Stuttgart und Umgebung, Deutschland

23 **Eduardo Moreno,**
CNIO, Madrid, Spain



Appendix

Seminars, Events and Awards

September

13 — **Mª Luisa Iruela-Arispe,** Cell and Developmental Biology, UCLA, Los Angeles, USA

17 — **Armando del Río,** Columbia University, New York, USA

21 — **Wolfgang Schamel,** Max Planck Institute for Immunology and University of Freiburg, Germany

21 — **Harald Kranz,** Gene Bridges, Heidelberg, Germany

23 - 24 — *International Symposium "New insights into cell & tissue interactions in cardiovascular developmental and disease"*

27 — **Charles Lowenstein,** University of Rochester School of Medicine and Dentistry, USA

October

04 — **Paul Riley,** UCL-Institute of Child Health, London, UK

05 — **Antonio Maraver,** CNIO, Madrid, Spain

06 — **Sasha Belenkov,** Perkin Elmer, Milan, Italy

07 - 08 — *First International Definiens Symposium*

15 — *METOCARD Course "Integration of the emergency extrahospital services and sanitary transport in clinical essays"*

18 — **Gianluigi Condorelli,** Institute of Biomedical Technologies, National Research Council, Milan, Italy

20 — **Michel Ovize,** Claude Bernard University, Lyon, France

21 — *Cicerone Conference "What you need to know about Cardiovascular Research"*

25 — **Stefan Offermanns,** Max-Planck-Institute for Heart and Lung Research, Bad Nauheim, Germany

26 — **Christiana Ruhrberg,** University College London, UK

November

02 — **Klaus Ley,** La Jolla Institute for Allergy and Immunology, California, USA

08 — **Benjamin Caballero,** Johns Hopkins Bloomberg School of Public Health, Baltimore, USA

16 — **Bin Zhou,** Albert Einstein College of Medicine, New York, USA

19 - 20 — *Cardiovascular Physiopathology Course "From the symptom to the genes"*

19 — **Tom Misteli,** National Cancer Institute, National Institutes of Health, Bethesda, USA

22 — **Antonio Vidal-Puig,** Institute of Metabolic Science, Cambridge, UK

25 - 26 — *Annual Meeting of the Celular Theraphy Net (TERCEL)*

29 — **Ziad Mallat,** Addenbrooke´s Hospital, Cambridge, UK

December

13 — **Erwin Wagner,** CNIO, Madrid, Spain

20 — **Abel Sanchez-Aguilera,** Children's Hospital Boston, USA

Appendix

Seminars, Events and Awards

Awards

Cardiovascular Developmental Biology

Award: ASH Scholar Award 2009-2010. American Society of Hematology.
Awarded to: **Simón Méndez-Ferrer**

Award: Extraordinary Ph D Thesis prize 2010 from the Universidad Autónoma de Madrid.
Awarded to: **Luis Luna Zurita** (José Luis de la Pompa Group)

Award: Elected Member of the Working Group on Developmental Anatomy and Pathology of the European Society of Cardiology.
Awarded to: **José Luis de la Pompa**

Vascular Biology and Inflammation

Award: Premio de Investigación de la Comunidad de Madrid "Miguel Catalán" a la carrera investigadora en Ciencias.
Awarded to: **Francisco Sánchez-Madrid**

Epidemiology, Atherotrombosis and Imaging

Award: Honorary Degree, International University of Catalonia, Barcelona, Spain. 2010.
Awarded to: **Valentín Fuster**

Award: Honorary Degree, Menéndez Pelayo International University, Santander, Spain. 2010.
Awarded to: **Valentín Fuster**

Award: Dr. Leon Dumont Prize 2010, Sociedad Belga de Cardiología.
Awarded to: **Vicente Andrés**

Award: Premio Impulsa Ciencia y Academia, Fundación Príncipe de Gerona.
Awarded to: **Borja Ibáñez**

Award: Joint Runner Up Prize in the Young Investigator Award Poster Competition, Heart Failure Winter Research Meeting on Translational Heart Failure Research, European Society of Cardiology.
Awarded to: **José Javier Fuster**

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 Institue) of the Ministerio de Ciencia e Innovación (Spanish Science and Innovation Ministry), 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 Goverment 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 has recently been extended until 2020.

Shortly after the agreement was signed, on January 24, 2006, this group of companies was formally constituted as the ProCNIC Foundation. Through its creation, some of the largest companies in the country have made a long-term commitment to biomedical research which 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 is based on a public-private partnership of a broad, socially-committed nature. In this innovative PPP, state funding is complemented by financing through the ProCNIC Foundation (http://www.fundacionprocnic.es).

New companies have since joined the ProCNIC Foundation, and there are now 13 members: Acciona, Banco Santander, BBVA, Endesa, Fundación Abertis, Fundación Ramón Areces, Gas Natural, Grupo Prisa, Inditex, La Caixa, Repsol YPF, Fundación de Investigación 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 ProCNIC Foundation not only provides the CNIC with money; it also contributes its accumulated managerial and business expertise. Representatives of the ProCNIC Foundation sit on the CNIC's Board of Trustees, and actively participate in the management, planning and decision taking related to the Center. In this way, some of the most important organizations in the private sector in Spain have committed themselves to a direct involvement in biomedical research and the fight against cardiovascular diseases.

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

Appendix

Funding

Public Funding

Private Funding

Appendix

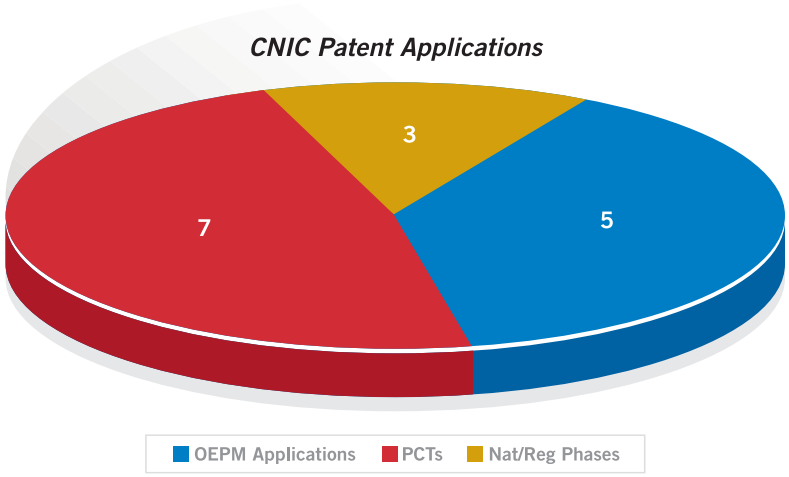
Funding

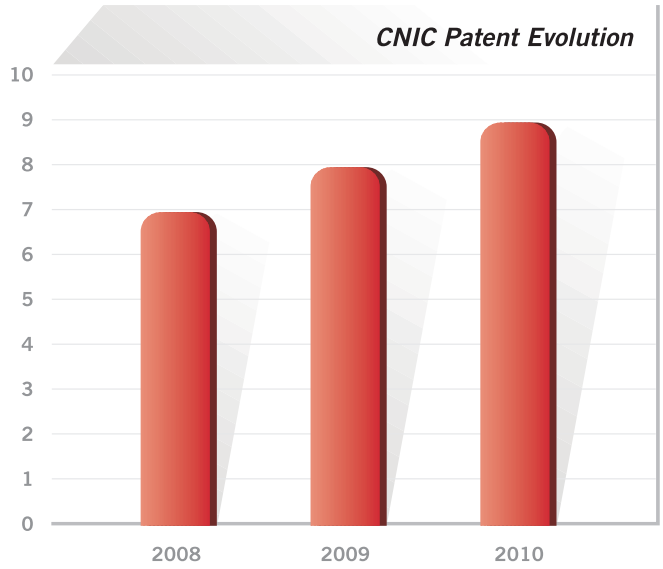
Technology Transfer

A coherent policy of technology transfer is essential for ensuring efficient translational research. Patents and their licensing are the basic tools of technology transfer, and the CNIC therefore encourages its researchers to identify findings with potential for development and application so that they can be assessed for possible patent protection.

Seven invention disclosures were assessed in 2010, of which five were protected by filing new Spanish patent applications. Four previous applications were extended to international patent applications, three through the Spanish patent office and one through the European patent office. Furthermore, protection of intellectual property rights related to the Polypill project is being extended to national and regional phases in collaboration with Ferrer.

This brings the total number of patent families on which the CNIC is an applicant or co-applicant to fifteen: five Spanish applications, seven international applications under the patent cooperation treaty, and three files that have been extended to different national or regional patent offices. Figures for the last three years (see figure) show that the CNIC increased its technology portfolio every year, demonstrating a clear appreciation by CNIC researchers of the importance of patent protection for transferring knowledge from bench to bedside. We hope that this trend will continue in the coming years.





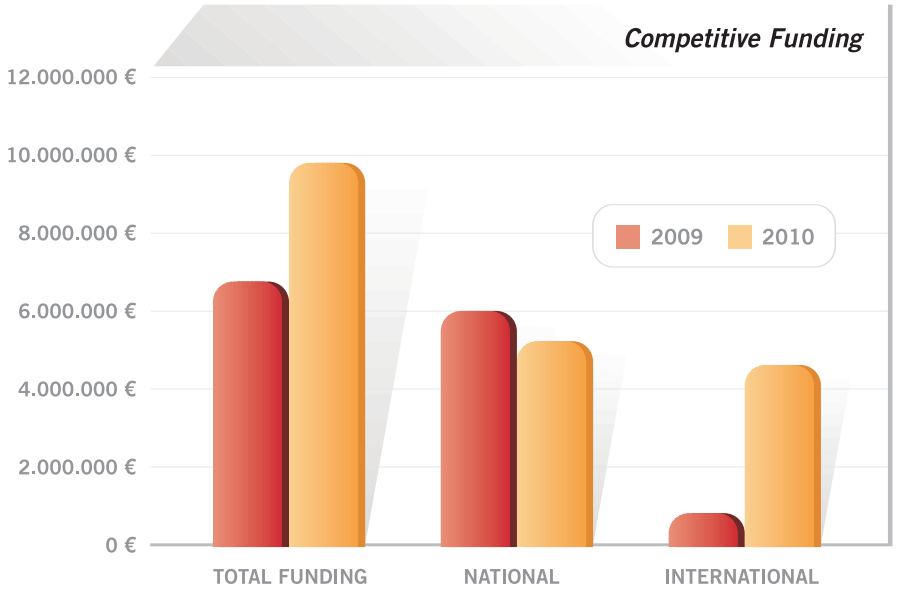
Appendix

Funding

Competitive funding

A total of 49 grant applications (8 European and 41 Spanish) were granted last year, providing more than €9.5 million of external funding to the CNIC. Of this, more than €4.6 million came from international sources and more than €5.1 million from national calls. Most of this funding was for research projects (~ €7.7 million), and most of the rest was funding for contracts and fellowships.

Fellowships awarded last year include 3 "Ramón y Cajal" contracts, 2 Juan de la Cierva fellowships and 1 "Sara Borrell" award. CNIC researchers also garnered a total of 9 fellowships under the FPU and FPI programmes (Spanish PhD fellowships). In addition, 2 Marie Curie International Reintegration Grants, 1 European Reintegration Grant and 1 Intra-European Fellowships for Career Development were awarded. During 2010, the CNIC also began negotiations for a COFUND project to recruit young group leaders.



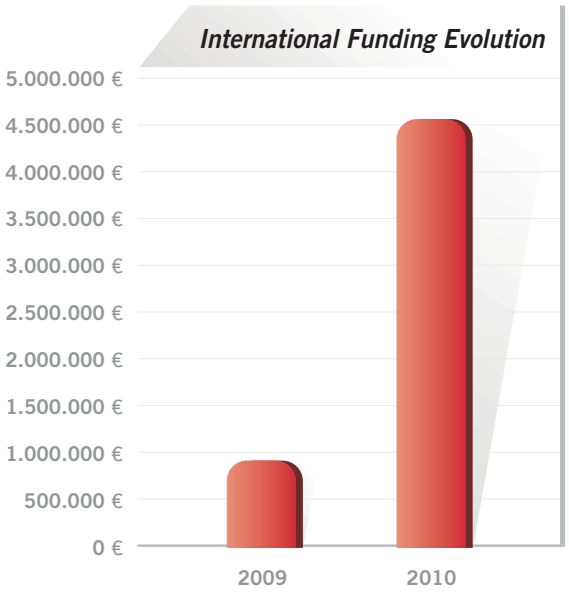
Appendix

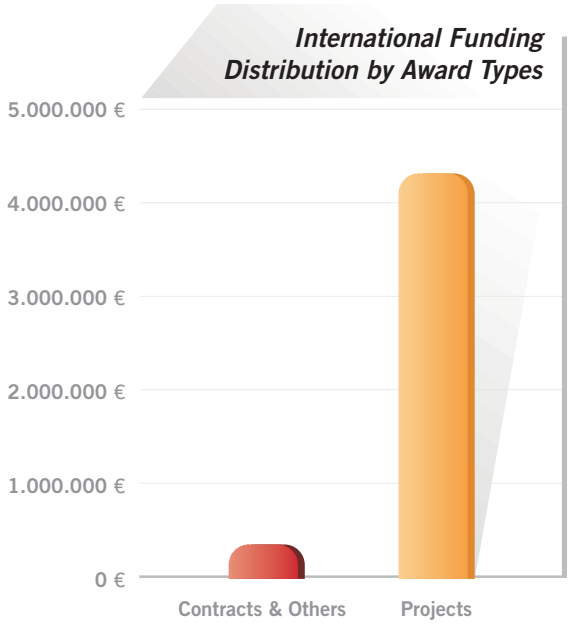
Funding

International Funding

The €4.5 m of international funding awarded to the CNIC in 2010 represents an increase of more than 5 fold over the level of funding in 2009. These funds correspond to 7 research projects: 3 Marie Curie awards; 2 European Research Council Starting Grants; 1 coordinated Cooperation project (CARE-MI); and 1 travel-grant from the Federation of Laboratory Animal Science Associations. The CNIC also received funds for a European Science Foundation funded COST project, which are not included in the total indicated above.

This brings the number of international projects in which the CNIC participates to 17. This includes CNIC coordination of the European Commission FP7 projects CARE-MI (a large scale integrating project) and FOCUS (a small-medium scale project), and participation in Hyperimage (FP7) and Heart Repair (FP6).



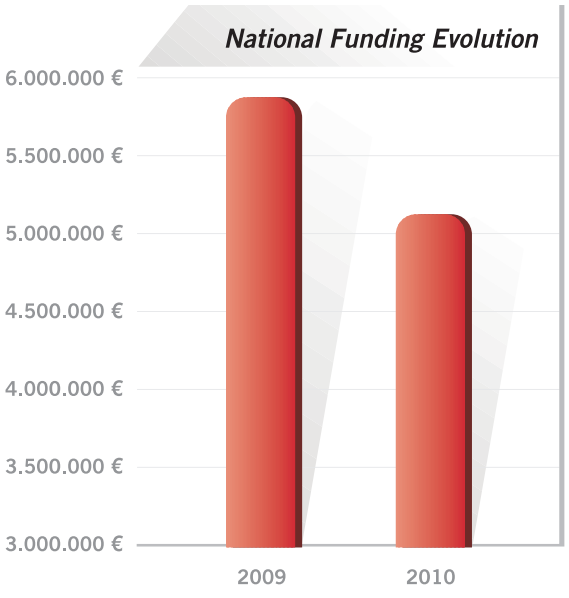


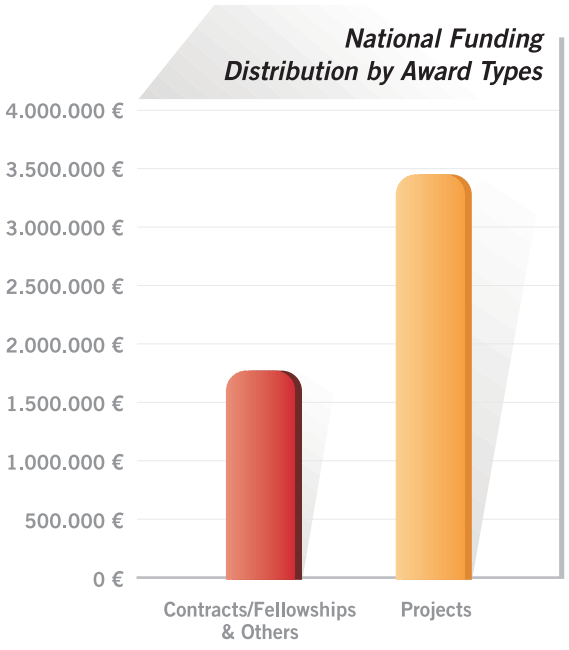
Appendix

Funding

National Funding

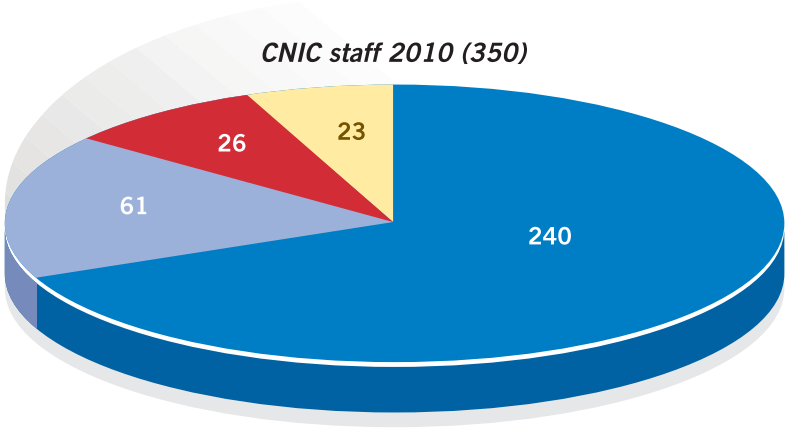
Although the national funding obtained in 2010 was approximately €0.7 million less than in 2009, the number of proposals approved was almost the same. National projects awarded last year included an INNPACTO Project, supporting cooperation between the CNIC and partners in the private sector.

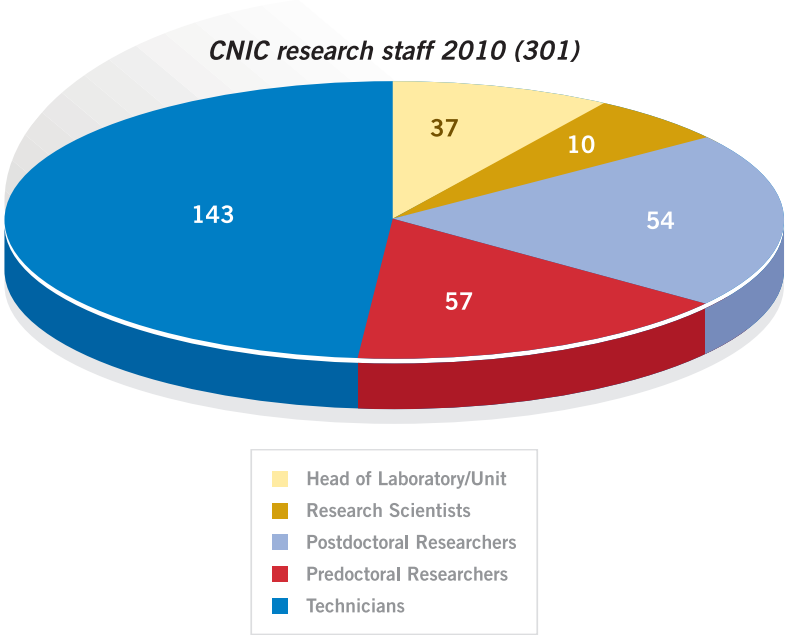




Appendix

Staff Figures





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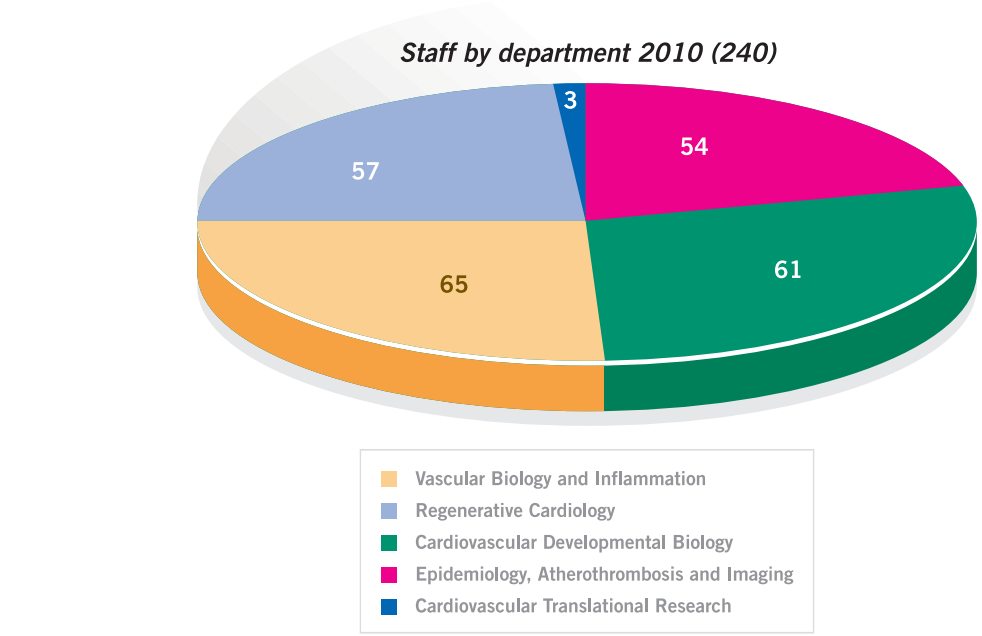
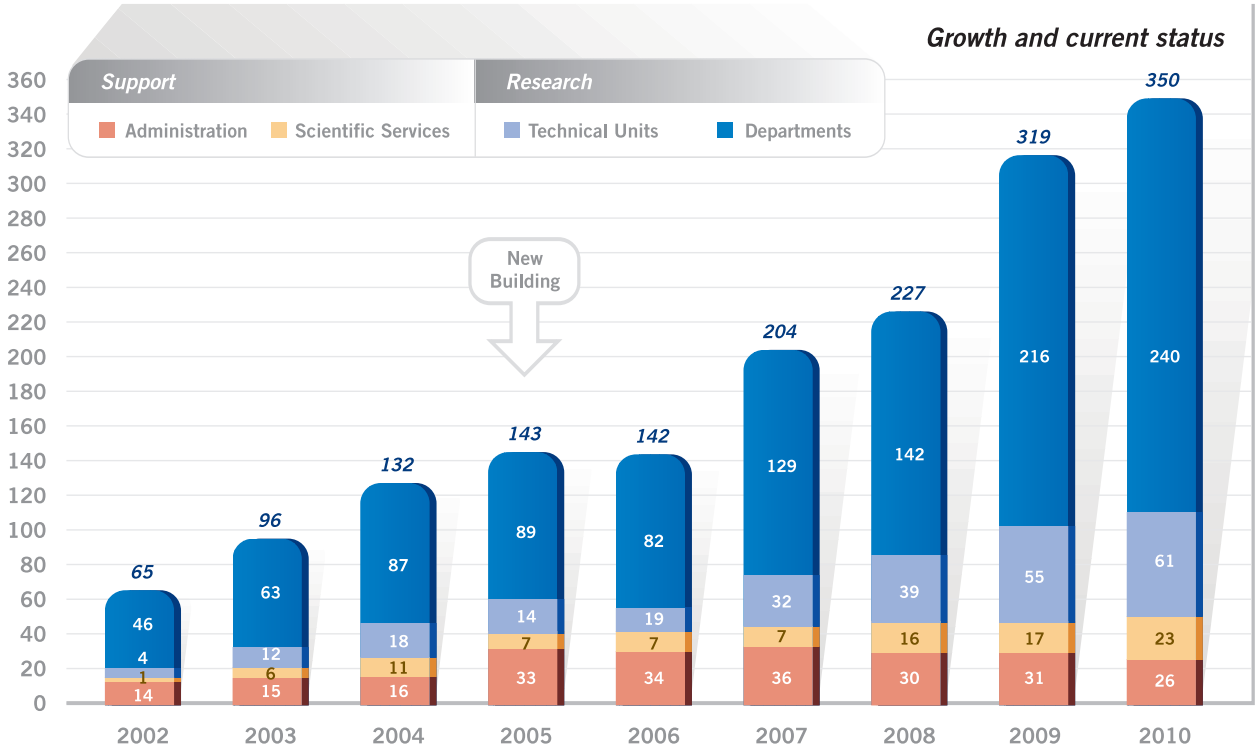
Staff Figures





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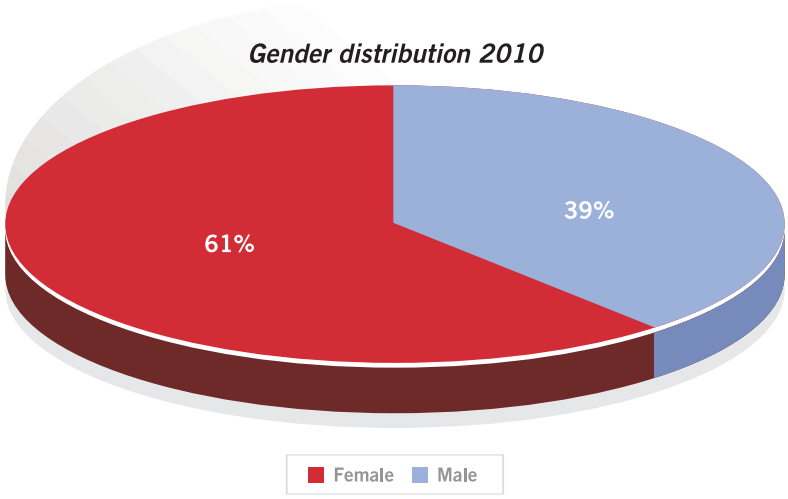
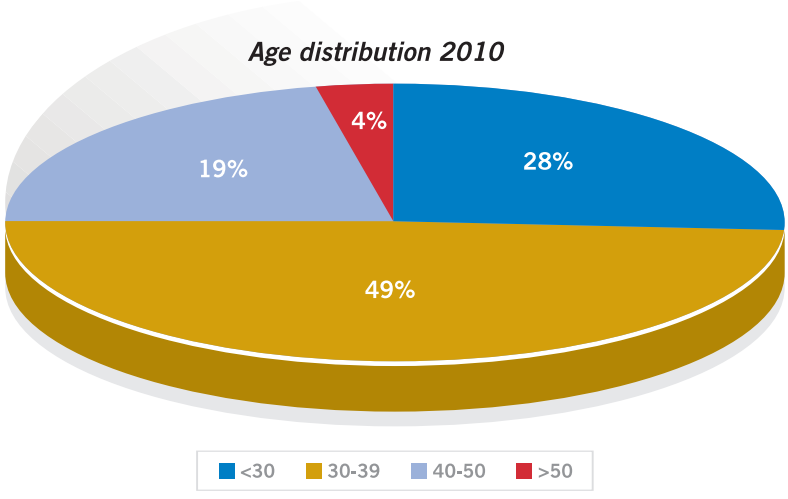
Staff Figures





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