Over the past few years the CNIC has established itself as a center of research excellence in the international scientific arena through its scientific productivity and its innovative organizational and funding pattern.

The CNIC’s impressive record is built on our diverse activities, which in addition to a dynamic research base include training programs, technological innovation and partnerships with the healthcare sector and industry. Our success is also the fruit of an open institutional environment that brings together investigators from diverse disciplines to work together to achieve the Center’s goals through innovation, efficiency, cooperation and enthusiasm. The CNIC’s management structure was praised as a model for other Spanish enterprises last June when Prime Minister Mariano Rajoy attended the annual governing council meeting of the Pro-CNIC Foundation, the panel of private companies whose continued support is so vital.

Another key event that attracted much media attention last year was the approval of the CNIC Polypill for commercialization in 22 countries, including Spain. Production of the CNIC Polypill was inaugurated in September in a ceremony presided over by Spanish State Secretary of Social Services and Equality, Susana Camarero. The results of the Focus Polypill trial were the focus of two key studies published last year in the *Journal of the American College of Cardiology*, and were a centerpiece of the European Society of Cardiology meeting held in Barcelona.

Also coming to fruition last year was the METOCARD-CNIC study, which examined the benefits of early intervention after a heart attack with the beta-blocker metoprolol. The positive follow-up findings were published in the *Journal of the American College of Cardiology*, and the groundbreaking advance signaled by this trial won strong praise at the ACC meeting. The AHA considered the METOCARD-CNIC trial one of the 10 most relevant studies in cardiovascular field worldwide, and invited CNIC researchers to present a dedicated session at the AHA meeting in Washington DC.

The Center’s commitment to pioneering translational research was reaffirmed with a new project called TAN SNIP. This transnational study pools the expertise of the CNIC, the Icahn School of Medicine at Mount Sinai, the Framingham Heart Study, and the VU University Medical Center in Amsterdam, and enjoys financial backing from AstraZeneca. The goal of this transatlantic network is to develop tools for stepwise non-invasive imaging for cardiovascular prognosis and prevention.

Delivering on our commitment to improving public cardiovascular health also involves us in a range of public educational programs, which form an essential part of the Center’s mission. The CNIC’s public profile was reinforced in February through a partnership with Spanish broadcaster *Radio Televisión Española* (RTVE) and *Fundación para el Conocimiento MADRID+D* (an initiative of the Madrid regional government). This health awareness campaign consists of a series of televised conversations with RTVE staff about the importance of taking care of cardiovascular health.
The CNIC’s outstanding research productivity continued last year, as detailed in the pages of this report. And this strength was rewarded by the Center’s continued success in securing competitive funding. National funds secured last year exceeded €6 million, while international funding exceeded €4 million. It is especially pleasing to see the CNIC’s success in obtaining European Research Council funding. Rui Benedito was awarded an ERC Starting Grant to study the formation of new blood vessels, and Susana González and Simón Méndez were awarded ERC Consolidator Grants for the study of heart muscle aging and stem cell niche biology, respectively.

CNIC researchers also won recognition in the form of prestigious prizes and honors. Rui Benedito was awarded the Princesa de Girona prize for his work on angiogenesis, and on International Women’s Day Guadalupe Sabio was garlanded by the Madrid regional government as one of the outstanding Spanish women scientists for her work on stress signaling in cardiovascular diseases. Another young investigator, Ana García Álvarez, received a Young Researcher Award from The European Society of Cardiology for her work on pulmonary circulation and right ventricular function. And one of us (VF) was deeply honored to receive the title of Marquis from Don Juan Carlos I of Spain, in one of his last acts as King, for “outstanding and unceasing research efforts and ... educational outreach work”.

The Center’s research profile was broadened and deepened with three key additions. Jorge Alegre-Cebollada leads the Single-Molecule Mechanobiochemistry laboratory, investigating the links between protein changes and the mechanical properties of the heart. José Jalife reinforces our work in the field of arrhythmias, and his Cardiac Arrhythmia laboratory maintains a formal collaboration with the University of Michigan. José María Castellano takes on a crucial role as the new Coordinator of the Clinical Research Unit. We are very pleased to welcome these three experts to the CNIC team.

2014 also saw a new training initiative, made possible through a strategic alliance with the Fundación Interhospitalaria para la Investigación Cardiovascular (FIC). The program, called FICNIC, was launched in March and is tailored for young cardiologists. Our training partnership with the Fundación La Caixa continues, with an injection of funding last year for four new grants under the La Caixa-Severo Ochoa International PhD Program.

As the consolidation of our project continues, it will be important to maintain an agile organizational infrastructure that fosters fluid cooperation in multidisciplinary projects. This openness and flexibility is an essential foundation for ensuring the best use of knowledge and resources, and thus lead to improved results and their scientific and social translation.
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The Department of Cardiovascular Development and Repair seeks to understand how the cardiovascular system is built, maintained and in some instances repaired. Our research programs examine the molecular and cellular basis of cardiovascular development, cardiovascular homeostasis and repair, and the role of stem-cell biology in these processes.

A. Cardiovascular Developmental Biology
We study how cardiac lineage specification occurs and how proliferation and patterning of the different cardiac regions that will form the mature heart are regulated. We want to unravel how alterations to these mechanisms lead to cardiovascular disease and how they can be manipulated to repair the diseased heart.

**Program Coordinator:** José Luis de la Pompa

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

**Program Coordinator:** Miguel Manzanares

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

**Program Coordinator:** José Antonio Enríquez
We are interested in understanding the cellular basis of developmental processes and how this is controlled by transcription factor networks (TFN). To study the cellular basis of developmental processes we have developed genetic methods in the mouse that allow us to trace cell lineages in clonal analysis or functional mosaics.

Using mosaic methods, we have described the involvement of cell competition in the selection of the fittest pluripotent cells during embryonic development. We recently found that cardiomyocytes and other cardiac lineages remain susceptible to induced cell competition during fetal and adult life, without compromising heart function. This finding opens the door to exploration of the roles of cell competition in cardiac repair and plasticity. We have also developed an in vitro embryonic stem cell competition model in which we can track the endogenous levels of Myc, a major determinant of cell competitive ability. This model is allowing us to study in great detail the live dynamics of cell competition and its cellular/molecular mechanisms.

Using clonal analysis methods, we have continued our efforts to trace the cellular history of the cardiovascular system. Starting with the initial specification steps, we have addressed the origin and relationships between the earliest blood and vascular cells. Unexpectedly, we found that the hemangioblast model of a common origin of vascular and blood cells does not hold for the in vivo situation in the early mouse embryo. Instead, we found initial independent specification of the two lineages followed by activation of an endothelial hemogenic program in the pre-circulation mouse yolk sac. In this area, we are now developing new methods for live analysis of early mouse development, which are allowing us to move toward live analysis of cellular contribution to early cardiovascular development.

In our work on TFNs, we are investigating the role of the TALE-Hox network in cardiac development and cardiomyocyte pool turnover in the adult heart. We study the molecular interaction and regulation of this TFN in two classical models of patterning; the limb and the main embryo axis. During this last year we proposed a new model for limb P-D specification involving an epigenetic timing mechanism.
Another research line of our lab, led by Silvia Martín Puig, is the study of the role of hypoxia in the homeostasis of the cardiovascular system, with a particular focus on heart development and disease. We have generated several gain- and loss-of-function mouse models of the hypoxia pathway paying special attention to early cardiovascular populations that will contribute to the different functional cell types and structures composing the mature heart. Using these genetic tools we are investigating whether changes in oxygen tension influence the migration, proliferation or differentiation of cardiac progenitors and regulate mammalian cardiogenesis. Our data indicate that hypoxia, through the VHL/HIF axis, is essential for the proper formation of the ventricular chambers, maturation of the myocardium, and the correct development of the coronary vasculature. We are currently unraveling the mechanisms underlying the phenotypic alterations found in the mouse models generated. The molecular characterization of these phenotypes will help to elucidate the participation of the VHL/HIF axis in congenital heart disease and may have therapeutic applications.
1. Cardiovascular Development and Repair
A. Cardiovascular Developmental Biology Program


*Equal contribution

Martin-Puig S, Tello D and Aragones J. The PHD-HIF oxygen sensing pathway in cardioprotection. *Front. Physiol.* (Cardiac Electrophysiol.) (accepted) Oxygen homeostasis in the cardiovascular system

Distribution of HIF1alpha protein within the developing ventricular chambers of the early mouse heart. Left panel: HIF1alpha (red) is expressed in most of the primitive ventricular cardiomyocytes labeled by troponin T (green) in an E9.5 heart section. Right panel: Myocardial HIF1alpha intensity map generated with Cell Profiler Software and representing differential HIF1alpha protein expression levels (low, medium, high) in ventricular cardiomyocytes.

**MAJOR GRANTS**
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- Ministerio de Economía y Competitividad (BFU2012-310862013-15)
- Ministerio de Ciencia e Innovación. FIS (CP09/00100). IP: S. Martin-Puig
- European Commission FP7. Marie Curie Career Integration Grant (276891). IP: S. Martin-Puig
- Ministerio de Ciencia e Innovación (SAF2011-29830). IP: S. Martin-Puig
- Comunidad de Madrid (S2010/BMD-2542). IP: S. Martin-Puig
Regeneration and aging

RESEARCH INTEREST

Although recent advances have overturned the old view of the human heart as an inert postmitotic organ, it is clear that the human heart’s capacity to proliferate, rejuvenate and regenerate is very limited. This presents a problem for strategies to treat damaged hearts after infarction, one of the leading causes of death worldwide.

Our group aims to develop strategies to enhance cardiac regeneration. Toward this goal, we are characterizing the subpopulation of cardiac cells capable of regeneration, and using the knowledge generated to explore to promote the repair of injured hearts. We have eliminated and reactivated telomerase, an anti-aging enzyme, in adult cardiac cells in order to assess the role of this enzyme in the re-expression of cardiac embryonic genes after infarction and in heart regeneration. A key element of our strategy is the comparison of animal models that differ greatly in their regeneration capacity: from the zebrafish, which can restore up to 20% its heart after injury, through the newborn mouse, whose heart possesses transient regenerative potential, to the adult mouse, in which heart regeneration capacity is very limited. Through these efforts, we hope to achieve a more complete knowledge of the role of endogenous cardiac progenitor cells and telomerase in heart rejuvenation and regeneration, which could eventually lead to the development of improved regeneration therapies.

Telomere length measurements in cardiomyocytes. Staining of heart sections reveals telomeres (red), nuclei (blue) and the cardiomyocyte marker troponin I (green).
1. Cardiovascular Development and Repair
   A. Cardiovascular Developmental Biology Program

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

Direct comparison of 2D and 3D telomapping. Better telomere and nuclei segmentation and more accurate results are obtained when a Z stack of confocal images is analyzed instead of the classical 2D maximum projection image. This work was done in collaboration with Hind Azegrouz of the Cellomics Unit.

MAJOR GRANTS
- Ministerio de Economía y Competitividad (SAF2012-38449)
- Ministerio de Economía y Competitividad. FIS. RETICS (Red de Investigación Cardiovascular RD12/0042/0045)
- Asociación Española contra el Cáncer PI: Tania Aguado

Selected Publications
Schneider RP, Garrobo I, Foronda M, Palacios JA, Maríon RM, Flores J, Ortega S, Blasco MA. TRF1 is a stem cell marker and is essential for the generation of induced pluripotent stem cells. *Nat Commun* (2013) 4:1946
Development of the epicardium and its role during regeneration

RESEARCH INTEREST

Unlike adult mammals, zebrafish have the capacity to regenerate their hearts in response to several types of injury. In the laboratory, we use cryoinjury to induce a cardiac tissue damage with the aim of mimicking the consequences of tissue loss upon myocardial infarction. Our results show that cardiac fibrosis is reversible and occurs as an intermediate step during regeneration. We aim to unravel the endogenous mechanisms of myofibroblast and extracellular matrix regression, as this might have implications for the design of clinical antifibrotic strategies. We recently conducted a detailed analysis to establish whether the regeneration we observe also is accomplished at a functional level. For this, we set up echocardiography in the zebrafish in order to study ventricular pumping efficiency. Our results reveal that cryoinjury transiently impairs ventricular fractional volume shortening but that pumping efficiency was completely recovered at late stages postinjury. However, in many cases ventricular wall contraction showed long-term alterations. Echocardiography thus allows a deeper understanding of the mechanisms of cardiac regeneration.

One of the first layers to reestablish during regeneration is the epicardium, the outer layer covering the myocardium. We are interested in how the epicardium forms during embryonic development. Using live imaging in zebrafish embryos we are studying the mechanisms through which the proepicardial cells emerge from the pericardial wall and attach to the myocardium. We found that proepicardium formation is dependent on pericardial fluid flow forces, which are triggered by the beating heart. Our current effort is dedicated to understanding the mechanosensory pathways underlying these events.

*Equal contribution


MAJOR GRANTS

- Ministerio de Economía y Competitividad (BFU2011-25297)
- Comunidad de Madrid (P2010/BMD-2321)
- Tercel (Red de Terapia Celular) (PI: M. Torres)
- European Commission. European Research Council Starting Independent Researcher Grant (ERC-337703 2013)
Molecular genetics of angiogenesis

RESEARCH INTEREST

Blood vessels are an important therapeutic target in cardiovascular diseases and cancer. The knowledge accumulated during the past several years in the field of vascular biology has allowed the use of VEGF and its major endothelial receptors as targets in numerous therapies designed to stimulate or inhibit the growth of blood vessels, leading to significant improvements in the treatment of many vascular related diseases. But VEGFs and their receptors do not work alone, and their different functions can also be controlled by other mechanisms which compensate or override VEGF function. One such mechanism is the Notch signaling pathway, which is a key regulator of the initial arterial differentiation program and also plays a fundamental role during developmental and pathological angiogenesis. We are studying in high detail the role of several molecular mechanisms that lie downstream of VEGF and Notch signaling, and that are involved in specific aspects of vascular proliferation, differentiation, maturation and quiescence. In the last year we generated new mouse models, optimized several in vitro angiogenesis assays, and performed quantitative gene expression analysis in vitro and in vivo to define the molecular regulation of endothelial proliferation in different contexts.
1. Cardiovascular Development and Repair
A. Cardiovascular Developmental Biology Program

Endothelial sprouts derived from mouse embryoid bodies in vitro. We use this assay to screen for the function of several genes in endothelial sprouting and proliferation.


MAJOR GRANTS
- Ministerio de Economía y Competitividad, Europa Excelencia 2013 (MIN/SAF1301)
- Ministerio de Economía y Competitividad, Plan Nacional (SAF2013-44329-P)
- Ministerio de Economía y Competitividad, Programa Ramón y Cajal (MIN/RYC1301)
RESEARCH DEPARTMENTS 1. Cardiovascular Development and Repair
A. Cardiovascular Developmental Biology Program

Intercellular signaling in cardiac development & disease

RESEARCH INTEREST

The heart is the first organ to form and function in the developing vertebrate embryo. Understanding the molecular mechanisms that regulate the cellular proliferation, differentiation and patterning processes that give rise to the adult heart is essential for understanding cardiac disease.

In the last year we have focused our efforts on the role of various intercellular signals in chamber and valve development and disease (cardiomyopathy and valve disease) and cardiac repair. Our ultimate goal is to identify new molecular markers for cardiac disease or processes eventually amenable to therapeutic intervention. To address these questions we have used mouse and zebrafish genetics, hiPSC, biochemistry, next-generation sequencing (NGS) applied to mouse and human samples, and imaging analysis.

We find that during ventricular chamber development the Notch signaling pathway emits signals from the inner layer of the heart, the endocardium, that promote trabeculation—the formation of a network of endocardium-covered myocardial ridges that protrude into the ventricular lumen, increasing the surface area for oxygen exchange within the growing ventricles. When the chambers reach a critical size, the task of nourishing the cardiomyocytes embedded in the thick ventricular walls is taken over by the developing coronary vasculature, and the trabeculae compact and contribute to increasing the mass of the outer and highly proliferative compact myocardium layer. Trabecular compaction is also dependent on Notch signaling, as the whole process of chamber development is tightly regulated, with various Notch ligands acting at different stages to activate the Notch1 receptor in the endocardium. Alterations of these developmental processes result in cardiomyopathy (Figure 1). We are currently validating the results of an NGS analysis in familial patients with left ventricular non-compaction cardiomyopathy, performed in collaboration with clinical colleagues at the CNIC and various hospitals.

We have also studied the interplay of Notch and Bmp2 signaling in valve development. Besides its involvement in the promotion of epithelial mesenchyme transition (EMT) to give rise to the valve primordium (Figure 2), Notch is also required for the fine tuning of mesenchymal cell proliferation, extracellular-matrix (ECM) secretion and remodeling processes associated with valve sculpting. EMT is coordinated by Notch and Bmp2, and a new gain-of-function model shows that Bmp2 must be tightly regulated throughout valve development, with Bmp2 overexpression in the myocardium disrupting valve morphogenesis and chamber development (Figure 3).

Through our collaboration with clinical colleagues we have undertaken a NGS study to try to identify the gene expression profile associated with aortic valve disease in patients with calcified tricuspid or bicuspid aortic valves. We are currently carrying out functional studies to verify the role of the identified genes in this disease, using valve endothelial and interstitial cells obtained from surgical samples.

Using gain- and loss-of-function models in the regenerating zebrafish heart, we have shown that Notch plays a crucial role in the modulation of cardiomyocyte proliferation and differentiation and the inflammatory and fibrotic response associated with cardiac damage (Figure 4).

We believe that advancing knowledge of the molecular mechanisms underlying cardiac development and disease will help us to identify novel diagnostic or therapeutic strategies to treat the diseased heart.

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SCIENTIFIC REPORT 2014
Figure 1. Working model of Notch function in murine ventricular chamber development. During an early (initiation) phase Dll4-Notch activation leads to trabeculae formation. At a later (maturation) phase, Jag1 and Jag2 are able to activate Notch from the myocardium and regulate cardiomyocyte proliferation and differentiation and myocardial compaction. M-Fng plays an important role in the temporal regulation of ligand specificity. Disruption of the signals mediated by the late-acting ligands leads to cardiomyopathy.

Figure 2. The Notch ligand Dll4, but not Jag1, is required for EMT. Amira 3D reconstructions and lateral views of confocal microscopy images of an atrio-ventricular canal explant, cultured on collagen for 3 days. Mesenchyme cells are labeled red, nuclei blue and cardiomyocytes green.
1. Cardiovascular Development and Repair
A. Cardiovascular Developmental Biology Program


**MAJOR GRANTS**

- Ministerio de Economía y Competitividad. FIS RETICS (TERCEL: RD12/0019/0003 and RIC: RD12/0042/0005)
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- Ministerio de Economía y Competitividad, Juan de la Cierva contract (JCI-2012-12260) PI: Mauro Sbroggio
- Ministerio de Economía y Competitividad (SAF2010-17555)

![Figure 3. Bmp2 overexpression in the developing heart disrupts cardiac development. Whole mount views of E14.5 embryos and H&E stained cardiac sections of wild-type (WT, A, A’) and Nkx2.5-Cre;Bmp2 ΔN/Δ embryos. Note the dysmorphology and increased cellularization of the trasgenic cushion (B’). The general views of the heart show that chamber structure is also disrupted.](image1)

![Figure 4. Notch signaling mediates cardiomyocyte proliferation and fibrotic tissue deposition in the cryoinjured zebrafish heart. Endocardial Notch signaling attenuates the expression of sarcomere assembly genes and regulates intermediate-early growth gene activation, two requirements for cardiomyocyte proliferation. In the injured area Notch weakens the inflammatory response and favors ECM deposition by inducing ECM molecule expression and blocking ECM degrading proteases. Tgf-β signaling may be one mediator of these functions as its activation in endocardial cells partly depends on Notch signaling.](image2)
RESEARCH INTEREST

Our group studies how the niche maintains and regulates stem cells and how its dysregulation can contribute to disease. Hematopoietic stem cells (HSCs) traffic between bone marrow and circulating blood, which is the basis of for lifesaving clinical transplantation. Our previous work showed that HSC numbers in blood are regulated by the brain, which regulates bone marrow nestin+ mesenchymal stem cells through peripheral nerves. We recently found that HSC-niche mesenchymal stem cells might be different from those that form the skeleton, instead sharing a common origin with peripheral nerves and supporting glial cells (Figure 1). Thus, tight regulation of peripheral stem-cell niches in vertebrates might build upon the developmental relationships among its cellular components. Moreover, we have shown that damage to this regulatory network is essential for the appearance of myeloproliferative neoplasms, diseases that were previously thought to be driven solely by mutated HSCs (Figure 2). Our recent data has also uncovered a selective regulation by sex hormones of the maintenance, survival and proliferation of normal and leukemic hematopoietic progenitors. These results might explain gender differences in blood cancer incidence and also offer a new way of targeting leukemic stem cells with clinically approved drugs (Figure 3).
Figure 2. Neuropathy of the hematopoietic stem-cell niche is essential for myeloproliferative neoplasms. Model illustrating HSC niche alterations and rescue in myeloproliferative neoplasms (MPN). HSC, hematopoietic stem cell; SNS, sympathetic nervous system; MSC, mesenchymal stem cell; NA, noradrenaline; AR, adrenergic receptor; C, control (disease-free mice). (Arranz L et al. Nature 2014)

Figure 3. Estrogen signaling selectively induces apoptosis of hematopoietic progenitors and myeloid neoplasms without harming steady-state hematopoiesis. Treatment of leukemic mice with the selective estrogen receptor modulator tamoxifen can block the development of myeloproliferative neoplasms and sensitize acute myeloid leukemia to conventional chemotherapy (Sánchez-Aguilera A et al. Cell Stem Cell 2014)


MAJOR GRANTS
- Comunidad de Madrid. Convocatoria de Programas de I+D en Biomedicina. (S2011/BMD-2542)
- Ministerio de Economía y Competitividad (RYC-2011-09209) PI: Joan Isern
- Ministerio de Ciencia e Innovacion (RYC-2009-04703)
- Ministerio de Economía y Competitividad (RYC-2011-09726) PI: Abel Sánchez-Aguilera
- Ministerio de Economía y Competitividad (SAF-2011-30308)
- European Commission FP7. Marie Curie Career Integration Grant (294262)
- European Commission FP7. Marie Curie Career Integration Grant (294096) PI: Abel Sánchez-Aguilera
- Ministerio de Economía y Competitividad (BFU2012-35892) PI: Joan Isern

Selected Publications
RESEARCH DEPARTMENTS
1. Cardiovascular Development and Repair
B. Stem Cell Biology Program

Pathophysiology of adipose and cardiac tissues

RESEARCH INTEREST

Cell-based therapy is a promising approach for many diseases, including ischemic heart disease. Our work focuses on cardiac mesoangioblasts, committed vessel-associated progenitors that can restore heart structure and function to a significant, albeit partial, extent in a mouse model of myocardial infarction. Low-intensity pulsed ultrasound (LIPUS) is a non-invasive form of mechanical energy that can be delivered into biological tissues as acoustic pressure waves, and is widely used for clinical applications including bone fracture healing. We hypothesized that the positive effects of LIPUS on bone and soft tissue, which include increased cell differentiation and cytoskeleton reorganization, could be applied to increase the therapeutic potential of mesoangioblasts for heart repair. During this year, we showed that LIPUS stimulation of cardiac mesoangioblasts isolated from mouse and human heart results in significant cellular modifications that provide beneficial effects to cells, including increased malleability and improved motility and invasiveness. Additionally, LIPUS stimulation increased the number of binucleated mesoangioblasts and induced cardiac differentiation to an extent comparable with 5-azacytidine treatment. Administration of LIPUS-stimulated mesoangioblasts in vivo resulted in greater retention and incorporation into cardiotoxin-damaged hearts. Taken together, these results provide functional evidence for the potential of LIPUS as a useful tool in heart cell therapy.

MAJOR GRANTS
- Ministerio de Economía y Competitividad (SAF2010-15239)

Top Canonical Pathways
- Cardiomyocyte differentiation via BMP Receptors
- Chemokine Signaling
- Regulation of Actin-based Motility by Rho
- Factors Promoting Cardiogenesis in vertebrates
- PAK Signaling

Main canonical pathways activated after treatment with LIPUS

Cardiac mesoangioblasts after treatment with LIPUS

Selected Publications


RESEARCH DEPARTMENTS

1. Cardiovascular Development and Repair
   B. Stem Cell Biology Program

SCIENTIFIC REPORT 2014

RESEARCH INTEREST

We are interested in the gene regulatory networks that control the early stages of mammalian development and underlie cardiovascular disease. Our research focuses on understanding how cis-regulatory elements located in the non-coding portion of the genome influence the spatial and temporal expression of nearby genes, as well as how their activity is modulated by chromatin structure. We are also exploring how these elements are the target of variation that results in increased risk of human disease. Uncovering the regulatory basis of cardiovascular diseases is one of our major goals.

We are exploring how initial decisions and lineage choices occur in the mammalian embryo, before it implants in the maternal uterus and when pluripotent cell fate is established. We have shown how different signaling pathways act together to activate gene expression in the outer layer at the blastocyst stage, thus distinguishing the embryonic from the extraembryonic lineage. A critical event in this process is the regulation of the expression of CDX2 by the Notch and Hippo pathways through a specific enhancer.

By exploring the findings of genome-wide association studies, we have found that regulatory elements distal to the PITX2 gene lie in a genomic region associated with an increased risk of atrial fibrillation. We are exploring the genomic architecture of this and other atrial fibrillation associated loci, finding unsuspected interactions with other genes in these regions. Using mouse genetic models, we are conducting a genome-wide study of how chromatin structure crucially regulates proper gene expression in the heart, and how this could underlie certain cases of human cardiovascular disease.

Collection of mouse blastocysts showing expression of a fluorescent reporter driven by a Cdx2 enhancer (red), endogenous CDX2 expression (green), and nuclei (blue).
Diagram showing how different states of the Notch and Hippo pathways result in differential expression of the Cdx2 gene between the outer blastocyst cells, which will form the trophectoderm (TE, the precursor of the placenta), and the cells of the inner cell mass (ICM), which will give rise to the embryo proper and later to all adult lineages.

Differential expression of the Pitx2 and Hcn4 genes in arrhythmogenic regions of the developing 14.5 dpc mouse heart. While Pitx2, which encodes a transcription factor that has been linked to atrial fibrillation, is expressed in the pulmonary veins (PV) and the left atrium (LA), the ion-channel-encoding gene Hcn4 is expressed in the right and left superior vena cava (RSVC, LSVC), the atrioventricular node and His bundle (AVN-His), and the sinoatrial node (SAN). Mis-expression of both of these genes has been linked to the occurrence of atrial fibrillation.

Major Grants
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Selected Publications


Epigenetic regulation in cardiac aging and disease

**RESEARCH INTEREST**

Adult stem cells participate in the natural homeostasis of adult tissues through their ability to both self-renew and differentiate into multiple lineages to regenerate tissue in response to injury signals. During aging, the proliferation and differentiation capacity of tissue-specific stem cells decreases, and they lose their potential to regenerate tissues after damage. PGC-mediated alteration of the epigenetic status of hematopoietic stem cells (HSCs) is proposed as one of the driving forces behind many age-related HSC changes and is often found to be misregulated in human malignancies. Protection of the transcriptional “stemness” network is thus essential for maintenance of a healthy HSC compartment throughout life. A key unanswered question in the field is whether the functional decline in adult stem cells is related to reversible chromatin modifications. We propose that changes to the chromatin state can restore the regenerative capacity of stem cells. To investigate this hypothesis, we are exploring the role of the epigenetic Polycomb-mediated silencing mechanism in stemness maintenance, with particular emphasis on the self-renewal capacity and the microenvironment of HSCs, a key adult stem cell population with diverse regenerative capacities. Understanding molecular mechanisms by which Polycomb members control stem cell fate will provide new insights into hematopoietic stem cell biology and will also increase understanding of neoplastic transformation.

We are also interested in the emerging role of different classes of chromatin regulators and how their dysregulation in the adult heart alters specific gene programs, with subsequent development of major cardiomyopathies. Dilated cardiomyopathy (DCM) represents the third most common cause of heart failure but has been poorly modeled in nonhuman species. We propose that epigenetic remodeling could provide an important means of modulating the transcriptional reprogramming of cardiac gene expression in this condition. Understanding the action of Polycomb factors will allow the development of strategies to control physiological and pathological gene expression.

**MAJOR GRANTS**

- Ministerio de Ciencia e Innovación (SAF2010-15386)

**Selected Publications**


Nuclear receptor signaling

RESEARCH INTEREST

Macrophages are myeloid cells that can be found in almost all tissues, making important contributions to homeostasis and protection against injury. Projects in our laboratory focus on elucidating the transcriptional control of macrophages in different tissues, especially in the heart, adipose tissue and bone, with special emphasis on possible medical applications in the treatment of metabolic and cardiovascular diseases.

A special interest of our laboratory is the study of the transcriptional regulation of macrophage functions by nuclear hormone receptors, especially retinoid X receptors (RXRs). Our most recent studies have underscored the importance of RXR signaling in the development of macrophage-associated pathologies, such as insulin resistance and osteoporosis. We have discovered that RXRs are involved in adipose tissue and bone homeostasis. Macrophage RXR deficiency exacerbates inflammation and insulin resistance in vivo, and leads to increased bone mass due to the formation of non-resorbing osteoclasts. Our results demonstrate that RXRs control osteoclast progenitor proliferation and their differentiation into active osteoclasts through dual mechanisms that converge on the upregulation of the transcription factor MAFB. Since RXRs are druggable targets for human diseases, our basic findings open new perspectives on the use of RXR ligands as potential regulators of pathologies where metabolic and bone homeostasis is disrupted due to altered macrophage differentiation and function. We are now extending these studies to the role of RXR macrophages in tissue homeostasis and injury in the heart.

Tissue-resident macrophages. (A) Heart macrophages: Cross sectional image of C57BL/6 mouse heart left ventricle stained for nuclei (blue), CD68 (red), and CD31 (white). (B) Adipose tissue macrophages: H&E stained V-WAT sections from high-fat-diet-fed mice, showing crown-like structures formed by macrophages. (C) Bone osteoclasts: TRAP-positive osteoclasts in vitro differentiated from bone marrow cells.
RXR/MAFB signaling in osteoclastogenesis. RXR homodimers sustain Mafb transcription in osteoclast progenitors, which allows a proper proliferative response to M-CSF and activation of mature osteoclasts, leading to bone resorption.

The RXR ligand bexarotene protects against post-ovariectomy bone loss. Representative µCT scans of the distal femur in control and bexarotene (BXR)-treated female mice that underwent sham surgery or ovariectomy (OvX).

**MAJOR GRANTS**

- Ministerio de Economía y Competitividad (SAF2012-31483)
- Fundación TV3 Marató
- European Union. Marie Curie Action Initial Training Network (ITN) (FP7-PEOPLE-2013-ITN, "CardioNext" 608027)

**Selected Publications**


Molecular regulation of heart development and disease

RESEARCH INTEREST

Heart failure is a major cause of death and hospitalization worldwide, especially among the elderly. Despite advances in medical management, there is no cure for heart failure and prognosis is still very poor. A major cause of heart failure is myocardial infarction, in which blockade of a coronary artery causes massive cardiomyocyte death due to lack of oxygen and nutrients. Due to the very limited regenerative capacity of the mammalian heart, dead cardiomyocytes are not substituted by new contractile cells, but by a collagen scar that prevents cardiac rupture. Although initially effective, this response progressively leads to dilatation of the left ventricle, remodeling of the heart, and, eventually, heart failure and death.

The molecular mechanisms underlying heart remodeling are poorly understood. In particular, the role of alternative splicing in this pathological response is almost unknown. Alternative splicing is the main mechanism driving protein diversity and is the main research interest in our lab. The study of alternative splicing at a global level requires the development of advanced bioinformatic pipelines. Our bioinformaticians have developed new analysis tools that considerably increase the precision with which we detect alternatively spliced genes in RNA-Seq experiments. We have also developed a new database of RNA binding proteins (ATtRACT), the largest to date. Using these tools, we have identified splicing factors that play regulatory roles in the infarcted heart. We are now analyzing the role of these factors through different gain- and loss-of-function approaches in mice.

The calcineurin splicing variant CnAβ1 represents a good example of how alternative splicing can dramatically change the function of a protein. Due to retention of an intron, CnAβ1 has a unique C-terminal domain that has no similarity with any other known protein. This unique domain drives CnAβ1 to the Golgi apparatus and is necessary for the activation of the Akt signaling pathway. Unlike other calcineurin isoforms, which play a pathological role in the heart, CnAβ1 improves cardiac function after myocardial infarction by reducing infarct expansion. In embryonic stem cells, where CnAβ1 is highly expressed, it regulates differentiation towards the mesodermal lineage. We are now investigating its antifibrotic effects. As part of a new European Network (CardioNext), also involving other CNIC groups, we are exploring the therapeutic potential of CnAβ1 in a preclinical myocardial infarction model in the pig.

ATtRACT database. ATtRACT is the largest database of RNA binding proteins and their associated nucleotide motifs. It incorporates search engines for RNA binding proteins and identification of motif enrichment in a series of sequences. Left, Home page of the ATtRACT database. Center, ATtRACT can run MEME and TOMTOM programs to search and identify new and known motifs. Right, Visualization of search results in ATtRACT.
Protein structure of CnAβ1 and CnAβ2. CnAβ1 and CnAβ2 are produced from the same gene. However, they have opposite functions due to their different C-terminal domains. The figure shows a 3D prediction of the CnAβ1 and CnAβ2 structures. CnAβ2 has 3 alpha helices that act as an autoinhibitory domain (red) by preventing exposure of the catalytic domain (light yellow) in the absence of intracellular calcium increases. CnAβ1 has two alpha helices (cyan) that act as an autoinhibitory domain and in addition promote localization of CnAβ1 in the Golgi apparatus and activation of the Akt signaling pathway.

FineSplice analysis pipeline. FineSplice is a program developed in our group to reduce the number of false-positive exon-exon junctions detected by RNA-Seq read aligners like TopHat. FineSplice increases precision when aligning a read and reduces the detection of false positive junctions from 10% to 1%. The figure and additional details can be found in Gatto et al., Nucl. Acids Res. 2014.

MAJOR GRANTS
- Comunidad de Madrid (GRUPOSCAM10, “Fibroteam” S2010/BMD-2321)
- Ministerio de Economía y Competitividad (SAF2012-31451)
- Ministerio de Ciencia e Innovación. FIS (CP08/00144)

Selected Publications
RESEARCH INTEREST

We study of the mammalian oxidative phosphorylation (OXPHOS) system, including its role in metabolic integration at cellular and organismal levels and its homeostatic response in health and disease. Part of our work is dedicated to cataloguing the set of nuclear encoded genes required for OXPHOS system homeostasis. We are also using high-throughput proteomics to define the mitochondrial protein interactome and posttranslational modifications in healthy, heart-stressed and metabolically altered animals.

A major program in the lab is directed at defining the role of genetic variability in mitochondrial function and in lifelong physiology, longevity, predisposition to disease, and the response to clinically relevant drugs. For this program we use conplastic mice—animals with the same nuclear genome but different non-pathological mtDNA haplotypes—as well as a variety of genetically defined healthy animals. Using these models, we are investigating phenotypic variations in angiogenesis regulation and vessel morphology and function (Fig1); deciphering adaptation of mitochondrial fuel use to suit the cell type or functional status; analyzing ROS-dependent activation of the Fgr pathway (Fig2); and defining supercomplex reorganization in the respiratory chain (Fig3). We are also developing noninvasive in vivo imaging methodologies to evaluate mitochondrial function upon heart disfunction. The long-term aim is to define new targets for the diagnosis or treatment of cardiovascular diseases.

Functional genetics of the oxidative phosphorylation system

Figure 1. Cardiac mitochondria. Transmission electron micrographs of C57BL6 mouse ventricular myocytes (x 40000). a) Intermyofibrillar mitochondria—longitudinal rows of mitochondria located within myofibrils. b) Cardiac muscle damaged by 27 days of administration with isoproterenol; vacuolated and disrupted intermyofibrillar mitochondria are evident.

Figure 2. ROS dependent activation of mito-Fgr pathway. Acín-Pérez et al., 2014, Cell Metab
**MAJOR GRANTS**

- Ministerio de Economía y Competitividad (BFU2013-50448)
- Ministerio de Economía y Competitividad (SAF2012-32776)
- Marie Curie Initial Training Networks (ITN). Mitochondrial European Educational Training (GA Nº 317433).
- Comunidad de Madrid. Programa de Biomedicina (S2011/BMD-2402).
- Ministerio de Economía y Competitividad (RyC 2011-07826). PI: Rebeca Acín
- European Commission. Marie Curie Career Integration Grant. PI: Rebeca Acín

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**Selected Publications**


*LAE and LS co-corresponding authors

RESEARCH DEPARTMENTS
Vascular Biology and Inflammation
Research in the **Department of Vascular Biology and Inflammation** (DVBI) is focused on the interactions between cells of the vascular system. The groups in the department work along the following strategic research lines: 1) Vascular Wall Remodeling; 2) Inflammation and Autoimmunity; and 3) Cell Biology and Signaling in Metabolism and Disease. DVBI research groups use a wide variety of techniques, including animal, tissue, cellular and molecular models, to investigate normal vascular function and the key steps in the vascular alterations that underlie cardiovascular diseases. Cardiovascular proteomics is also a major interest.
Gene regulation in cardiovascular remodelling and inflammation

RESEARCH INTEREST

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

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

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

We are dissecting signaling pathways involved in vascular wall remodeling, a major feature of vascular diseases such as atherosclerosis, aneurysm and restenosis. We have set up animal models of these pathologies, and have generated mice deficient for AngII-target molecules that are regulated by CN. Some of these animals are totally resistant to these diseases and we are working to elucidate the molecular and cellular mechanisms underlying this protection.
Histological analysis of aortas before (left) and after (right) treating mice with pathological stimuli. Masson’s Trichrome staining shows fibrosis in blue, cellular cytoplasm in red and nuclei in purple.

Cross-sectional images of myocardium from mice treated with saline (left) and AngII (right) stained with wheat germ agglutinin-FITC, which stains cardiomyocyte cell membranes.

MAJOR GRANTS
- Ministerio de Economía y Competitividad (SAF2012-34296)
- Ministerio de Economía y Competitividad. FIS RETICS (Red de Investigación Cardiovascular: RD12/0042/0022)
- Fundació La Marató TV3 (264/C/2012) (PI: Sara Martínez)

Selected Publications


Lymphocytes exert their functions by communicating with other cells. They form specific immune synapses with antigen-presenting cells (APCs) and also secrete soluble mediators. During synapsis, exosomes—nanovesicles produced by multivesicular bodies (MVBs)—act as “shuttles” that transfer specific mediators, including microRNAs (miRNA), to the target cell. Formation of the immune synapse is triggered by T cell receptor (TCR) stimulation. Its downstream signaling pathways engage actin and tubulin-based cytoskeletal networks that control the spatial and molecular architecture of the IS. One of these elements is the centrosome or MTOC (microtubule-organizing center). The MTOC governs directed secretion toward the APC by juxtaposing the Golgi and endosomal compartments at the T:APC contact zone. MVBs form part of this assembly for intracellular transport and sorting. Our group aims to define the connection between TCR activation and the reorganization of the tubulin cytoskeleton and organelle transport, the delivery of specific mediators to the APC and other target cells, and the functional consequences of this mode of cell-cell communication in the regulation of the immune inflammatory response.

The group pursues three main lines of investigation. 1) Regulation of immune synapse formation and function. We are exploring protein multiplexing at the MTOC, specifically the role of MTOC folding complexes, and the post-translational modifications of Ser/Thr kinases and the tubulin deacetylase HDAC6. We address the molecular mechanisms that control mitochondria transport during leukocyte-endothelial adhesion and extravasation and maturation of the IS. We are also analyzing the role of mitochondrial components in the biogenesis and secretion of exosomes and their impact on macrophage and dendritic cell function. 2) Fine tuning of T cell biology by miRNAs and exosomes. The production of exosomes by different T cell subsets is being examined with the aim of identifying characterizing specific miRNAs delivered to target cells. We also investigate the molecular mechanisms underlying the specific sorting of proteins and miRNAs to exosomes. This information may allow engineering of immune cells to produce exosomes able to specifically modulate the immune response. 3) Immunoregulatory molecules and miRNAs in inflammatory diseases. We are analyzing the role of different immunoregulatory molecules such as CD69, galectins, aminocaid transporters and HDAC6 in animal models of atherosclerosis and psoriasis in humans in order to identify the molecular basis of these inflammatory diseases.
MAJOR GRANTS

- European Commission. ERC Advanced Investigators Grant (ERC-2011-AdG 20110310) (GENTRIS)
- Ministerio de Economía y Competitividad (SAF2011-25834)
- Ministerio de Economía y Competitividad. FIS RETICS (RIC: RD12/0042/0056)
- Redes de Excelencia de la Comunidad de Madrid (P2010/BMD-2332)
- Ministerio de Economía y Competitividad. FIS (PI11/00939) PI: Gloria Martínez del Hoyo

Selected Publications


Miro-1 controls polarity of mitochondria and the microtubule organizing center during lymphocyte chemotaxis

Miro-1 links mitochondria and microtubule Dynein motors to control lymphocyte migration and polarity.
Integrin signaling

RESEARCH INTEREST

Caveolae are actin-linked plasma membrane nano-invaginations, abundant in mechanically stressed tissues. They are involved in signaling, viral entry, membrane trafficking and lipid metabolism; however, controversy surrounds these actions, and the precise functions of caveolae and their main proteins caveolins (Cav 1-3) and cavins (1-4) remain unresolved. Cav-deficient mice show tissue abnormalities, and caveolar disorders are associated with lipodystrophy and muscular dystrophy, cardiovascular disease and cancer, suggesting a role as homeostatic regulators. Preliminary evidence suggests that caveolae sense mechanical cues. We have shown that Cav1 can modulate cell shape and responses via force-dependent remodeling of the 3D microenvironment. Elongated cancer associated fibroblasts (CAFs) form stiff, parallel-fiber networks through which cancer cells move rapidly, promoting local invasion and subsequently distant metastasis. By performing image-and-RNAi-based high content screening in co-cultures of tumor cells and CAFs (Figure 1), we aim to identify networks of stromal genes involved in biomechanical matrix stiffening.

Our works shows that stromal-Cav1 drives not only pathological remodeling of the tumor microenvironment, but also physiological remodeling, for example in the mammary gland and the skin. We are now addressing the role of Cav1 and the phosphorylation of its Tyr14 in cardiac remodeling after acute myocardial infarction using the LAD (left anterior descending) artery permanent ligation model (Figure 2).

Our recent work also establishes Cav1, through the suppression of a MEK-ERK1/2-Snail1 pathway, as a major checkpoint in the transition from an epithelial to a mesenchymal identity in the peritoneum. The efficacy of a MEK pharmacological inhibitor in counteracting the EMT/fibrosis developed in Cav1−/− mice during peritoneal dialysis (PD) warrants further translational studies in other chronic inflammatory diseases.

We have previously established links between caveolae-dependent membrane trafficking and directional cell migration and invasion through Rho family GTPases, including RhoA and Rac1. Rac1 has been detected in the nucleus, but the function of nuclear Rac1 remains elusive. We now provide insight into the molecular mechanism of Rac1 nucleocytoplasmic shuttling. Rac1-driven nuclear actin polymerization controls nuclear membrane organization and shape. Dysregulation of this mechanism in cancer leads to Rac1 nuclear accumulation, promoting nuclear deformation and cell invasion through narrow spaces (Figure 3).

Coculture of breast cancer cells and cancer-associated fibroblasts (CAFs). Cav1 (green) is silenced upon lentiviral infection (marked by CherryFP, in red). Silencing and CherryFP expression are restricted to the fibroblast cell line (1069sk) via the integrin alpha-11 promoter. Breast cancer cells (MCF7 cell line) are labeled with a pan-cytokeratin antibody (magenta).
Collagen fiber remodeling after myocardial infarction imaged by second harmonic generation (SHG). Native (non-fixed) hearts from wild-type mice were imaged for SHG. Whereas collagen fibers are highly ordered under basal conditions (left), after permanent ligation of the LAD strong, disordered collagen deposition is observed (right).

Scheme showing that Rac1 nucleocytoplasmic shuttling drives nuclear shape changes. Dysregulation of this mechanism leads to Rac1 nuclear accumulation, deformation, and cell invasion through narrow spaces.

MAJOR GRANTS
- European Commission. Marie Curie Actions Initial Training Network (ITN) (Horizon 2020, “BIOPOL”)
- European Union. Marie Curie Actions Intra-European Fellowships (FP7-PEOPLE-2013-IEF)
- WorldWide Cancer Research (UK) (formerly known as AICR) (AICR 15 – 0404)
- Ministerio de Economía y Competitividad (SAF2011-25047)
- Ministerio de Economía y Competitividad. Consolider COAT (CSD2009-00016)
- Fundació La Marató TV3 (674/C/2013)

Selected Publications


RESEARCH DEPARTMENTS 2. Vascular Biology and Inflammation

Cardiovascular proteomics

RESEARCH INTEREST

Our group works on the development of high-throughput quantitative approaches for the dynamic analysis of the deep proteome and their application to cardiovascular projects. We have recently developed a generic integration algorithm (SanXoT) that serves as the basis for systems biology analysis of high-throughput quantitative proteomics experiments. By using a novel redox proteomics technology (GELSILOX) recently developed in our laboratory, we have also demonstrated that ischemia-reperfusion increases oxidation of Cys sites in mitochondrial proteins and that this effect is inhibited by ischemic or pharmacological preconditioning. These studies are being extended to models of aging, hypertrophy and animal models of deletion or overexpression of several protein factors. We have developed a novel data-independent mass spectrometry scanning technique (DiS) that improves on the performance of conventional shotgun approaches and also allows in-silico-targeted quantification of any suspected peptide, including post-translationally-modified species (PTM). We are using an extension of this technique (Blue-DiS) to generate an extremely detailed structural map of components of mitochondrial oxidative phosphorylation supercomplexes in several models, which include characterization of PTMs that may act as molecular determinants of assembly. We have also used advanced interactomics to study the supramolecular structure of human T-cell-derived exosomes. We have found evidence that the network of intramolecular interactions with tetraspanins may act as a sorting machinery that determines the protein composition of exosomes. In an extension of these studies, we have contributed to the discovery of a novel molecular mechanism by which miRNAs are selectively transported from cell to cell within exosomes.

Our peptide identification workflow uses postscoring filtering schemes to take full advantage of the high mass resolution provided by modern orbitraps, avoiding identification artifacts due to anomalous behavior of scores in conventional approaches.
Our layered statistical model reveals that HDL proteome composition is highly variable among different individuals, but remains stable in the same individual, allowing the systematic detection of protein alterations resulting from atheroma plaque rupture.

MAJOR GRANTS

- Ministerio de Economía y Competitividad (BIO2012-37926)
- Ministerio de Economía y Competitividad. FIS Proteored (PT13/0001/0017)
- Ministerio de Economía y Competitividad. FIS RETICS (RIC: RD12/0042/0056)
- European Commission: 7th Framework Programme for Research (FP7-PEOPLE-ITN-2013)
- Progeria Research Fund Specialty Award (USA)

Selected Publications


*Co-corresponding authors
Matrix metalloproteinases in angiogenesis and inflammation

RESEARCH INTEREST

In adults, angiogenesis, the formation of new vessels from pre-existing ones, is often coupled to inflammation, and its deregulation can result in failed tissue repair after acute injury or in the progression of chronic inflammatory disorders. One of the first steps in the transition from a quiescent to an angiogenic vasculature is the remodeling of the basement membrane and the perivascular extracellular matrix, a process involving the action of matrix metalloproteinases (MMPs). We have lately identified i) a combinatorial proteolytic program driven by the protease MT1-MMP in TNFα-activated endothelial tip cells; ii) a molecular complex formed by EMMPRIN (an MT1-MMP substrate) that provides local ATP for actomyosin contractility and endothelial cell junction stability; and iii) a requirement for MT4-MMP in aortic vessel wall development and function.

Our interests include the initiation events of angiogenesis in inflammation, the therapeutic potential of MT1-MMP and MT4-MMP in animal models of inflammatory pathologies, the visualization of the dynamics and cell-cell interactions during inflammation-induced angiogenesis, and the intimate nature of macrophage/endothelial cell crosstalk in inflammatory angiogenesis. Our studies in these areas are conducted with 2D and 3D angiogenic models, genetically modified mouse lines, and animal models of inflammatory disease. Cutting edge technologies employed in the lab include high-resolution and super-resolution confocal microscopy, 3D reconstruction and image analysis, bioinformatics and biomathematics, and novel lentivirus-based gene therapy strategies. These approaches are being applied to gain greater understanding of how angiogenesis occurs in the inflammatory context and to identify new molecules or cell subsets whose modulation might impact the angiogenic process.

In this way we aim to contribute to the goals of improving physiological wound healing and tissue repair in diseases such as myocardial infarction and ameliorating the symptoms and progression of chronic disorders, such as inflammatory bowel disease, that involve alterations to vascular function.

Analysis of endothelial cell junction dynamics during capillary formation. (A) Staining of endothelial cells (VE-cadherin, red) coating a Cytodex bead and embedded in a fibrin gel allows investigation of endothelial junctions during the formation (left) and maturation (right) of 3D capillaries in response to angiogenic factors. (B) The ‘Patching Algorithm’ (Bentley et al., Nat Cell Biol 16, 309, 2014) is a new image analysis tool that allows the classification of single endothelial cell junctions (VE-cadherin, green; left) depending on their stage of remodeling (active to inactive; right).
Ex vivo image analysis of the vasculature in the infarcted heart. Confocal microscopy imaging of thick sections from the pig heart are used for 3D image analysis with MatLab to extract parameters of the whole vasculature (geometrical, fractal) and single vessels (diameter, length) in ischemic versus non-ischemic tissue. This analysis allows the classification and visualization of vascular responses to ischemia.

Imaging vascular responses and cellular crosstalk in inflammation-driven angiogenesis. Confocal microscopy imaging of whole-mount intestine and 3D reconstruction with Imaris software show the changes in organization and structure of the mucosal vascular plexus (green) in a mouse model of chemical-induced colitis (inflammation: 7 days of treatment with 1% DSS) compared with control (homeostasis). Note also the altered association of vessels (green) with monocytes/macrophages (red) in the inflamed tissue. Nuclei in blue; lower panels show orthogonal views.

MAJOR GRANTS
- Ministerio de Economía y Competitividad (SAF2011-25619)
- FIS RETICS (Red de Investigación Cardiovascular: RD12/0042/0023)
- Comunidad Autónoma de Madrid, ANGIOBODIES 2.0 (S2010/BMD-2312)
- Fundación La Marató TV3 (165/C/2012)
- European Union (PITN-GA-2013-608027) (CardioNext) (Coordinator)

Selected Publications


B lymphocyte biology

RESEARCH INTEREST

B lymphocytes protect the organism against infection by producing highly specific antibodies. Misregulation of B lymphocytes and antibody generation can lead to health conditions such as immune deficiencies, autoimmunity and cancer. Our lab focuses mostly on the biology of B cells in germinal centers, the site of secondary antibody diversification. Somatic remodeling of antibody genes in germinal centers involves two key molecular events, somatic hypermutation and class switch recombination. Both of these events are initiated by the enzyme activation-induced deaminase (AID). However, when AID targets non-antibody genes its activity can damage DNA, causing mutations and chromosome translocations.

We are interested in understanding the regulation of germinal center events, above all the function of AID and the role of microRNAs in this environment. Our work over the past few years has highlighted the importance of microRNAs in autoimmunity and the link between germinal centers and cancer and AID regulation (J Exp Med 2008, Immunity 2010, Curr Opin Immunol 2011, Immunol Rev 2013, Blood 2014). In addition, we have deciphered some other molecular events that regulate AID activity (PloS One 2008, J Exp Med 2012). We are currently exploring the molecular regulation of germinal center B cells in health and disease.

AID activity promotes antibody diversification and oncogenic lesions. The antibody genes of B cells in germinal centers undergo somatic diversification through somatic hypermutation (SHM) and class switch recombination (CSR), thus allowing the generation of high-affinity and effector-versatile antibodies. SHM and CSR are initiated by activation-induced deaminase, an enzyme that deaminates cytosines on the DNA of antibody genes. However, this same activity can target other genes, promoting mutations and chromosome translocations with lymphomagenic potential.
AID promotes genotoxic damage in pancreas. We have generated a mouse model to express AID in pancreas and address its oncogenic potential in a non-B-cell context. We introduced an AID-GFP-encoding cassette preceded by a transcriptional stop flanked by two loxP sites into the endogenous Rosa26 locus and crossed these mice with p48-Cre$^{+/+}$ mice to promote specific expression of AID in pancreas. Pancreatic explants from AID$^{+/+}$ (R26AID$^{+/+}$) and control (R26AID$^{+/-}$) mice were stained with anti γ-H2AX antibody to detect double-strand breaks.

MAJOR GRANTS
- Ministerio de Economía y Competitividad (SAF2013-42767-R)
- European Commission. European Research Council Starting Independent Researcher Grant (ERC-BCLYM 2007)

Selected Publications
Immunobiology of inflammation

RESEARCH INTEREST

We use innovative approaches to study the immune and inflammatory responses to infection and tissue damage. The general research question addressed in the lab is how myeloid cells initiate and modulate immunity and inflammation by sensing damaged-self and non-self. We believe that this research has the potential to lead to the development of new vaccines and immunotherapy strategies. We are interested in how damaged-self and non-self is detected by dendritic cells (DCs) and macrophages, how this sensing modulates the function of myeloid cells, and what relevance this interaction has in vivo in models of infection, inflammation and cancer (Fig. 1).

Infection is frequently associated with tissue damage, but knowledge is limited about how the immune and inflammatory response to infection are affected by concomitant sensing of cell death by myeloid cells. Some myeloid C-type lectin receptors (CLRs) have been identified as receptors for necrotic cells that couple to Syk signaling, potentially triggering innate and adaptive immune responses. DNGR-1 (CLEC9A), expressed on DCs, and Mincle (CLEC4E), expressed broadly in myeloid cells, detect ligands exposed upon necrosis and potentially modulate signals from other pattern-recognition receptors during infection.

We preferentially focus on the following specific areas of research (Fig. 1):

1. CLEC9A as a model CLR that detects tissue damage and modulates immunity.
2. The specialized functions of Batf3-dependent DCs.
3. Mincle as a model CLR that senses ligands both in damaged-self and in non-self.
4. New avenues of research into additional functional effects of sensing by myeloid cells: we are interested in the effects of sensing non-self and damaged-self on the metabolism of myeloid cells, and particularly the role of oxidative phosphorylation activity in myeloid cells in inflammation and immunity.

Our recent results reveal that Batf3-dependent DCs are crucial for generation of Th1 immunity through the production of IL-12 (Fig. 2 and Martínez-López et al. 2014). This has been dissected in the gold standard model of infection by the eukaryote parasite parasite Leishmania major, which induces tissue damage and also mimics many tissue-derived danger signals. Using Batf3−/− mice, we are currently analyzing the role of this DC subset in models of atherosclerosis, asthma and cancer.
Batf3-dependent DCs are crucial for generation of Th1 immunity against Leishmania major. (A, B) Batf3-deficient mice develop an exacerbated L. major cutaneous pathology (A) Lesion diameter (measured with a digital calliper) in WT and Batf3−/− mice was tracked for 12 weeks after intradermal infection in the ear pinnae with L. major. (B) Parasite load in the ears of WT and Batf3−/− mice at different times after infection with L. major (each circle represents one sample). (C) Batf3 deficiency impairs local Th1 immunity to L. major. Ear cells were obtained 2, 3 and 7 weeks after infection, restimulated with anti-CD3 and anti-CD28 antibodies, and stained for IFN-γ. (D) Draining lymph node cells from infected ears were stained for CD11c, MHC-class-II, CD8α, CD103 and intracellular IL-12p40. Plots show frequency (top) and absolute numbers (bottom) of IL-12p40 staining in CD103+CD11c+MHC class II+ DCs. Data are presented as mean + SEM. * p < 0.05; ** p < 0.01; *** p < 0.001 (unpaired two-tailed Student’s t test).

MAJOR GRANTS

- Ministerio de Economía y Competitividad (SAF2013-42920-R)
- European Commission. European Research Council Starting Independent Researcher Grant (ERC-StG-260414)
- Research cooperation agreement with MedImmune (Cambridge, UK)
- Ministerio de Economía y Competitividad (RYC2009-04235)
- ERS/EU Marie Curie Post-doctoral Research Fellowships (RESPIRE 2 - 3708-2013).

Selected Publications

Martínez-López M, Iborra S, Conde-Garrosa R, Sancho D. Batf3-dependent CD103+ dendritic cells are major producers of IL-12 that drive local Th1 immunity against Leishmania major infection in mice. Eur J Immunol doi: 10.1002/eji.201444651

Iborra S, Sancho D. Signalling versatility following self and non-self sensing by myeloid C-type lectin receptors. Immunobiology doi: 10.1016/j.imbio.2014.09.013


Stress kinases in diabetes, cancer and cardiovascular disease

**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 clinical outcomes include cardiovascular disease and type 2 diabetes. Moreover, metabolic syndrome may be a predisposing factor for the development of some types of cancer, such as hepatocellular carcinoma.

The high cardiovascular risk associated with metabolic syndrome and type 2 diabetes suggests that common mechanisms are involved in the etiology of these conditions, and that agents acting on the same therapeutic targets might improve disease parameters in both. 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 group investigates the involvement of SAPKs in the development of cancer, diabetes, cardiac hypertrophy and atherosclerosis induced by obesity. Our research is conducted with a number of disease models in combination with whole-body and tissue-specific knockout mice, and has shown that the p38γ/δ isoforms control IL6 and TNF production in myeloid cells. We are now studying how the regulation of inflammation by these kinases affects the development of metabolic syndrome. We are also studying the function of these kinases in other tissues, such as muscle, heart, the central nervous system and adipose tissue, in order to elucidate the role of these kinases in the development of different diseases associated with obesity (steatosis, diabetes, cardiovascular diseases and some types of cancer).

**MAPK pathway**

A general feature of MAPK pathways is a canonical cascade consisting of MAPK kinase kinases (MAP3K), a MAPK kinase (MAP2K) and a MAPK. The MAPK family can be divided into three main pathways: ERK (extracellular signal-regulated kinase), JNK (c-Jun N-terminal kinase), and p38. Numerous stimuli, including growth factors, inflammatory cytokines and a wide spectrum of cellular stresses, can activate MAPK signaling pathways. Once activated, MAPKs can phosphorylate several downstream targets, including other protein kinases, cytosolic substrates, and transcription factors.
Obesity related diseases
Obesity is one of the leading causes of life-threatening diseases and can compromise health and shorten life expectancy. In our group we study several of themes such as diabetes, cancer, and heart disease.

MAJOR GRANTS
- Ministerio de Economía y Competitividad (SAF2013-43506-R)
- European Commission. European Research Council Starting Independent Researcher Grant (ERC-StG-260464)
- Comunidad de Madrid. INMUNOTHERCAN (S2011/BMD-2326)
- Ministerio de Economía y Competitividad (RYC-2009-04972)

Selected Publications


Innate and adaptive immune responses are implicated in immune-mediated damage and repair of the heart. Although a moderate immune response is needed for tissue repair after an insult, an exacerbated immune response or autoimmunity against heart-derived antigens induce irreparable damage to the myocardium. Elucidation of the factors that determine the balance of the immune response is therefore critical for efforts to control the severity of cardiovascular diseases (CVD). MicroRNAs (miRNAs) have emerged as one of the most innovative tools for the development of strategies to stop both, inflammation and CVD. Several emerging studies link pro-inflammatory helper 17 T (Th17) cells, and anti-inflammatory regulatory T (Treg) cells with acute coronary syndrome, congestive heart failure and other CVDs. Other studies, in which ischemia is induced by right femoral artery ligation, show that Treg and Th17 cells modulate postischemic neovascularization in an antagonistic manner, revealing a possible role of these cell types in vascular diseases. However, the role of miRNAs derived from Th17 or Treg cells has received scant attention, leaving an open window for the development of novel diagnostic and therapeutic tools. Our group has revealed the role of the leukocyte antigen CD69 in the control of cardiomyopathy. CD69 is a master regulator of Th17/Treg balance, necessary for the control of Th17-mediated heart inflammation and failure. We are now investigating Th17/Treg-related miRNAs in murine models of CVDs and in patients with the aim of identifying their putative target genes, an essential step toward elucidating their potential as novel therapeutic molecules and diagnostic tools in these diseases.
Neovascularization after hindlimb ischemia. (A) Blood perfusion after femoral aorta surgery was measured using a laser Doppler perfusion imaging system. (B) Quantitative analysis of the blood flow ratio between ischemic and non-ischemic hindlimbs.

MAJOR GRANTS
- Ministerio de Economía y Competitividad (SAF2011-27330)
- Comunidad de Madrid. Redes de Excelencia (S2010/BMD-2332)
- Ministerio de Economía y Competitividad. FIS RETICS (RIC: RD12/0042/0056)

Selected Publications


RESEARCH DEPARTMENTS 2. Vascular Biology and Inflammation

Single-molecule mechanobiochemistry

RESEARCH INTEREST

In our laboratory, we generate new knowledge about the mechanical properties of the myocardium and its regulation, with the potential to uncover new targets for the treatment of heart disease. Contractility of cardiac muscle depends on the concerted action of sarcomeric proteins with a mechanical function—actin, myosin, titin and several other associated proteins. Mutations in these proteins lead to different forms of life-threatening cardiomyopathy. However, the molecular mechanisms leading from genotype to pathogenic phenotype remain unknown. We are currently testing the hypothesis that missense mutations in elastic sarcomeric proteins can induce mechanical phenotypes that result in the development of disease. We also want to understand how the elasticity of the myocardium is tuned by posttranslational modifications of sarcomeric proteins. To bridge the gap between mechanics and biochemical regulation of sarcomeric proteins, we apply our double expertise in protein biochemistry and single-molecule force-clamp spectroscopy by atomic force microscopy (AFM). The mechanisms of cardiac regulation we examine cannot be studied by classic biochemistry techniques alone, and have remained unexplored. By implementing advanced single-molecule manipulation techniques, we are helping to establish mechanobiochemistry as a new field of scientific research.

A single molecule experiment by AFM. A single polyprotein made from 8 repetitions of a titin domain is pulled using an AFM tip. In the picture, one of the domains has been mechanically unfolded, exposing buried cysteines that are being posttranslationally modified by an oxidorreductase enzyme.
Detection of redox posttranslational modifications in titin. Preliminary mass spectrometry results showing the redox state of cysteine residues in titin domains (in collaboration with the CNIC Cardiovascular Proteomics group). Top: Gray lines represent identified peptides in the primary sequence of full-length titin. We obtain 70% coverage of the titin sequence. Bottom: Three domains are highlighted (PDB codes are indicated). The paired cysteines detected in domain I1 are oxidized, suggesting that they form a disulphide bond in native tissue. Results with domain I67 suggest that a disulphide bond is formed between residues 74 and 85. Oxidized cysteines are also found at positions that are incompatible with disulphide bond formation, suggesting that they are the target of other redox posttranslational modifications, such as S-glutathionylation.

MAJOR GRANTS
- European Union / CNIC: CNIC IIF (International Incoming Fellowships for Young Group Leaders) (FP7-PEOPLE-2010-COFUND-267149)

Selected Publications


RESEARCH DEPARTMENTS
Atherothrombosis, Imaging and Epidemiology
Our department combines basic science, clinical data, and population-level analysis in order to better understand the occurrence, natural history and prognosis of cardiovascular disease, and to develop therapeutic alternatives. Our programs include studies into the molecular and cellular mechanisms underlying atherosclerosis, restenosis and aging; the role of neutrophils and other myeloid leukocytes in various aspects of the inflammatory response; cardioprotection during myocardial infarction; and complex cardiac arrhythmias including studies in animal models and humans using latest-generation advanced imaging techniques.

The department works closely with the Advanced Imaging Unit, bringing in expertise in imaging, nanomedicine, radiochemistry and metabolomics. The department also coordinates epidemiological studies on the distribution and progression of atherosclerosis and the genetic, environmental, lifestyle, and social determinants in human populations.
RESEARCH INTEREST

Our group has developed research applications for noninvasive, high-resolution and high-sensitivity imaging technologies to support translational research and population studies in preclinical atherosclerosis. We collaborate with other CNIC investigators, offering support in translational research in the use of basic cardiovascular imaging techniques (Figure 1). Our main noninvasive bioimaging population studies (Figure 2) for early detection and progression of atherosclerosis, PESA and AWHS (see Multidepartamental Projects) are clinical trials designed to identify new imaging, lifestyle habits and omics factors associated with the presence and progression of atherosclerosis in middle aged adult populations.

In 2014 our group performed more than 4000 preclinical echocardiogram procedures in mouse and zebrafish models to support research projects from all CNIC departments. This work has contributed for more than 40 studies and preclinical sub-studies to elucidate pathological phenotypes by basic CV imaging. Our collaborations include work on several mouse models of cardiovascular disease, ischemia-reperfusion in acute myocardial infarction, myocardial regeneration in zebrafish, mouse models of atherosclerosis and aging, models of systolic, diastolic and diabetic heart failure, and models of non-compact cardiomyopathy, left ventricular hypertrophy, aortic aneurysm, degenerative aortic valve, aortic annular ectasia, mitral insufficiency, coronary aneurysm, acute pulmonary hypertension, and chronic pulmonary hypertension associated with smoking or scleroderma. In many of these projects our basic CV imaging analysis and our clinical expertise have been crucial to identifying the preclinical cardiovascular phenotype and have been the key to identifying the focus of clinical translation to human disease.

The PESA study has moved into Phase II follow-up at 3 years for 4000 participants aged 40-54 years. In this second phase, we look for changes in the systemic extent of atherosclerosis and its association with factors that were detected in the recruitment phase, three years earlier. The studies include the analysis of systemic subclinical atherosclerosis through multi-territory 2D/3D ultrasound and the detection of coronary calcium CT. We also continue to analyze the influence of lifestyle, omics, known risk factors, analytical blood and urine, as well as changes in timing. To analyze the prognostic value of local plaque inflammatory markers, a group nearly 1000 participants with atherosclerosis at baseline has been evaluated by 18FDG PET/MRI. Follow-up at 3 and 6 years enables us to determine which factors predict the progression of atherosclerosis in a middle aged population, and long-term monitoring will reveal new prognostic markers for events.

Our research into cardiovascular disease is based on a simple principle: create to understand, create to treat.

Our major area of interest is arrhythmogenic right ventricular cardiomyopathy (ARVC). This heart muscle disease is characterized by right ventricular anatomical abnormalities and ventricular arrhythmias that can lead to sudden cardiac death, especially in young athletes. To be able to study the effect of exercise on hearts of mice carrying the most prevalent ARVC-associated mutation in plakophilin-2 (PKP2), we used AAV to express the R735X mutant in wild-type mice. Our work shows that injected AAV-R735X animals develop an overt ARVC phenotype when subjected to endurance training, supporting the recommendation for exercise cessation recommendation in carriers of this mutation.

Preclinical imaging for translational research in mouse models of left ventricular dysfunction and of pulmonary hypertension. Upper panels show M-mode echocardiogram of (a) control and (b) depressed left ventricular systolic dysfunction. Lower panels show pulsed Doppler of pulmonary flow in (c) control mice, with relative symmetric peak velocity time (PVT) compared with ejection time (ET), and (d) in mice with pulmonary hypertension, with relative shorter peak velocity time.
MAJOR GRANTS

- European Commission FP7 (241559 FOCUS).
- Departamento de Salud y Consumo of the regional government of Aragon, General Motors Spain and CNIC (AWHS).
- AHA Grant (14SFRN20490315). PI: Fuster V.
- NIH Grant Programs in Excellence in Nanotechnology. (298201000045-C-0-1). Fuster V Co-Inv
- NIH Grant Project: (U01HL-114200-02)
- Grenada Heart Project (Louis Mayer FDTN/W. Eisenberg)
- Project Astra Zeneca. (ISSBR1L0246/ D5130L00081)

Selected Publications


RESEARCH INTEREST

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

A major interest of the group is cardioprotection during myocardial infarction (MI). We have established models of MI in rodents and large animals, and we are using these to study the mechanisms underlying the beneficial effects of various cardioprotective strategies, mainly related to modulation of the adrenergic system. We are also interested in the myocardial response to pulmonary hypertension. We have developed small and large animal models of pulmonary hypertension and use imaging technology to evaluate the response to different therapies.

In the clinical setting, our team is a key participant in the METOCARD-CNIC trial, which uses magnetic resonance imaging to evaluate the effectiveness of a cardioprotective strategy based on beta adrenergic modulation in patients suffering a myocardial infarction. Last year we reported the primary outcome of the trial, which shows that this strategy can reduce infarct size in patients undergoing primary angioplasty. During 2014 we analyzed the follow-up data of this trial to evaluate the effect of the therapy on long term myocardial performance and clinical events. The METOCARD-CNIC trial serves as the platform for a future large trial in myocardial infarction patients that will test the effect of the therapy on hard clinical endpoints. This trial will be coordinated by us and will have more than six European partners. Finally, we are evaluating diffuse fibrosis within the myocardium by novel magnetic resonance imaging sequences, and are currently recruiting patients with different cardiomyopathies for this endeavour.

Impact of early i.v. metoprolol administration during myocardial infarction on long term left ventricular performance. Results from the METOCARD-CNIC trial. Follow-up left ventricular ejection fraction (LVEF) categories (A) and indications for implantable cardioverter defibrillators according to treatment allocation (B).
Edematous reaction after myocardial infarction follows a bimodal pattern. The development of myocardial edema is a well-known phenomenon occurring after ischemia/reperfusion (myocardial infarction). This edematous reaction was long assumed to be stable for at least 1 week. Using advanced cardiac magnetic resonance and histopathology, we have challenged this classical dogma, demonstrating that post-ischemia/reperfusion edema is bimodal. An initial wave of edema abruptly appears upon reperfusion and almost completely disappears at 24 hours. A deferred wave appears later and increases progressively until day 7.

Major Grants
- Ministerio de Economía y Competitividad - EXPLORA CIENCIA (SAF2013-49663-EXP)
- Ministerio de Economía y Competitividad - Acciones de Dinamización Europa Investigación (EJIN2013-50881)
- Ministerio de Economía y Competitividad. ISCIII-FIS (PI13/01979)
- Ministerio de Economía y Competitividad. ISCIII-RETICS (RiC, RD12/0042/0054)
- European Commission FP7-ICT-2011-8 (LIPHOS-317916)
- Maratón, Fundación TV3 (REF: 70/C/2012)
- CNIC Translational Grants (01-2009)
- European Commission FP7-PEOPLE-2013-ITN (CARDIONEXT).

Selected Publications


Imaging cardiovascular inflammation and the immune response

**RESEARCH INTEREST**

Our group studies various aspects of the biology of leukocytes, the cells that mediate organismal immunity but that are also the major culprits of vascular disease. We begin our studies with the cells that form the basis of the immune system, the hematopoietic stem cells, and search for the factors and cells that regulate their maintenance in the bone marrow. We currently focus on how two such molecules, ESL-1 and TGFβ, work together to ensure normal proliferation of stem cells. We are also working out the processes inside blood vessels in which leukocytes and platelets work together to promote inflammation. We have identified a surprising mechanism by which neutrophils (the leukocyte subset that underlies most cases of vascular disease) actively scan and search for circulating platelets to initiate inflammation. We continue to make progress in our understanding of the basic biological behavior of neutrophils in the absence of inflammation, in an attempt to identify new functions for these leukocytes. Finally, we are interested in the functions of a population of macrophages that reside within the healthy myocardium, and which may play an important role in supporting heart function.

**Regulation of the niche by hematopoietic cells**

Whole-mount images of the sternal bone marrow, showing the distribution of CXCL12-producing cells (green), which are strongly inhibited by ESL-1-deficient hematopoietic cells.

**Macrophages residing within the healthy myocardium**

Sectional image of the heart of a mouse showing macrophages (red and green) located between myocardial fibers delimited by laminin (white).
Neutrophils scan the circulation to initiate inflammation
3D reconstruction of a live inflamed venule showing the distribution of neutrophils (green) and a specific receptor (red) that traps activated platelets and relays signals to the cells, which then initiate inflammation.

**MAJOR GRANTS**

- Ministerio de Economía y Competitividad (SAF2013-49662-EXP)
- Ministerio de Economía y Competitividad (ERA-NET Infect-ERA 2014 #143 BActInfectERA)
- Comunidad de Madrid (P2010-BMD-2314)
- Ministerio de Economía y Competitividad (SAF2012-31142)

**Selected Publications**

  *Equal contribution


Molecular and genetic cardiovascular pathophysiology

RESEARCH INTEREST

The World Health Organization estimates that cardiovascular disease (CVD) will by 2020 be the main health and socio-economic problem worldwide, in part due to the progressive aging of the world population. Atherosclerosis, vascular calcification (VC) and heart failure contribute significantly to CVD-related morbimortality in the elderly. These anomalies, and the aging process itself, are much accelerated in Hutchinson-Gilford progeria syndrome (HGPS), a rare genetic disorder caused by the expression of progerin, a mutant form of lamin A. The most serious aspect of HGPS is extensive atherosclerosis, VC and cardiac electrophysiological alterations which are associated with early death (average lifespan: 13.5 yr, range: 8-21 yr), predominantly as a result of myocardial infarction or stroke. Progerin is also expressed at low level in aged tissues of non-HGPS individuals, suggesting a role in normal aging. Understanding how this mutant form of lamin A causes CVD and premature aging may therefore shed light on normal aging.

Our research is currently focused on 5 areas: 1. Identifying mechanisms through which wild-type lamin A/C regulate CVD; 2. Unraveling mechanisms of VC in the setting of normal and premature aging; 3. Identifying tissue-specific and systemic mechanisms through which progerin promotes atherosclerosis, VC, cardiac alterations, and aging; 4. Generating a porcine model of HGPS using CRISPR/Cas9 technology; and 5. Unraveling gender- and tissue-specific molecular mechanisms common to premature and physiological aging and specific to each process.

Differential Hox expression contributes to the maintenance of phenotypic differences between smooth muscle cells (SMCs) from atheroprone (aortic arch) and atheroresistant (thoracic aorta) regions through regulatory feedback mechanisms involving inflammatory mediators (e.g. reciprocal inhibition between HOXA9 and NF-κB).
Progeroid Zmpste24<sup>−/−</sup> mice exhibit mislocalization of connexin 43 (Cx43) and severe cardiac conduction abnormalities, and develop arrhythmias.

Reduced systemic and local levels of ATP and pyrophosphate (PPI) in a mouse model of HGPS accelerate vascular calcification, and this effect is ameliorated by treatment with PPI.

MAJOR GRANTS
- European Commission FP7-ICT-2011-8 (LIPHOS-317916)
- Progeria Research Foundation (Innovator Award PRF 2012-42)
- Progeria Research Foundation (Established Investigator Award PRF 2014)
- Ministerio de Economía y Competitividad. Modalidad Retos Investigación (SAF2013-46663-R)
- Ministerio de Economía y Competitividad. FIS RETICS (RIC, RD12/0042/0028)
- Ministerio de Economía y Competitividad (SAF2010-16044)
- Ministerio de Economía y Competitividad. FIS RETICS (RECAVA, RD06/0014/0021)
- Ministerio de Economía y Competitividad. FIS (CP11/00145) PI: J.M. González Granado

Selected Publications


Andrés V. Vitamin D puts the brakes on angiotensin II-induced oxidative stress and vascular smooth muscle cell senescence. Atherosclerosis (2014) 236:444-47

Advanced development in arrhythmia mechanisms and therapy

**RESEARCH INTEREST**

The laboratory focuses on investigating the mechanisms underlying complex cardiac arrhythmias that occur in highly prevalent cardiovascular diseases in the general population, as well as in specific subsets at particular risk of sudden cardiac death. Atrial fibrillation (AF), ventricular fibrillation (VF) and infarct scar-related ventricular tachycardia (VT) represent three of the most prevalent cardiac rhythm disorders, in which the capacity of current therapeutic strategies to accurately eliminate or prevent the arrhythmogenic substrate is limited. Our goal is to achieve in-depth insight into the mechanisms of these three complex arrhythmias through the use of appropriate experimental and numerical models, and for this insight to be used to improve patient care and develop new and more specific therapies.

We use a translational approach to study infarct scar-related VT in pigs and clinical infarct-related reentrant VT. High-resolution MRI images, both in humans (in vivo) and animals (ex vivo) provide the structural details to construct patient and animal-specific 3D anatomical models of the ventricles. Electrophysiologically realistic numerical simulations can be incorporated in the 3D model to induce and characterize reentrant VTs. Computational simulations are validated and compared with electrophysiological data and outcomes obtained during the electrophysiological study and ablation procedure, either in animals or in humans.

Both in-hospital and out-of-hospital cardiac arrest due to VF are associated with high mortality rates and significant cerebral disability. However, early prognosis in comatose survivors after cardiac arrest due to VF is unreliable, especially in patients undergoing mild hypothermia. We are currently involved in developing a reliable risk-score to enable early prediction of cerebral performance and survival.

In AF, we aim to characterize differences in the transcriptome, ion channel density and function, and molecular and gross anatomical structure of relevant regions of the atria of pigs with paroxysmal, persistent and long-standing persistent AF. Such differences provide information essential for the understanding of AF maintenance and patterns of electrical activation observed during the arrhythmia.

Sample of ventricular tachycardia reentry patterns on one patient-derived model with dilated pathology with high gray zone. Loose anchoring around regions of high gray zone causes the focal activation to shift throughout simulation. Beginning on the inferior wall, the main spiral shifts further along the lateral wall as simulation progresses.
Upper panel. VF tracing with superimposed 3-s sliding windows that were used to obtain spectral components from the DC shock up to the available signal length. The VF spectrum was obtained for each segment and the spectral components were estimated. Bottom panel. Sequential spectral components along 3-s segments (‘t’ seconds prior to the DC shock) in a patient with predicted and observed favorable neurological performance using a spectral based predictive algorithm.

MAJOR GRANTS

- Eugenio Rodríguez Pascual Foundation.
- Spanish Society of Cardiology (Electrophysiology & Arrhythmia Division).
- Salud 2000 Foundation.
- Spanish Society of Cardiology (General Division – not CNIC)

Selected Publications


Atherothrombosis and cardiovascular epidemiology

RESEARCH INTEREST

We are a multidisciplinary group pursuing highly innovative research that covers the major risk factors for CVD, including diet, exercise, genetics and epigenetics, metabolic factors, the environment, and psychosocial factors. We are also developing expertise in the analysis of high-throughput data and in the evaluation of novel and established cardiovascular risk factors in studies of populations with subclinical measures of atherosclerosis. Through these approaches and by conducting and coordinating various high-quality and high-impact research studies both in primary and secondary prevention, we are making significant contributions to the understanding and control of the current epidemic of CVD.

The CNIC’s major population studies include PESA (Progression of Early Subclinical Atherosclerosis), AWHS (Aragon Workers Health Study), TANSNIP (Trans-Atlantic Network to Study Stepwise Noninvasive Imaging as a Tool for Cardiovascular Prognosis and Prevention), IMJOVEN, and the Fuster-CNIC-Ferrer Polypill project. The polypill study was finalized in 2014 and showed that the polypill strategy met the primary endpoint for adherence to secondary prevention following an AMI—increased self-reported and directly measured medication.

We are also developing educational programs from early in life to adulthood to promote healthy habits for cardiovascular prevention. The Program SI! (in collaboration with the SHE Foundation) works with children from the ages of 3 to 16 and their proximal environment (family and school) with the aim of instilling appropriate lifestyle behaviors for CVD prevention. The 50/50 Project (in collaboration with the Spanish Observatory of Nutrition and the Study of Obesity) works with adults on peer-to-peer motivation to improve physical activity, healthy diet, smoking cessation, self-controlled blood pressure, and weight management.

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

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- Instituto de Salud Carlos III (P110/0021). PI: M Laclaustra
- Instituto de Salud Carlos III (PI11/00403). PI: JL Peñalvo
- Departamento de Salud y Consumo of the regional government of Aragon, General Motors Spain and CNIC (AWHS).
- NIH Grant (5U01 HL-114200-03). PI: Fuster V.
- AHA Grant (14SFRN20490315). PI: Fuster V.
- Astra Zeneca. (ISSBR110246/ D5130L00081) PI: Fuster V.

Selected Publications


TRANSLATIONAL PROJECTS

AWHS
PESA, Grupo Santander and Fundación Botín
Polypill/CNIC-Ferrer
METOCARD-CNIC trial
TANSNIP
AWHS

The Aragon Workers Health Study (AWHS) is an ongoing project conducted in collaboration with the Instituto Aragonés de Ciencias de la Salud (IACS) and the General Motors factory in Zaragoza. The AWHS has been designed to evaluate the trajectories of traditional and emergent CVD risk factors and their association with the prevalence and progression of subclinical atherosclerosis in a population of middle-aged men and women in Spain. The study examines the development of cardiovascular disease and its risk factors by monitoring factory workers at their annual medical checkups.

The AWHS is an observational, prospective cohort study including more than 5000 participants. Recruitment began in 2009 and all workers at the factory fulfilling the inclusion criteria and willing to participate have now made their initial visit. Current planned follow-up will continue to 2018.

The initial visit consisted of a clinical examination, biochemical and hematologic tests and sample collection. Sample aliquots of serum, plasma, whole blood, DNA, and urine have been frozen and stored. All laboratory procedures conform to the ISO9001:2008 quality standard. After inclusion, workers’ health data and biochemistry tests are collected at each annual health check-up.

In 2011, a screen was begun to detect subclinical atherosclerosis among 40-54-year-old participants, based on vascular 2D and 3D ultrasound in carotid, aorta and ilio-femoral arteries and on measurement of coronary artery calcification by computed tomography (CT). At the end of 2014, more than 2500 participants had been studied and the screen was concluded.

In 2012, the study’s general methods were published* in an open access journal to support a more focused future publication of the ongoing research subprojects and to provide a clear description of the study to support fund-attracting strategies.


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

- Insulin resistance and inflammatory response to oxidative stress: Study of determinants and interactions (ISCIII CP08/112)
- Identification of the genetic determinants of mitochondrial DNA content in a working population, and its relationship with oxidative stress and subclinical atherosclerosis (ISCIII PI10/21)
- Cadmium exposure, metallothionein levels, and kidney disease in a General Motors assembly plant (Johns Hopkins NIOSH Education and Research Center Research Project Award)
- DNA methylation and the association of cadmium exposure with chronic kidney disease in a population-based occupational study (Johns Hopkins NIEHS Center in Urban Environmental Health Award)
- Polymorphism APOA2 -265T>C in relation to dietary patterns and cardiovascular risk factors (ISCIII PI11/403)

Selected Publications


## AWHS

Prevalence of subclinical atherosclerosis in the initial 587 AWHS participants completing all imaging procedures

<table>
<thead>
<tr>
<th></th>
<th>Age 40 - 50</th>
<th>Age 50 - 56</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>47.0 (2.5)</td>
<td>52.9 (2.5)</td>
<td>50.0 (3.6)</td>
</tr>
<tr>
<td>Carotid plaque</td>
<td>46 (18.5)</td>
<td>194 (35.7)</td>
<td>240 (30.3)</td>
</tr>
<tr>
<td>Femoral plaque</td>
<td>101 (39.6)</td>
<td>317 (56.0)</td>
<td>418 (50.9)</td>
</tr>
<tr>
<td>Coronary calcium</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Agatston score &gt;1 to 100</td>
<td>48 (20.0)</td>
<td>159 (30.5)</td>
<td>207 (27.2)</td>
</tr>
<tr>
<td>Agatston score &gt;100</td>
<td>8 (3.3)</td>
<td>59 (11.3)</td>
<td>67 (8.8)</td>
</tr>
</tbody>
</table>

From Casasnovas et al. BMC Cardiovascular Disorders 2012 12: 45
Strategies to identify individuals with subclinical alterations indicating increased risk of cardiovascular events have been boosted by the development of basic noninvasive imaging techniques (2D/3D vascular ultrasound and coronary calcium score by computed tomography) and advanced imaging techniques (magnetic resonance imaging and positron emission 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. Most studies to date have examined populations composed of individuals above the age of 60 years. Atherosclerotic disease in this group has already several decades of evolution and may be too advanced for prevention of future events.

The PESA CNIC-Grupo Santander and Fundación Botín is an ambitious clinical trial designed to identify new imaging and biological factors associated with the presence and progression of early phases of atherosclerosis. PESA has recently completed the prospective enrolment of 4184 healthy subjects aged 40 to 54 years (2635 men and 1549 women) who have undergone a multi-territory screening for subclinical atherosclerosis by noninvasive 2D/3D ultrasound in the carotid, abdominal aorta and ilio-femoral arteries (Figure 1), together with coronary artery calcium score by computed tomography (Figure 2). Participants have additionally been assessed for a complete set of cardiovascular risk factors (including lifestyle and psychosocial factors) and have provided blood samples for advanced “omics” and future biobanking analyses. In addition, advanced imaging assessment by18FDG PET/MRI technology was performed at the CNIC Advanced Imaging Unit during 2013 and 2014 in 938 individuals in whom a significant plaque burden was detected by ultrasound and CT. The study has also received approval for research into the association between atherosclerosis initiation/progression and telomere dysfunction and progerin expression in circulating leukocytes, and leukocyte samples have been collected from a subgroup of 1456 PESA participants for this analysis.

All PESA participants are followed-up at 3 and 6 years to assess the evolution of early phases of atherosclerosis and to determine how the detection of subclinical disease may impact the risk of future cardiovascular events. By the end of 2014, more than 1500 participants had already undergone the 3-year follow up visit (visit 2).

In May 2014 the PESA CNIC-Santander project received renewed ISO 9001:2008 certification from Bureau Veritas. This certification gives external recognition of the management quality and of data control and traceability.

In September 2014, the analysis and initial results from the baseline imaging studies were concluded and the article entitled “Prevalence, Vascular Distribution and Multi-territorial Extent of Subclinical Atherosclerosis in a Middle-Aged Population: The PESA (Progression of Early Subclinical Atherosclerosis) Study” was submitted for publication.

Novel 3D vascular ultrasound technology used in the PESA study to detect atherosclerotic plaques in the carotid artery, allowing volumetric quantification of plaque burden.

Non-contrast computed tomography images, showing coronary artery calcification in (A) the left anterior descending artery, (B) the left circumflex artery, and (C) the right coronary artery in a PESA participant.
Polypill/CNIC-Ferrer

FOCUS Project

The Focus Project was supported by a grant from the Seventh European Framework program. Finalized in 2014, the results of the FOCUS Project were presented by Dr. Fuster at the European Society of Cardiology meeting in Barcelona and published in the Journal of the American College of Cardiology.

The FOCUS (Fixed Dose Combination Drug for Secondary Cardiovascular Prevention) project consisted of a cross-sectional study (phase 1) aimed at elucidating the factors that interfere with appropriate adherence to cardiovascular medication for secondary prevention after an acute myocardial infarction (AMI). Additionally, 695 patients from phase 1 were randomized into a controlled clinical trial (phase 2) to test the effect of a polypill (containing 100 mg aspirin, 40mg simvastatin, and 2.5, 5 or 10 mg ramipril) compared to the three drugs given separately. Tested outcomes were treatment adherence, blood pressure (BP) and serum low density lipoprotein cholesterol (LDL-C), as well as safety and tolerability over a 9-month follow-up.

In phase 1, a 5-country cohort (Argentina, Brazil, Italy, Paraguay, and Spain) of 2118 patients was analyzed. Patients were randomized to either the polypill or the three drugs separately for phase 2. The primary endpoint was treatment adherence measured at the final visit by the self-reported Morisky-Green questionnaire (MAQ) and pill count (patients had to meet both criteria for adherence at the in-person visit in order to be considered adherent).

In phase 1, overall CV medication adherence, defined as a MAQ score of ≥20, was 45.5%. In a multivariable regression model, the risk of being non-adherent (MAQ<20) was associated with younger age, depression, being on a complex medication regimen, poorer health insurance coverage, and a lower level of social support, with consistent findings across countries.

In phase 2, the polypill group showed improved adherence compared to the group receiving separate medications after 9 months follow-up: 50.8% vs 41% (p=0.019; intention-to-treat population) and 65.7% vs 55.7% (p=0.012; per protocol population) for the primary endpoint (attending the final visit with MAQ=20 and an 80-110% pill count) combined, to assess adherence. Adherence was also higher in the FDC group when measured by MAQ alone (68% vs. 59%, p=0.049). No treatment difference was found at follow-up in mean SBP (129.6 vs 128.6 mmHg), mean LDL-C levels (89.9 vs 91.7 mg/dL), serious adverse events (23 [6.6%] vs. 21 [6%]) or death (1, 0.2% in each group).

In conclusion, for secondary prevention following an AMI, younger age, being depressed and following a complex drug treatment are associated with lower medication adherence, while adherence is higher in patients with higher levels of insurance cover and social support. Compared with the three drugs given separately, the use of a polypill strategy met the primary endpoint for adherence—increased self-reported and directly measured medication—for secondary prevention following an AMI.
Polypill/CNIC-Ferrer

AETNA/CNIC/Mt. Sinai/ Ferrer Consortium to study the clinical and economic impact of non-adherence to cardiovascular medication in a US population.

In October 2013, Aetna partnered with the CNIC among others to carry out a series of retrospective analyses using the Aetna Database to study the effect on major adverse cardiovascular outcomes on a post MI population of more than 4000 patients. The results of this study were presented at the Registry Hotline at the European Society of Cardiology in Barcelona. A manuscript based on this study will be submitted for review and publication in January 2015.

The same study was carried out in a diabetic population. The results of this study have been submitted for a presentation at the next American College of Cardiology meeting in March 2015.

A third study with a similar outline has been carried out, but in an atherosclerotic vascular disease population (comprised of participants who have had MI, stroke or peripheral artery disease). The results of this study are being analyzed and a manuscript will be submitted in January 2015.

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Selected Publications


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

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.

ß-blockers are a class of drugs that have been used to treat cardiovascular conditions for several decades. ß-blockers reduce mortality when administered after an AMI, and are a class IA indication in this context. There is a lack of information on the infarct-limiting effect of ß-blockers in patients undergoing reperfusion (current state-of-the-art treatment for infarction). Based on strong pre-clinical data, CNIC lead the METOCARD-CNIC trial, the first randomized trial testing the effect of i.v. ß-blockers on infarct size in patients undergoing primary angioplasty.

METOCARD-CNIC was a multicenter randomized clinical trial comparing the effect of early and delayed metoprolol initiation on infarct size and clinical events. The trial has already been completed, with a total of 270 patients recruited by the emergency medical services (55%) and participating hospitals (45%). A total of 220 patients underwent a magnetic resonance imaging (MRI) scan five days after infarction, and 202 patients underwent follow-up MRI at six months. All patients underwent one year clinical follow-up. Studies of patients recruited in Madrid were performed at the CNIC’s human imaging facility, where the advanced imaging protocol is performed with a novel cutting-edge MRI system. MRI scan data were analyzed in a core laboratory at the CNIC.

The primary endpoint of the trial (infarct size as evaluated by MRI) was published in 2013 (Circulation 2013;128:1495-503), and the follow-up of the study was published in 2014 (J Am Coll Cardiol. 2014;63:2356-62). In summary, the administration of early metoprolol before reperfusion resulted in a very significant reduction of infarct size (see Figure). This effect was seen along with a significant increase in left ventricular ejection fraction. Follow-up MRI studies revealed that patients receiving metoprolol had a significant reduction in the incidence of severe heart dysfunction, along with a significant reduction in hospital readmission due to heart failure.

METOCARD-CNIC is the result of a multidisciplinary effort requiring close cooperation between investigators at the CNIC, hospitals across Spain, and, importantly, the emergency medical services. Hospitals participating in the METOCARD-CNIC trial are Hospital Clínico San Carlos, Puerta de Hierro, Hospital de la Princesa, Hospital 12 de Octubre and Hospital Quirón in Madrid, Hospital Meixoeiro in Vigo, Hospital Marqués de Valdecilla in Santander, and Hospital de León. Emergency medical services actively participating as co-investigators are SUMMA112, 061 Galicia, and SAMUR. The randomization center was located in the headquarters of SUMMA112 and was run 24/7 by trained full time staff.

CNIC is already working in the design of a multinational clinical trial, the MOVE ON! Trial. This trial will follow the same design of METOCARD-CNIC but will be powered to detect differences in clinical endpoints (mortality, heart failure, and arrhythmias). The MOVE ON! Trial will be conducted in several European countries.

Follow-up LVEF categories and indications for ICD according to treatment allocation

(A) Distribution of patients according to LVEF categories. B) Rate of formal indication (Class I recommendation in clinical guidelines) for an implantable cardioverter defibrillator. (from J Am Coll Cardiol 2014;63:2356-62: METOCARD-CNIC follow-up publication.)
METOCARD-CNIC trial

Members of the METOCARD-CNIC research group.

Selected Publications


TRANSLATIONAL PROJECTS

TAN SNIP: Trans-Atlantic Network to Study Stepwise Noninvasive Imaging as a Tool for Cardiovascular Prognosis and Prevention

The TAN SNIP study includes 4 projects whose unifying purpose is to develop a model for improved risk stratification based on the detection, quantification and characterization of subclinical atherosclerosis. The goal is for this improved risk stratification to enable novel targeted therapies and risk reduction strategies. This transatlantic network brings together expertise and resources, aligning (a) leaders from complementary fields (imaging, biomarkers, and population sciences), (b) existing patient cohorts, (c) state-of-the-art imaging resources and know-how, and (d) sophisticated biomarker platforms.

PURPOSE AND AIMS

Existing tools for characterizing atherosclerosis and determining the risk of its complications are inadequate. These deficiencies limit effective management across the spectrum of this common disease. Consequently, opportunities for early, cost-effective interventions in subclinical disease are missed, while treatments for high-risk populations with manifest disease are administered almost indiscriminately. This leads to high ‘numbers-needed-to-treat’ (NNT), unnecessary patient risk, wasted resources and unsustainable costs for health care providers.

AIM 1. In a relatively low-risk population (the PESA-CNIC cohort), we will study whether a personalized worksite-based lifestyle intervention, driven by imaging data (3D-ultrasound of carotid and ilio-femoral arteries, and coronary calcification) can engender behavioural changes, improved control of risk factors, and reduced progression of subclinical atherosclerotic plaque burden (SAPB).

Output: Non-pharmacological worksite lifestyle intervention validated against modification of conventional risk factors.

AIM 2. We will evaluate the predictive value of the Framingham risk score (FRS) and SAPB on the prevalence of microvascular/parenchymal brain changes (MPBC) and the incidence of cerebrovascular disease (CD) and cerebrovascular events (CVE) in asymptomatic individuals with a varying extent of CV risk factors and SAPB. This study will be carried out at the Icahn School of Medicine at Mount Sinai (ISMMS), New York. We will quantify SAPB by 3D-US of the carotid arteries (CA) and ilio-femoral arteries (IFA) in participants with no cardiovascular symptoms. MPBC will be detected through a combination of 1) MRI to assess microvascular perfusion and parenchymal changes in structural and functional brain connectivity and 2) 18F flumetamol/florbetapir-PET to quantify parenchymal amyloid deposition. CD will be assessed by standard neurophysiological tests.

Output: Understanding, achieved through cutting-edge imaging analysis, of the complex interaction between FRS, SAPB, MPBC and CD.

AIM 3. In an intermediate-risk population (the HRP-USA population) we will validate the added value of SAPB quantification (vascular 3-D ultrasound of carotid and ilio-femoral arteries) on top of classical risk factors (FRS) for predicting SAPB progression/regression and CVD events (CVDE).

Output: A cost-effective method to determine and monitor SAPB and determine its incremental value over FRS, and to increase understanding of the natural history of SAPB over a 5-year period.

AIM 4. In the 3 complementary cohorts detailed above, we will validate recent proteomic and metabolomic discoveries related to major atherosclerotic CVDE and the metabolic risk factors for CVD identified by the Framingham Heart Study (FHS). This will allow the development of improved CVD risk assessment methods that incorporate cutting-edge ‘omics’ discoveries made by the FHS as well as state-of-the-art imaging results from the TAN SNIP network in conjunction with established CVD risk factors.

Outputs: Validation of FHS-derived novel biomarker panels (‘omics’) in 3 populations at graded cardiovascular risk. Novel algorithms that incorporate ‘omics’, SAPB, and established FRS criteria to improve personalized risk assessment.
TAN SNIP: Trans-Atlantic Network to Study Stepwise Noninvasive Imaging as a Tool for Cardiovascular Prognosis and Prevention

AIM 1 (the PESA-CNIC cohort):

The study population for this part of the TAN SNIP study consists of participants in the PESA study: employees aged 40 to 54 years of the Banco de Santander Headquarters in Madrid (Spain). Two parallel randomized controlled trials (RCT) will be conducted within the PESA cohort population. One RCT will focus on a sample of employees with high imaging-defined CV risk, whereas the second RCT will be conducted on a sample with low imaging-defined CV risk. In both RCTs, the participants will be randomized to receive a comprehensive 3-year worksite lifestyle intervention or standard occupational health care. The worksite-based lifestyle intervention program will consist of three elements: (A) twelve 1-hour sessions of personalized lifestyle counseling ; (B) provision of a pedometer for self-monitoring of physical activity (Polar) ; and (C) use of a sit-to-stand workstation (optional). Data will be collected at baseline (concurrent with PESA Visit 1) and at follow-up at 1 year (T1), 2 years (T2), and 3 years (T3; concurrent with PESA Visit 3). Primary outcome measures are CVD risk assessment and MVPA. Secondary outcomes are physical activity, sedentary behavior, standing behavior, diet, smoking, vitality and quality of life and risk factor profiles, as well as specific changes in anthropometric measures, blood biomarkers, work-related outcomes (including work productivity and sickness absenteeism). A process evaluation and a cost-effectiveness study will be conducted during the intervention.

We predict that individual awareness of CVD risk stratification based on subclinical atherosclerosis imaging, accompanied by a comprehensive 3-year worksite-based lifestyle intervention, will lead to a reduction in the prevalence of CV risk factors related to lifestyle and an increase in MVPA, compared to standard practice. We further predict that the level of compliance with the worksite-based lifestyle intervention will be higher in the high imaging-defined CV risk group than in the low imaging-defined CV risk group.
Technology Transfer & Translational Research Platform

The Technology Transfer & Translational Research Platform runs initiatives that foster translational research at the CNIC, in Spanish clinical facilities, and with international partners. The Platform also identifies, promotes, and co-develops CNIC research with potential for industrial application by facilitating the granting of patents and their subsequent development or licensing.

The activity of the Technology Transfer & Translational Research Platform is divided into the following areas: Technology Development Unit, Technology Transfer Office, Projects Office, Biobank, and Translational Research & Epidemiology.
Advanced Imaging
Bioinformatics
Cellomics
Comparative Medicine
Genomics
Microscopy and Dynamic Imaging
Pluripotent Cell Technology
Proteomics
Transgenesis
Viral Vectors
Intravenous injection of dual (PET-MRI) single core nanoparticles. $^{68}$Ga was inserted into the iron oxide core of nanoparticles, which were intravenously injected into a rabbit.
MAJOR GRANTS

- Ministerio de Sanidad y Consumo (CIBERES CB06/06/1090)
- European Commission FP7-PEOPLE-2010-ITN (ITN-NET 264864) (NO CNIC)
- European Commission FP7-PEOPLE-2013-ITN (CardioNext PITN-GA-2013-608027)
- Fundación La Marató TV3 (70/C/2012) PI WP2, Borja Ibañez, Colaborador Jesus Ruiz Cabello
- Ministry of Economy and Competitiveness. FIS RETICS (Terapia Celular: RD12/001/0005) PI: Miguel Torres, Colaborador Jesus Ruiz Cabello
- Ministry of Economy and Competitiveness. Modalidad Generación Conocimiento (MAT2013-47303-P) PI: Fernando Herranz
- Ministry of Economy and Competitiveness. FIS (PI14/01427) PI: Jesús Mateo
- Madrid-MIT M+Visión (PRMIT2013) PI: Samuel España
- Madrid-MIT M+Visión (MIT14) PI: Teresa Arias

Selected Publications


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

In the last year, the Bioinformatics Unit has established four new pipelines using state-of-the-art algorithms for the analysis and interpretation of high-throughput biological data: 4C-Seq; alternative splicing detection from RNA-Seq data; expression profiling plus GO Enrichment and GSEA from RNA-Seq data; and SNP detection with STAR-GATK from RNA-Seq data.

To accommodate increasing demand, the Unit’s infrastructure grew in 2014 with the installation of a new 80 TB HPC storage EMCisilon Solution™ and, starting at the beginning of 2015, installed HPC clusters are being upgraded from 246 threads and 700 GB of RAM to 1736 threads and 5TB of RAM. Another main focus during 2014 and into the New Year has been the implementation of the Bioinformatics Unit Galaxy Platform. This platform enables CNIC researchers to analyze their data using the pipelines developed by the Bioinformatics Unit through Galaxy, bringing them closer to their data.

The Unit has also continued to provide customized advice and training to CNIC researchers in the analysis and interpretation of their experimental data. We also participated in the doctoral training at the Medical University of Białystok in Poland.

The Unit is closely involved in providing support and guidance to new PhD students working on bioinformatics projects at the CNIC, and co-directs 3 PhD projects.

**RESEARCH INTEREST**

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**Support Scientists:**
- Fátima Sánchez Cabo
- Carlos Torroja
- Manuel José Gómez Rodríguez

**Predoctoral Researchers:**
- Wencke Walter
  (from the Nuclear Receptor Signaling Laboratory led by Mercedes Ricote)
- Alberto Gatto
  (from the Molecular Regulation of Heart Development and Disease Laboratory led by Enrique Lara-Pezzi)
- Girolamo Giudice
  (from the Molecular Regulation of Heart Development and Disease Laboratory led by Enrique Lara-Pezzi)

**Head of Unit**
Fernando Martínez

**Bioinformatics Unit HPC:**
Summary of total CPU hours (number of CPU cores x wall time hours, excluding non-Bioinformatics Unit usage of the resource) from Jan 1 to Dec 1, 2014.

**Bioinformatics Unit Jobs:**
Summarizes the total number of running Bioinformatics Unit jobs (excludes non-Bioinformatics Unit usage of the resource) from Jan 1 to Dec 1, 2014.

Cellomics

RESEARCH INTEREST

The Cellomics Unit provides the CNIC with the two principal cell analytical techniques, flow cytometry and high content screening (HCS), and supports quantitative image-based research. The Unit has expanded its sorting capabilities with the acquisition of an automated magnetic sorter (Automacs). Daniel Jiménez, a young mathematician, computer and biomedical engineer with PhD training in biomedical image processing and analysis from UPM Madrid and BWH Boston, joined Cellomics Unit in November, replacing Gopal Karemore.

In 2014 we successfully completed a drug repurposing screen to identify FDA-approved drugs that regulate Cav1 expression in pancreatic cancer associated fibroblasts, in partnership with the Integrin Signaling group at the CNIC and the Clinical Research and Experimental Therapeutics Programs at the CNIO. A secondary/validation screen is currently in progress. The Unit has also developed an automated multiparametric assay for toxicity assessment (Fig. 1) that has been used for in vitro toxicity testing of nanoparticles in partnership with the Advanced Imaging Unit. Our Unit has also programmed a script for automatic scanning of high resolution Z stacks of zebrafish embryo hearts using the Opera imaging device. This work formed part of the development of an HCS assay in collaboration with the Development of the Epicardium and its Role during Regeneration group at the CNIC (Fig. 2). The Unit has also developed customized image analysis tools for a variety of purposes, for example quantification of tumor cell invasion in 3D matrices (Fig 3), detection of candida-infected cells and proliferating cells from immunofluorescence tissue images, and quantification of second harmonic microscopy images.

In vitro toxicity assay. EA.hy926 cells were treated with 0.5 mM taxol or staurosporine or left untreated (control). A) DAPI stain. B) Staining with a fluorescent substrate for activated caspases 3 and 7. C) Mitotracker. D) Cell Rox. The figure shows four images per field obtained from duplicate wells using the Opera automated imaging device.
Prescan-rescan script for automatic acquisition of high resolution 3D images from zebrafish embryos. Software was developed to allow the Opera System to automatically detect heart locations on 2D low resolution (4x) images and acquire 3D high resolution (20x) images from multiple zebrafish embryos on multiwell plates. Images show staining with fluorescent markers of epicardium (green) and myocardium (red) and nuclear DAPI (blue) as overlaid images obtained at the magnifications indicated.

Analysis of tumor cell invasion through 3D matrices. Tumor cells were loaded onto 3D collagen matrices and allowed to invade for 5 days, then fixed and stained with DAPI. A) DAPI staining raw image. B) Nuclear segmentation. C) Top layer estimation from spatial sampling and cubic interpolation of the maximum Z coordinate values of the nuclei centroids. D) Top layer and overlaid cell nuclei segmentation. E) Sub-pixel cell-by-cell Z penetration calculation (distance to top layer in microns). F) Classification of cells as non invaders (brown) and invaders (colors).

Selected Publications


Comparative Medicine

The Unit develops and manages laboratory animal models to reproduce the principal human cardiovascular diseases, working closely with the CNIC research teams and applying the 3 Rs. The Unit tries to refine these animal models by identifying factors that could interfere with research project aims, be source of non-representative data, or have a major impact of the animal welfare.

The Comparative Medicine Unit’s support for in vivo work at the CNIC is organized into five core work areas.

- **Animal Husbandry.** The Unit’s technicians, managers and veterinarians are trained to work under the facility’s SPF conditions and take charge of the daily husbandry of the animal colonies. The Unit enacts an environmental enrichment program to support species-specific behaviors to maximize animal welfare and wellbeing.

- **Pathology Core (PC).** The Histopathology Laboratory provides specialized hispathological services including animal necropsy, paraffin and OCT processing and sectioning, histochemical and immunohistochemical staining of tissue sections, digital scanning and image analysis, optical projection tomography with an OPT scanner 3001 and general support to CNIC researchers with phenotyping and histopathological evaluation of their animal models.

- **Phenotyping Core (PhC).** In this area, we have added new equipment to meet the needs of the CNIC research groups, including a coagulation analyzer and a metabolic cages system.

- **Veterinary Medicine and Experimental Surgery Core (VMESC).** The VMESC provides highly specialized expertise in the surveillance and monitoring of animal health status, disease follow-up, development of surgical animals models with emphasis on minimally invasive procedures, life support, and setting up of new experimental strategies that reproduce human cardiovascular diseases or acquisition of pathophysiological data. The VMESC team is run by two clinical veterinarians with extensive expertise in laboratory animal science and four veterinary specialist technicians.

- **Quality Control Core (QCC).** The QCC follows the recommendation the last FELASA report (Laboratory Animals 2014, Vol. 48(3): 178-192).

The Comparative Medicine Unit maintains ISO 9001 accreditation for all five core work areas.

1. Thoracic section of a 16.5 day mouse embryo, stained with H&E.
2. Section of a zebrafish whole-body stained with Acid Fuchsin Orange G.
3. Hamamatsu scanner for histological sample digitalization.
4. Immunostaining of the CD31 marker to highlight the newly formed vessels in the granulation tissue of a pig myocardial infarct.
**Genomics**

### RESEARCH INTEREST

During the last 4 years, the Genomics Unit has focused on second generation sequencing (NGS) technologies for genome analysis using an Illumina Genome Analyzer IIx sequencer. In 2014 the Unit expanded its sequencing capacity by acquiring a new Illumina HiSeq 2500 sequencer. In addition to its larger production power, the HiSeq 2500 delivers a lower cost per sample across most of the applications.

The Unit provides these cutting-edge genomic technologies to the scientific community at the CNIC and beyond, offering a wide variety of NGS applications (RNA Seq, Low input RNA Seq, small RNA-Seq, ChiP Seq, PCR Seq, Exome Sequencing, targeted resequencing, etc). On each sequencing project the Unit’s tasks include project consultation, sample quality check, sample library preparation, and data generation. At least 3 of the CNIC top scientific publications in 2014 include NGS experiments performed in the Genomics Unit.

The team is also very much committed to continuing to improve methods for low-input RNA Seq applications, in which RNA Seq can be performed from tiny amounts of starting biological material or even directly from cells.

We are also automating the newly incorporated NGS library preparation protocols by using an open liquid handling platform. Automation of this step avoids the bottleneck created by the high sample number typically included in sequencing runs, and also reduces the risk of human error during this step.

On request, the Unit continues to offer microarray analysis services using the Agilent microarray platform. Other services include DNA fragmentation using a Covaris E220 ultrasonicator, the maintenance and management of real-time PCR instruments (one AB 7000 and two ABI 7900HT machines) and a TaqMan array processing service.

In addition to providing these high-quality genomic services, the Unit performs its own research.

### MAJOR GRANTS

- Ministerio de Economía y Competitividad. FIS (PI10/01124)

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**Selected Publications**


RESEARCH INTEREST

2014 saw a major technological development with the installation of a nanoscopy platform and the acquisition of a single plane illumination microscopy (SPIM) system. The nanoscopy platform includes an innovative gated STED-3X microscope and a system for single molecule localization by ground state depletion (dSTORM). The platform allows a comprehensive approach to super-resolution imaging using a variety of fluorescent probes, ranging from spectral variants of the fluorescent protein to standard chemical dyes. The customized SPIM system joins the state-of-art confocal and multiphoton microscopes already in use, completing a large portfolio of imaging modalities capable of imaging individual biomolecules in cells as well as sub-micron compartments in living model organisms. To maximize the performance of this portfolio, we have also dedicated considerable effort to developing software packages and protocols suitable for handling and analyzing large 3D-image files.

The considerable technological expansion of the Unit has laid the groundwork for new collaborative research projects with internal and external partners that have been granted funding by national and international funding agencies. For example, in a project in partnership with the Institute of General Organic Chemistry (CSIC) and the San Raffaelele Foundation in Milan, the Unit is designing novel fluorophores for increased performance in super-resolution microscopy to reveal the sub-structure of IRE1 proteins rearranged into composite domains in the ER membrane as sensor of cellular stress.

The Unit continues its intensive individual theoretical and practical training activities and workshops, and contributes to the CNIC-JOVEN training plan through the ACERCATE, CICERONE and the Master Programs.

IRE1 domains in the endoplasmic reticulum of HeLa cells during the unfolded protein response. (A) Confocal image (top) and STED image (bottom). (B) single domain zoom in confocal (top) and STED (bottom) resolution. (C) 3D-STED gallery of IRE1 domains in a whole cell.

MAJOR GRANTS

- INFRA-MINECO-2013 - Plataforma Biomédica Avanzada CNIC en Nanoscopía multimodal

Selected Publications


Pluripotent Cell Technology

RESEARCH INTEREST

The Pluripotent Stem Cell Technology Unit provides knowledge, training, and state-of-the-art technological support with the culture and manipulation of mouse and human pluripotent stem cells. A suitable working environment for work with stem cells is provided by two supervised culture rooms, one devoted to human stem cells and the other to mouse embryonic stem cells (mESCs). In 2014, our staff continued to facilitate gene-targeting experiments by supply genetically modified mESCs under tight quality-control, an essential requirement for germline transmission and the generation of mutant mice. Taking charge of all key steps in the gene targeting protocol (electroporation of the targeting vector, screening, karyotyping, culture and the preparation of cells for blastocyst injection) the Unit contributed to the generation of six new mouse mutant lines in 2014. The Unit also applied its wide expertise in genetic modification to the generation of mouse stem cells modified using the CRISPR/cas system. The Unit supplies knockout stem cell lines for the development of research projects. On request, we can also assist CNIC researchers in fine-tuning differentiation protocols for a specific lineage.

Human induced pluripotent stem cells (hiPSC) are an extraordinarily valuable source of cells for basic and translational research, including drug development and disease modeling. The PCT Unit also dedicates effort to the transgene-free reprogramming of somatic cells obtained from cell banks. Several hiPSC lines have already been derived, characterized and banked. We also establish differentiation programs to specific lineages and provide the latest cutting-edge technology for genome editing.

Generation of mouse reporter line. A) Diagram showing the 5’ wild-type (wt) region of the gene involved in homologous recombination, the targeting vector and the screening strategy designed to detect recombination events. B) Detection of targeted clones by Southern blot.

iMARIS reconstruction of the direct differentiation of an embryoid body toward the cardiac lineage. Expression of fluorescent EGFP (green) is under the control of the cardiac TroponinT promoter.

Selected Publications


Phosphatidylcholine-coated iron oxide nanomicelles for in vivo prolonged circulation time with an antibiofouling protein corona. (Top) Production of phosphatidylcholine-coated superparamagnetic iron oxide nanoparticles (PC SPION) nanomicelles. (Bottom) Relative % weight of proteins with known antibiofouling properties (dysopsonins) and biofouling properties (opsonins) classified by their biological function in the coronas of micellar PC and P80 SPION incubated in vitro in rat serum. Modified from Groult et al. 2014.

Proteomics

RESEARCH INTEREST

The Proteomics Unit is dedicated to technological innovation and the development of new applications of interest to the research community. The Unit has been working on improvements to the separation and quantitative analysis of protein expression by shotgun proteomics using high-throughput technologies based on nanoHPLC coupled to mass spectrometry. The Proteomics Unit houses several nano-HPLC systems coupled to state-of-art mass spectrometers for ultra-deep proteome analysis. During 2014 substantial progress was made in quantitative proteomics approaches, mainly using stable isobaric labeling (ITRAQ and TMT). Particular improvements were made in the development of the chromatographic conditions for peptide separation, optimization of fragmentation parameters, statistical analysis of quantitative data, and systems biology interpretation of results using programs developed in house.

These approaches are being extended to the quantitative analysis of oxidative post-translational modifications, an area of paramount interest in the cardiovascular field, and for biomarker discovery in the clinical setting to the analysis of dozens of plasma samples, using depletion protocols of the most-abundant proteins.

One of the most interesting developments is a data-independent scanning acquisition mode that mixes targeted and shotgun approaches, based on signal-independent fragmentation. This novel approach is being explored in selected subproteomes to increase the coverage and number of identified peptides, allowing us to conduct post-acquisition targeted in-silico analysis of selected peptide sequences.

This robust analytical platform, together with our recognized experience in the field, enables us to manage large research projects that require qualitative and quantitative proteomic approaches to measure differential protein expression, characterize chemical and posttranslational modifications, and map protein-protein interactions in different biological systems.
LONP1 controls tumor bioenergetics by remodeling OXPHOS subunits. (Top) Heatmap showing protein abundance changes in LON and shLon cells in Complex I and Complex V subunits of enriched mitochondrial preparations, obtained with high-throughput iTRAQ quantitative proteomics. The relative abundance changes are expressed using the Z score in relation to each control. (Bottom) Model summarizing the functional relevance of Lon protease in reprogramming mitochondrial activity in cancer. Modified from Quirós et al. 2014.
Transgenesis

RESEARCH INTEREST

The Unit's work with mice (Mus musculus) is divided into three areas: rederivation of mouse strains, production of genetically modified mice, and assisted reproductive techniques (ART). Rederivation to establish colonies in the SPF zone of the animal facility is done by embryo transfer. Genetically modified mouse strains (transgenic mice) are produced using well established standard techniques, mainly microinjection of DNA and/or RNA into the zygote pronuclei or cytoplasm, or microinjection of embryonic stem cells (ESC) into 8-cell or blastocyst-stage mouse embryos. For assisted reproduction, valuable mouse strains are cryopreserved by freezing sperm and embryos, and other ARTs used include in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI).

Another important vertebrate model organism used in scientific research is the zebrafish (Danio rerio). The Unit cryopreserves sperm from this species and performs in vitro fertilization using fresh and frozen sperm.

In 2014, following the general trend toward production of transgenic animals by gene edition using engineered nucleases, the unit succeeded in the production of genetically modified mice using zinc finger nucleases (ZFN) and also the clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 system.

The Unit collaborates with several CNIC groups on specific aspects of their research programs, and Unit members participate in the CNIC’s training plans by providing theoretical and practical sessions.

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**MOUSE**

- Rederivation of mouse strains
  - Embryo transfer

**Production of genetically modified mice**

- Embryonic stem cell injection of mouse zygotes
- Somatic cell transfers to mice
- Production of mice derived from aggregation of eight-cell mouse embryos
- Microinjection of mouse embryonic stem cells (ESC) into eight-cell and blastocyst-stage mouse embryos

**Assisted Reproductive Technology in mouse**

- In vitro fertilization (IVF)
- Intracytoplasmic sperm injection (ICSI)

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**ZEBRAFISH**

**Assisted Reproductive Technology in zebrafish**

- Sperm cryopreservation
- In vitro fertilization (IVF)

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**Transgenesis Unit activities**

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**Pronuclear microinjection with DNA solution for the production of transgenic mice.** Left: Pronuclear microinjection of a mouse zygote (B6CBAF2 strain) anchored with a holding needle; the injection needle containing the DNA solution is visible at the bottom of the image. Right: Expanded pronucleus after microinjection.
Blastomere disaggregation of an 8-cell mouse embryo: genetic analysis of individualized blastomeres. A single 8-cell B6CBAF2 mouse embryo (left) is chemically treated to remove its surrounding zona pellucida (middle), and is then disaggregated to separate the eight blastomeres (right). The zona pellucida is a glycoprotein layer surrounding the cytoplasmic membrane of preimplantation mammalian embryos.

Production of a heteroplasmic mouse strain. One NZB cytoplast—the cytoplasm portion without nuclear genetic material—has been introduced by micromanipulation into the perivitelline space of a C57BL/6JOlaHsd zygote with two pronuclei. After an electrofusion process, cytoplast and zygote will fuse to produce a heteroplasmic zygote bearing C57BL/6JOlaHsd genomic DNA (gDNA) and a mix of mitochondrial DNAs (mtDNAs) from C57BL/6JOlaHsd and NZB origins.
Viral Vectors

RESEARCH INTEREST

The Viral Vector Unit continues to provide the non-clinical grade recombinant viral vectors (lentivirus and AAV) to CNIC researchers and to external collaborators in Spain, accommodating most investigators’ needs regarding virally mediated gene transfer.

We have implemented specific programs aimed at developing novel approaches to gene editing and novel applications of currently available tools. First, we have developed a simple system based on integrase-deficient lentivirus (IDLV) vectors to direct the insertion of genes into the safe AAVS1 locus, mediated by the HUH-site-specific recombinase Rep78 of AAV. By packaging Rep78 in lentiviral particles together with a construct encoding the minimal recognition site for the protein to promote site-specific recombination, we have obtained integration frequencies of up to 10% at the AAVS1 safe harbor locus in human cells, with no evidence of off-target insertions. Remarkably, this hit-and-run method limits the cytotoxic effects derived from long exposure to the protein, both in the producer and the target cells. Second, we have used the popular RGEN technology (RNA-guided endonuclease), also known as the CRISPR/Cas9 system, to demonstrate that it is possible to generate cells undergoing human-cancer-specific chromosomal translocations at high efficiency. We have generated chromosomal translocations identical to those observed in Ewing’s Sarcoma (ES) and Acute Myeloid Leukemia (AML), either in a well establish cell line (HEK293) or in primary cells; human mesenchymal stem cells (hMSC) in the case of ES and hematopoietic stem cells (HSC) in the case of AML. This work was conducted in close partnership with members of the Molecular Cytogenetics group at the CNIO.

Viral Vector Figure 1. Site-Specific integration by Rep78 in AAVS1 locus.

A. Strategy for generating lentivirus containing the recombinase Rep78 and control ORFs (eGFP, cre). The heterologous ORFs were cloned between the matrix (MA) and capsid (CA) ORFs of the gag-gene in the plasmid.
B. Southern blot analysis of G418R HEK293 clones showing single copy insertion in the AAVS1 locus in clone #11 (arrowhead). The diagrams indicate the expected sizes of the neo (internal probe, gray bar) and AAVS1 probes (external probe, green bar).
Viral Vector Figure 2. Strategy for engineering chromosomal translocations using CRISPR/Cas9.

A. Translocation strategy. Double-strand breaks are introduced by the sgRNAs (arrowheads) mapping to introns in ETO (purple) and RUNX1 (green).

B. FISH analysis of chromosome 8 (red) and 21 (green), verifying reciprocal chromosomal translocation in CD34+ human hematopoietic stem cells.

MAJOR GRANTS
- Ministerio de Economía y Competitividad (PI11/02041)
APPENDIX

Publications
Training Programs and Courses
Seminars, Events and Awards
Strategic Alliances
Funding
Patent Portfolio
Staff Figures
There were 210 CNIC publications in 2014, 186 of them in JCR-listed journals with an Impact Factor (IF). Of the total publications, 68% were produced through collaboration with foreign institutions, 26% with national institutions, and 6% were authored solely by CNIC researchers.

A CNIC scientist was a main author on 59% of the publications. The average IF for all the articles was 7.386.

**Articles with an IF**

- **Andrés V.**
  *Vitamin D Puts the Brakes on Angiotensin II-Induced Oxidative Stress and Vascular Smooth Muscle Cell Senescence.*
  Atherosclerosis (2014) 236: 444-7
  IF: 3.971

  *Neuropathy of Haematopoietic Stem Cell Niche is Essential for Myeloproliferative Neoplasms.*
  IF: 42.351

- **Aslibekyan S, Dashti HS, Tanaka T, Sha J, Ferrucci L, Zhi D, Bandinelli S, Borecki IB, Ascher DM, Arnett DK and Ordovás JM.**
  *PRKZ Methylation is Associated with Sunlight Exposure in a North American but not a Mediterranean Population.*
  Chronobiol Int (2014) 31: 1034-40
  IF: 4.350

  *Plasma Membrane Calcium ATPase Isoform 4 Inhibits Vascular Endothelial Growth Factor-Mediated Angiogenesis Through Interaction With Calcineurin.*
  IF: 6.338

  *A New Non-Canonical Pathway of Galpha Protein Regulating Mitochondrial Dynamics and Bioenergetics.*
  Cell Signal (2014) 26: 1135-46
  IF: 4.304

- **Berciano S and Ordovás JM.**
  *Nutrition and Cardiovascular Health.*
  IF: 3.342

- **Bilal U, Fernández E, Beltrán P, Navas-Acien A, Bolumar F and Franco M.**
  *Validation of a Method for Reconstructing Historical Rates of Smoking Prevalence.*
  IF: 4.780

- **Bilal U, Fernández E, Navas-Acien A, Bolumar F and Franco M.**
  *Five Authors Reply.*
  IF: 4.780

  *Metabolomics Reveals Metabolite Changes in Acute Pulmonary Embolism.*
  IF: 5.001
APPENDIX  
Publications 2014

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Castellano JM, Sanz G and Fuster V.

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de Haas HJ, Narula J and Fuster V.
From Molecular Imaging to Pathogenesis and Vice Versa. Circ Cardiovasc Imaging (2014) 7: S81-S IF: 5.795

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IF: 6.982

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IF: 2.590

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Moving Beyond Coronary Stenosis: Has the Time Arrived to Address Important Physiological Questions Not Answered by Fractional Flow Reserve Alone? Circ Cardiovasc Inter (2014) 7: 282-4
IF: 6.543

Ezkurdia I, Vázquez J, Valencia A and Tress M.
IF: 5.001

Fernández-Friera L, Ibáñez B and Fuster V.
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IF: 4.909

IF: 3.342

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García-Prieto J.*, García-Ruiz JM*,
Sanz-Rosa D*, Pun A, García-Alvarez A,
Davidson SM, Fernández-Friera I, Nuño-Ayala M, Fernández-Jiménez B, Bernal JA,
Izquierdo-García JL, Jiménez-Borreguero
J, Pizarro G, Ruiz-Cabello J, Macaya C,
Fuster V, Yellon DM and Ibáñez B. (*MG,
JMRG and DSR contributed equally)

beta Adrenergic Receptor Selective
Stimulation During Ischemia/ Reperfusion Improves Cardiac Function in Translational Models Through Inhibition of mPTP Opening in Cardiomyocytes.
Basic Res Cardiol (2014) 109: 422
IF: 5.904

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IF: 3.534

González-Granado JM*, Silvestre-Rojig
C, Rocha-Perugini V, Trigueros-Motos L,
Cibrían D, Morlino G, Blanco-Berrocal
M, Osorio FG, Freije JM, López-Otín C,
Sánchez-Madrid F* and Andrés V*. (*MGG,
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C, Cabrera JA, Valenciano J, de Prado
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L, Martín-Pérez D, Torroja C, Sánchez-Cabo
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Y, Erazo M, Martínez-Caro L, García A, de
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IF: 5.163
APPENDIX

Publications 2014


SCIENTIFIC REPORT 2014


APPENDIX
Publications 2014


APPENDIX

Publications 2014


**APPENDIX Publications 2014**

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Cuando no funciona el cribado para el riesgo cardiovascular elevado en la población general. Ges Clin San (2014) 16: 54


Laclaustra M. 
Cuando no funciona el cribado para el riesgo cardiovascular elevado en la población general. Ges Clin San (2014) 16: 54


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Peralta M, González-Rosa JM, Marques I and Mercader N. 

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Villa Del Campo C, Claveria C, Sierra R and Torres M. 

Wiley B and Fuster V. 
APPENDIX Publications 2014

Articles with a non-CNIC Main Author

Articles with an IF

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<th>Title</th>
<th>Journal</th>
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<th>Year</th>
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APPENDIX

Publications 2014


T Cells Kill Bacteria Captured by Transfection From Dendritic Cells and Confer Protection in Mice.

Cell Host Microbe (2014) 15: 611-22

IF: 12.609


NFkappaB2/p100 Is a Key Factor for Endotoxin Tolerance in Human Monocytes: A Demonstration Using Primary Human Monocytes from Patients with Sepsis.


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de Haas HJ, Arbutini E, Fuster V, Kramer CM and Narula J.

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IF: 11.861


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J Vasc Access (2014) 15: 45-50

IF: 1.017


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Publications 2014


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IF: 8.808


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IF: 5.114


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IF: 3.342

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IF: 5.089


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IF: 5.089


Retinoid X Receptor Alpha Attenuates Host Antiviral Response by Suppressing Type I Interferon.
Nat Commun (2014) 5: 5494
IF: 10.742

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SCIENTIFIC REPORT 2014


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IF: 5.014


IF: 6.014


IF: 1.018


IF: 4.672


IF: 4.041

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IF: 3.485


IF: 4.555


IF: 4.350


IF: 9.775


IF: 4.304


IF: 5.480


IF: 42.351


IF: 42.351


IF: 4.304


IF: 5.480


IF: 42.351


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The APPENDIX
Articles without an IF

Novel Association of the Obesity Risk-Allele Near Fas Apoptotic Inhibitory Molecule 2 (FAIM2) Gene With Heart Rate and Study of Its Effects on Myocardial Infarction in Diabetic Participants of the PREDIMED Trial.

Low/Negative Expression of PDGFR-alpha Identifies the Candidate Primary Mesenchymal Stromal Cells in Adult Human Bone Marrow.
Stem Cell Reports (2014) 3: 965-74

ATP-Dependent Lon Protease Controls Tumor Bioenergetics by Reprogramming Mitochondrial Activity.
Cell Rep (2014) 8: 542-56

Ringenberg J, Deo M, Figueirêse-Rama D, Pizarro G, Ibáñez B, Peinado R, Merino JL, Berenfeld O and Devabhaktuni V.
Effects of Fibrosis Morphology on Reentrant Ventricular Tachycardia Inducibility and Simulation Fidelity in Patient-Derived Models.
Clin Med Insights Cardiol (2014) 8: 1-13

Hand2 Is an Essential Regulator for Two Notch-Dependent Functions Within the Embryonic Endocardium.
Cell Rep (2014) 8: 2071-83

Creative Thinking as an Innovative Approach to Tackle Nutrition in Times of Economic Crises.
APPENDIX

Publications 2014

TOTAL AND AVERAGE CITES (until December 2014)

Times Cited
Average Citation per Item

PUBLICATIONS/GROUP

TOTAL PUBLICATIONS/GROUP
TOTAL IF PUBLICATIONS/GROUP
Training is one of the CNIC’s core activities, and the Center has devised a comprehensive training plan, **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 2014

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#### 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 informed choices about the possibility of pursuing a scientific career.
## Training Programs and Courses

### Fellowships in 2014

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<td>Universidad Rey Juan Carlos</td>
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APPENDIX  Training Programs and Courses

Recent Graduates

CARDIOVASCULAR POSGRADUATE Program

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

In the academic year 2014-2015, the CNIC collaborated in the Masters in Molecular Biomedicine, offering a module in Cardiovascular Disease. This optional module provides a broad overview of cardiovascular biology, including perspectives from basic, clinical and translational research.

Dates: 13 January-18 February 2015
Venue: CNIC
UAM MSc Students: 14
CNIC PhD students: 17

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 2014

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<td>Biomedicina Molecular (Autónoma de Madrid)</td>
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<td>Suárez, Javier</td>
<td>Univ. Alcalá de Henares (Madrid)</td>
<td>Dianas Terapéuticas en Señalización Celular: Investigación y Desarrollo (Alcalá de Henares)</td>
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</table>

PREDOCRATIONAL (PhD) Program

The PREDOCRATIONAL Program provides a unified framework for all researchers at the CNIC who are working towards a doctoral degree. All predocotoral 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
## Training Programs and Courses

> **Graduate students at the CNIC who obtained their PhD degrees in 2014**

<table>
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<tr>
<th>Name</th>
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<th>CNIC Department</th>
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<td>Mercedes Ricote Pacheco</td>
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<td>Vascular Biology and Inflammation</td>
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<td>Casanova, Maria</td>
<td>Characterization of a population of aged neutrophils and its effect on hematopoietic niches.</td>
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<td>Atherothrombosis, Imaging and Epidemiology</td>
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<td>Cardiovascular Development and Repair</td>
<td>Mercedes Ricote Pacheco</td>
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<td>Escolano, Amelia</td>
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<td>Cardiovascular Development and Repair</td>
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## Training Programs and Courses

### > Graduate students studying for their PhD theses at the CNIC during 2014

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## Training Programs and Courses

> **Graduate students studying for their PhD theses at the CNIC during 2014**

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## Training Programs and Courses

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## Training Programs and Courses

> **Graduate students studying for their PhD theses at the CNIC during 2014**

<table>
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<td>Universidad de León</td>
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<td>CICERONE Program 2011-2012</td>
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LA CAIXA-SEVERO OCHOA INTERNATIONAL PhD Program

The la Caixa Foundation is a non-profit organisation funded by the third largest bank in Spain, the Caja de Ahorros y Pensiones de Barcelona (la Caixa). Since 1982, the la Caixa Foundation has run various fellowship programs to enable Spanish students to study postgraduate courses in Spain and abroad. Thanks to this support, thousands of students have been able to pursue their studies.

The la Caixa Foundation funds fellowships at the CNIC in recognition of the Center’s status as one of the Spanish centers of excellence named in the first edition of the Severo Ochoa Award. In 2014 the la Caixa Foundation provided support for four highly qualified graduate students to carry out their experimental work towards obtaining a PhD degree at the CNIC within an International PhD Program.

> la Caixa Fellowships, 2014

<table>
<thead>
<tr>
<th>Name</th>
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<tr>
<td>Alonso Herranz, Laura</td>
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APPENDIX  Training Programs and Courses

Graduates & Medical Professionals

RES@CNIC Program

The aim of the Res@CNIC Program is to offer medical professionals, during the first years of their specialization period as resident interns, the opportunity to learn about and become familiar with the latest techniques in cardiovascular research being used in the CNIC’s laboratories, under the guidance of a CNIC scientist. Residents participating in RES@CNIC also receive training in theoretical aspects of cardiovascular research through a taught module run by experts. The Program also seeks to create links and collaborations so that on conclusion of their MIR specialization period, these professionals will have the chance to undertake research projects in their respective National Health System centers in collaboration with the CNIC.

RES@CNIC was launched in 2012. Students selected for the third call will join the CNIC during January and February 2015.

> Selected Candidates for the third call

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<tr>
<th>Candidate</th>
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<td>Jesús J. Borreguero</td>
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<td>Pérez Nogales, Eliú</td>
<td>C. H. Insular (Las Palmas de Gran Canaria)</td>
<td>Miguel Torres</td>
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<td>Sobrino Balandrán, Adolfo</td>
<td>H.G.U. Gregorio Marañón (Madrid)</td>
<td>Jesús Ruiz-Cabello</td>
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<td>Valandrón Suárez, Isabel</td>
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<td>Varela Barca, Laura</td>
<td>H.U. Ramón y Cajal (Madrid)</td>
<td>José Antonio Enríquez</td>
</tr>
</tbody>
</table>
APPENDIX  Training Programs and Courses

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 2014

<table>
<thead>
<tr>
<th>Name</th>
<th>Hospital</th>
<th>CNIC Department</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ayaon Albarrán, Ali</td>
<td>Hospital Clínico San Carlos, Madrid</td>
<td>Atherothrombosis, Imaging and Epidemiology</td>
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<tr>
<td>Marina Breysse, Manuel</td>
<td>Hospital General Universitario de Ciudad Real</td>
<td>Atherothrombosis, Imaging and Epidemiology</td>
</tr>
</tbody>
</table>

FICNIC Program

The CNIC has partnered with the Fundación Jesús Serra (FJS) and the Fundación Interhospitalaria para Investigación Cardiovascular (FIC) to create this new program, aimed at promoting training in translational cardiovascular research. The program offers training fellowships to medical professionals specializing in cardiology or cardiovascular surgery.

The FICNIC Program is intended for medical professionals during the final year of their resident intern physician (MIR) specialization period or cardiologists or cardiovascular surgeons within three years of completing their specialization.

> Fellowships in 2014

<table>
<thead>
<tr>
<th>Name</th>
<th>Hospital</th>
<th>CNIC Supervisor</th>
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<tr>
<td>Fernández Jiménez, Rodrigo</td>
<td>Hospital Clínico San Carlos, Madrid</td>
<td>Borja Ibáñez</td>
</tr>
</tbody>
</table>
**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 question key researchers and opinion leaders in the field. Since 2012 editions of the Jornada CICERONE have been run in collaboration with the Fundación Interhospitalaria para la Investigación Cardiovascular and takes place in the Hospital Clínico San Carlos, Madrid.

*Dates: October 24-25, 2014*

*Attendees: 98*

**CARDIOVASCULAR PATHOPHYSIOLOGY Course: “From symptoms to genes”**

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

*Dates: November 21-22, 2014*

*Venue: CNIC Lecture Hall*

*Attendees: 101*
APPENDIX Training Programs and Courses

VASCULAR BIOLOGY Course

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

Dates: July 21-22, 2013
Attendees: 190

Research Professionals

CNIC International Postdoctoral Program

The CNIC International Postdoctoral Program (CNIC IPP) is aimed at supporting transnational mobility of postdoctoral researchers and to broaden and deepen their individual competence, particularly in terms of acquisition of complementary skills needed to become an independent group leader in the future. The program offers fellowships for researchers who hold a PhD Degree at the time of the application deadline.

The CNIC-IPP is supported by CNIC and the European Commission under the FP7 Marie Curie Actions- PEOPLE- COFUND Programme.

> Fellowships in 2014

<table>
<thead>
<tr>
<th>Name</th>
<th>CNIC Department</th>
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<tr>
<td>Sánchez Álvarez, Miguel</td>
<td>Vascular Biology and Inflammation</td>
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</tbody>
</table>
APPENDIX  Training Programs and Courses

CNIC International Incoming Fellowships for Young Group Leaders

The CNIC IFF program aims to increase the mobility within Europe of experienced researchers in the cardiovascular research area. The program has been designed to support transnational mobility of researchers and to broaden and deepen their individual competencies, particularly in terms of acquisition of complementary skills needed to attain or strengthen a senior independent position in biomedical research.

The CNIC IIF is supported by the CNIC and the European Commission through the COFUND Programme, within the Marie Curie Actions theme in FP7. The EC contributes 40% of the total cost of the program.

> Fellowships in 2014

<table>
<thead>
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APPENDIX Seminars, Events and Awards

January

13  Ajay Chawla
   Cardiovascular Research Institute / University of California
   San Francisco, USA

24  Marco Tripodi
   Roma University
   Italy

27  Mike Murphy
   MRC Mitochondrial Biology Unit, Medical Research Council
   Cambridge, UK

February

7   Mariona Graupera
    Institut d’Investigació Biomèdica de Bellvitge (IDIBELL)
    Barcelona, Spain

10  Christer Betsholtz
    Uppsala University / Karolinska Institutet
    Stockholm, Sweden

24  Emmanouil Dermitzakis
    University of Geneva Medical School
    Switzerland

March

7   Francisco Real
    Centro Nacional de Investigaciones Oncológicas (CNIO)
    Madrid, Spain

10  Brian Hendrich
    Wellcome Trust-MRC Stem Cell Institute, Department of Biochemistry, University of Cambridge
    UK

20  Lluis Morey
    Centre for Genomic Regulation (CRG)
    Barcelona, Spain

24  Joseph Vita
    Journal of the American Heart Association
    Boston, USA

31  Ari Helenius
    ETH Zürich, Institute of Biochemistry
    Switzerland

April

10  Andrés Santos
    E.T.S.I. Telecomunicaciones, Universidad Politécnica de Madrid
    Spain

May

5   Gerd Heusch
    Direktor des Institutes fuer Pathophysiologie,
    Universitaetsklinikum Essen
    Germany

8   2014 Weinstein Cardiovascular Conference

16  Florent Ginhoux
    Singapore Immunology Network (SIgN), Agency for Science, Technology and Research (A*STAR)

19  Josef Penninger
    Institute of Molecular Biotechnolgy (IMBA)
    Vienna, Austria

June

2   Gwendalyn J. Randolph
    Washington University School of Medicine
    St. Louis, USA

9   Stefan Schulte-Merker
    Hubrecht Institute
    Utrecht, The Netherlands

13  Ángel R. Nebreda
    Institute for Research in Biomedicine
    Barcelona, Spain

17  Mª Ángeles Moro
    Universidad Complutense de Madrid
    Spain

18  David del Álamo
    EMBO Journal
    Heidelberg, Germany

26  Pere Puigserver
    Harvard Medical School
    Boston, USA
APPENDIX  Seminars, Events and Awards

July
3  Jacob Fog Bentzon
   Aarhus University
   Denmark
4  Inflammation MACS® Day – CNIC
9  Katrien De Bock
   KU Leuven
   Belgium

September
4  José Jalife
   University of Michigan Medical School
   USA
22 Michael A. Gimbrone
   Harvard Medical School / Center for Excellence in Vascular Biology,
   Brigham & Women’s Hospital
   Boston, Massachusetts, USA
26  La Noche de los investigadores
    Bioinformática: unir investigación cardiovascular básica y aplicada es el futuro

October
1  John E. Dick
    Princess Margaret Cancer Centre, University Health Network / University of Toronto / Ontario Institute for Cancer Research
    Canada
3  Multicolor panel design for flow cytometry Workshop
6  Luis Serrano
    Centre for Genomic Regulation (CRG)
    Barcelona, Spain
16 Fifth Madrid Zebrafish Club Meeting
23 Jennifer Nichols
   Wellcome Trust Centre for Stem Cell Research, University of Cambridge
   UK
24 Jornada Cicerone “What you need to know about Cardiovascular Research”
27 Miriam Merad
   Mount Sinai Hospital
   New York, USA

November
7  IV CNIC Conference “Energy homeostasis and metabolic disease”
11 Semana de la Ciencia “Ven a CNIC: Visita interactiva a sus departamentos para conocer la investigación cardiovascular”
13 TRANSCARDIO14 “Primer Encuentro Español de Ciencia Translacional en Cardiología”
17 Goran K Hansson
   Karolinska Institutet
   Stockholm, Sweden
21  VIII Curso de Fisiopatología Cardiovascular “Del síntoma a los genes”
26 Nicholas Robert Forsyth
   Institute for Science and Technology in Medicine, Keele University
   UK
27  Salim Seyfried
    Max-Delbrück-Center for Molecular Medicine
    Berlin, Germany

December
1  CNIC Phd Day - 2014 Conference
    Towards Leadership Development in Science
Awards

**Cardiovascular Development and Repair Department**

**Award:** Premio Fundación Princesa de Girona Investigación Científica  
**Awarded to:** Rui Benedito

**Award:** Premio Nacional de Fin de Carrera de Educación Universitaria.  
**Awarded to:** Héctor Sánchez

**Award:** Segundo premio del Área de Ciencias Biológicas y Biomédicas en el Certamen Arquímedes.  
**Awarded to:** Laura Alonso

**Vascular Biology and Inflammation Department**

**Award:** Estrella de la Comunidad de Madrid en el Día Internacional de la Mujer.  
**Awarded to:** Guadalupe Sabio

**Epidemiology, Atherothrombosis and Imaging Department**

**Award:** Included in the 25 outstanding figures of the last 25 years. Readers of Spanish national newspaper El Mundo.  
**Awarded to:** Valentin Fuster, MD, PhD

**Award:** Appointment Editor-in-Chief, American College of Cardiology. American College of Cardiology  
**Awarded to:** Valentin Fuster, MD, PhD

**Award:** 2014 Frontiers in Science Award. American Association of Clinical Endocrinologist  
**Awarded to:** Valentin Fuster, MD, PhD

**Award:** Title of Marquis for his “outstanding and unceasing research efforts and his educational outreach work”. King Juan Carlos I of Spain  
**Awarded to:** Valentin Fuster, MD, PhD

**Award:** Opening keynote lecturer of six International meetings. Chest World Congress and The One Century Celebration of the Mayo Clinic Cardiovascular Dept.  
**Awarded to:** Valentin Fuster, MD, PhD

**Award:** “XII Premio Nacional de Investigación CMC - Barclays” al trabajo “Effect of early metoprolol on infarct size in ST-segment elevation myocardial infarction patients undergoing primary PCI: the METOCARD-CNIC trial”. Colegio Oficial de Médicos de Córdoba – Barclays.  
**Awarded to:** Borja Ibañez et al., Circulation. 2013;128:1495-1503

**Award:** Young Researcher Award. The European Society of Cardiology Working Group on Pulmonary Circulation and Right Ventricular Function.  
**Awarded to:** Ana García Álvarez

**Award:** Award to the 2nd best communication. International Symposium NEUTROPHIL 2014 (Montreal, Canadá).  
**Awarded to:** María Casanova Acebes

**Award:** Mención de Alumno Distinguido por la Universidad de Extremadura. Universidad de Extremadura.  
**Awarded to:** Federico Sierra Rodríguez de la Rubi

**Award:** Premios Jóvenes Jaén 14ª Edición en la Categoría de Universidad. Instituto Andaluz de la Juventud (IAJ) de la Consejería de Igualdad, Salud y Políticas Sociales. Junta de Andalucía.  
**Awarded to:** Ana Victoria Lechuga Vieco
STRATEGIC ALLIANCES: The CNIC consolidates and expands its alliances to investigate, train, innovate and transfer

The central aim of biomedical research is to translate knowledge generated in basic research laboratories into improved and innovative clinical practice, and reciprocally to stimulate research into questions raised in healthcare centers. Excellence in this area requires an integrated network based on close contacts with a wide range of institutions in different sectors.

In the last year, the CNIC has signed 37 interinstitutional agreements to create or consolidate partnerships.

In the education sector, the CNIC has expanded its academic network by signing agreements with universities in Spain (Francisco de Vitoria de Madrid, San Pablo CEU de Madrid, Barcelona, Castilla-La Mancha, and Extremadura) and abroad (Università degli Studi di Napoli, Italy). These agreements mostly establish student exchange programs and short visits for practical work in the CNIC’s laboratories.

To strengthen the CNIC PhD Program, the Center has also consolidated its partnership with the Fundación La Caixa, receiving a major injection of funding for new PhD fellowships within the “La Caixa-Severo Ochoa International PhD Program”.

The Center has also reinforced its relationships with the Fundación Interhospitalaria de Investigación Cardiovascular, establishing a new training program called the “FICNIC Program”, aimed at young cardiologists.

Links with the clinical sector have been consolidated through the signing of new agreements with Spanish hospitals such as Hospital Clínico San Carlos (Madrid), Hospital Puerta de Hierro (Madrid), and Hospital Clínico de Barcelona.

The CNIC’s international projection is greatly strengthened by a new agreement signed with the Icahn School of Medicine (Mount Sinai, New York) and the private sector (AstraZeneca) to create a transatlantic network for the study of cardiovascular prognosis and prevention by the use of non-invasive imaging tools (TAN SNIP Project).

Finally, visibility of CNIC’s scientific activities has been reinforced through the establishment of new collaborations with media organizations such as Radio Televisión Española (RTVE) and the Fundación para el Conocimiento Madrimasd (Comunidad de Madrid).
Public-Private Partnership

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

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

Shortly after the agreement was signed, on January 24, 2006, the group of companies was formally constituted as the ProCNIC Foundation, signaling the most significant act of business sponsorship in recent years in terms of the amount of funding provided, its social significance, the group of companies involved, and the anticipated outcomes.

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

On November 5 2013, Fundación Mapfre—the non profit organization set up by Spanish insurance giant MAPFRE—became the fourteenth partner in the Pro-CNIC Foundation, through an agreement signed by Pro-CNIC president Luis de Carlos and MAPFRE President Antonio Huertas, in the presence of Dr. Fuster. The other thirteen Pro-CNIC members are Acciona, BBVA, Endesa, Fundación Abertis, Fundación Mutua Madrileña, Fundación Botín, Fundación Ramón Areces, Fundación Repsol, Gas Natural Fenosa, Grupo Prisa, Inditex, la Caixa, and Telefónica. These full members are joined by ProCNIC International Collaborator Mitsubishi. This unique PPP 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 does more than provide 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 base 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

Private Funding

Fundación proCnic

Competitive Funding

International Competitive Funding

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</tr>
<tr>
<td>2014</td>
<td>4,500,000</td>
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</tbody>
</table>
Funding

International Competitive Funding by department

National Competitive Funding
Since 2004 the CNIC has attracted more than €25 million from international competitive sources, and around €50 million from national funds.

With just 33 research groups, the CNIC participated in 29 projects funded under the European Commission’s Seventh Framework Programme (FP7). This included two key CNIC-coordinated projects funded under the Cooperation FP7-Health Programme:

- **CARE-MI. PI: Antonio Bernad Miana. Funding: €1.5 million**
- **FOCUS. PIs: Ginés Sanz y V. Fuster. Funding: €0.5 million**

Twenty projects were awarded to CNIC groups under the People (Marie Curie) FP7 Programme. These include two coordinated ITN projects (one of them in the Innovative Doctorate Training category, the first such project coordinated by a Spanish institution), and two COFUND projects (which bring a total funding of €4 million).

- **ITN-IDT-2013. CardioNext. PI: AG Arroyo. Funding: €2.7 million**
- **ITN-2011. CardioNet. PIs: Enrique Lara-Pezzi, Miguel Torres, José Luis de la Pompa. Funding: €1 million**

The scientific competitiveness of the CNIC research groups is highlighted by their participation in European Research Council (ERC) funded projects. The ERC funds scientific projects that enable Europe’s brightest minds to tackle research challenges, and the CNIC contributes to the achievement of this goal through 5 ERC projects awarded under FP7 and one recently awarded under H2020 (under negotiation).

- **ERC Advanced Grant. GENTRIS: Mechanisms of MTOC guidance and Genetic Transfer at the Immune Synapse: novel modes of Immunomodulation. PI: Francisco Sánchez Madrid. Funding: €1.5 million**
- **ERC Starting Grant. CLR Sensing Necrosis - Immune functions of myeloid Syk-coupled C-type lectin receptors sensing necrosis. PI: David Sancho. Funding: €1.3 million**
- **ERC Starting Grant. OBECAN: Role of obesity in the development of hepatocellular carcinoma. PI: Guadalupe Sabio Buzo. Funding: €1.5 million**
- **ERC Starting Grant. BCLYM: Mechanisms of mature B cell lymphomagenesis. PI: Almudena R. Ramiro. Funding: €1.6 million**
- **ERC Starting Grant. ZebraHeart: Novel insights into cardiac regeneration through studies in the zebrafish. PI: Nadia Mercader Huber. Funding: €1.5 million**
**CNIC patent portfolio 2014**

Twenty two inventions are currently being filed, ten of them in cooperation with other institutions. Of these, four are licensed. The following table lists those patents available for licensing:

<table>
<thead>
<tr>
<th>TITLE</th>
<th>INVENTORS</th>
<th>APPLICANTS</th>
<th>PATENT APPLICATIONS</th>
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<td>Method and system for generating MR images of a moving</td>
<td>Javier Sanchez Gonzalez, Nils Dennis Nothnagel, Borja Ibáñez Cabeza,</td>
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<td>object in its environment</td>
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<td>Method of predicting or prognosticating neurological</td>
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<td>performance in patients which have suffered a cardiac</td>
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<td>arrest and optionally comatose status due to ventricular</td>
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<td>P-selectin glycoprotein ligand-1 (PSGL-1) for the treatment</td>
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<td>Single core radionuclide-metal oxide nanoparticles: a new</td>
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<td>biocompatible nanosystem for dual hot spot imaging</td>
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<td>Micellar nanoparticles containing antitumoral glycosides</td>
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<td>Fernández-Mayoralas Álvarez, Manuel Nieto Sampedro, Lorenzo Romero</td>
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<td>AAV vectors for the treatment of ischemic and non-ischemic</td>
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<td>Stable episomes based on non-integrative lentiviral</td>
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<td>Secuencias nucleotídicas motivo que dirigen la</td>
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<td>Uso de agonistas selectivos de receptores beta-3 adrenérgicos para el tratamiento de hipertensión pulmonar</td>
<td>Borja Ibañez Cabeza, Valentín Fuster Carulla y Ana García-Álvarez</td>
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<td>Methods of using the Calcineurin A variant CnAB1 for the treatment of cardiac hypertrophy</td>
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<td>Nanopartículas recubiertas de gelatina</td>
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<td>Caveolin-1 in tumor-associated fibroblasts as biomarker for tumor progression</td>
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<td>Selective peptides that inhibit the biological activity of calcineurin</td>
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Patent Applications:
ES - Spain
PCT - Internacional
EP - Europe
US - USA
CNIC staff 2014 (418)
- Scientific Departments
- Technical Units
- Administration
- Scientific Services

CNIC research staff 2014 (372)
- Head of Laboratory/Unit
- Research Scientists
- Postdoctoral Researchers
- Predoctoral Researchers
- Technicians
**Staff Figures**

**Staff by department 2014 (318)**

- Imaging, atherotrombosis and epidemiology
- Vascular Biology and Inflammation
- Cardiovascular Development and Repair
- Translational Platform

**Gradual growth current status**

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Gender Distribution 2014

- Men: 60%
- Women: 40%

Age distribution 2014

- < 30: 32%
- 30-39: 19%
- 40-50: 8%
- > 50: 41%