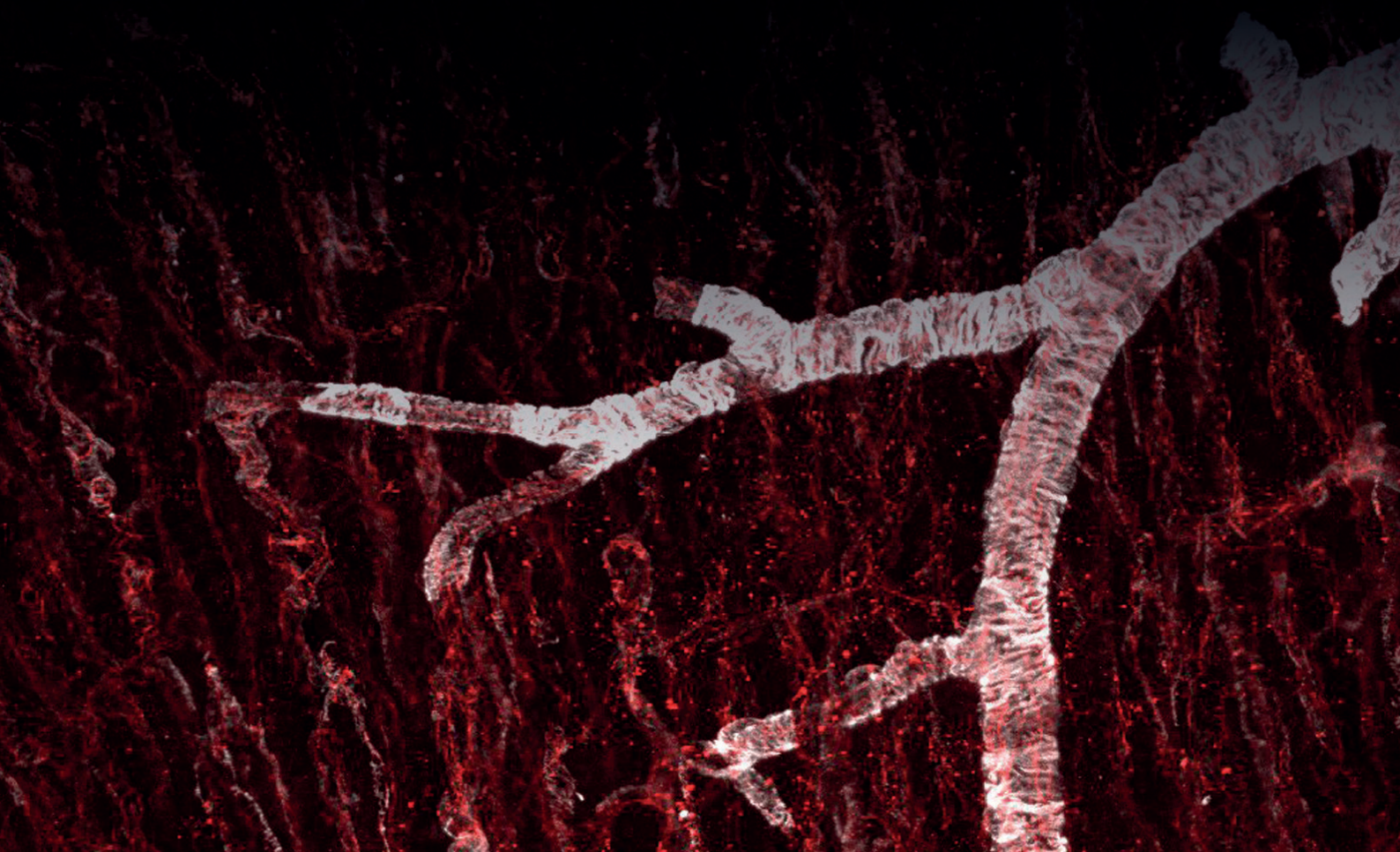
RESEARCH AREAS

1. Myocardial Pathophysiology
2. **Vascular Pathophysiology**
3. Cell and Developmental Biology



2

RESEARCH AREAS

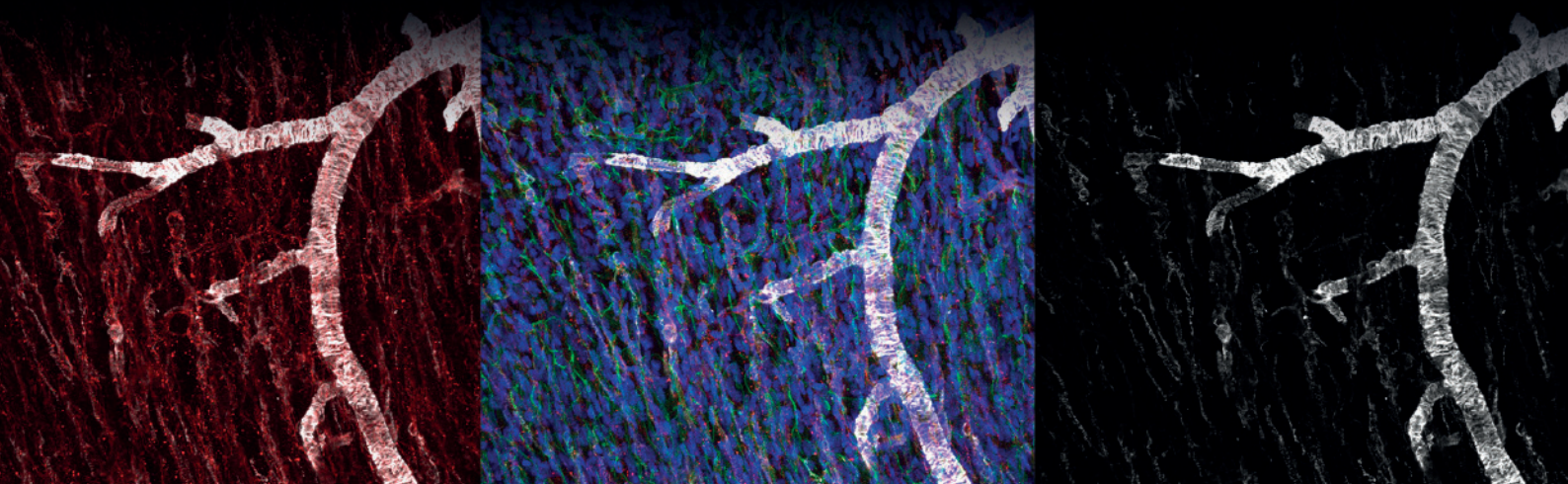
Vascular Pathophysiology

Area coordinators:



Almudena Ramiro *Antonio Fernández-Ortiz*

Research in the Vascular Pathophysiology Area (VPA) focuses on the biology of the cardiovascular system in health and disease, using a multidisciplinary and transverse approach, embracing molecular and cellular biology as well as translational and clinical research. The work in the VPA is broadly divided into 2 programs: Cardiovascular Biology and Signaling & Inflammation. VPA research groups use a wide variety of techniques, including animal, tissue, cell and molecular models, to investigate normal vascular function and the key steps in the vascular alterations that underlie cardiovascular diseases. VPA groups work on translational and clinical research through several research projects, including Secure and PESA. We also have a major interest in cardiovascular proteomics. The VPA hosts three technical units: Genomics, Proteomics/Metabolomics, and Bioinformatics.





Molecular and genetic cardiovascular pathophysiology

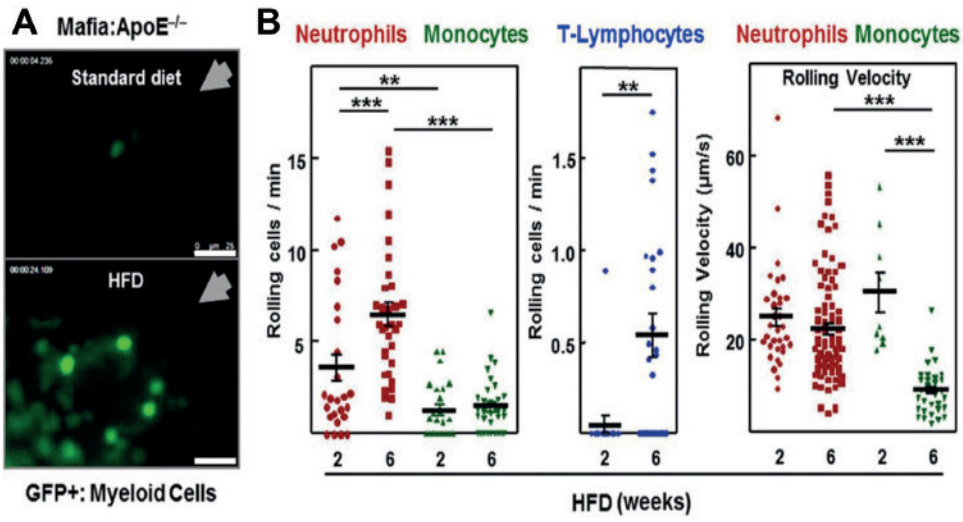
Head of Laboratory
Vicente Andrés García



RESEARCH INTEREST

The World Health Organization has estimated that cardiovascular disease (CVD) will by 2020 be the main health and socio-economic problem worldwide, in part due to the progressive aging that the world population is experiencing. Atherosclerosis and heart failure contribute significantly to CVD-related morbimortality in the elderly. These anomalies and the aging process are greatly 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 and cardiac electrophysiological alterations that are associated with early death (average lifespan: 13.5 yr, range: 8-21 yr), predominantly from 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 currently focuses on: 1) Identifying mechanisms through which wild-type lamin A/C regulates CVD; 2) Identifying tissue-specific and systemic mechanisms through which progerin promotes atherosclerosis and aging, and developing novel therapeutic strategies; 3) Generating a porcine model of HGPS using CRISPR/Cas9 technology to accelerate translational research in HGPS; and 4) Unraveling molecular mechanisms common to premature and physiological aging and specific to each process.



New method for high-resolution imaging of intravascular atherogenic inflammation in live mice allows simultaneous tracking of inflammatory leukocytes and platelets within the carotid artery of atherosusceptible Mafia:ApoE$^{-/-}$ mice. A) Myeloid leukocytes (green) rolling at the bifurcation of the carotid artery in mice fed standard chow or high-fat diet (HFD) for 10 days. Scale bars, 25 µm. Arrows show the direction of blood flow. B) Number of rolling cells (left and middle) and rolling velocity (right) of neutrophils (red), monocytes (green), and T lymphocytes (blue). Data from 18 to 94 fields from 4 mice. Lines show mean ± SEM. ***P*<0.01 and ****P*<0.001 (From: Chèvre et al. Circ Res. 2014;114:770-779).

Postdoctoral Researchers:
Lara del Campo Milán
José María González Granado *(Miguel Servet Programme)*
Álvaro Macías Martínez
Cristina Rius Leiva
José Rivera Torres

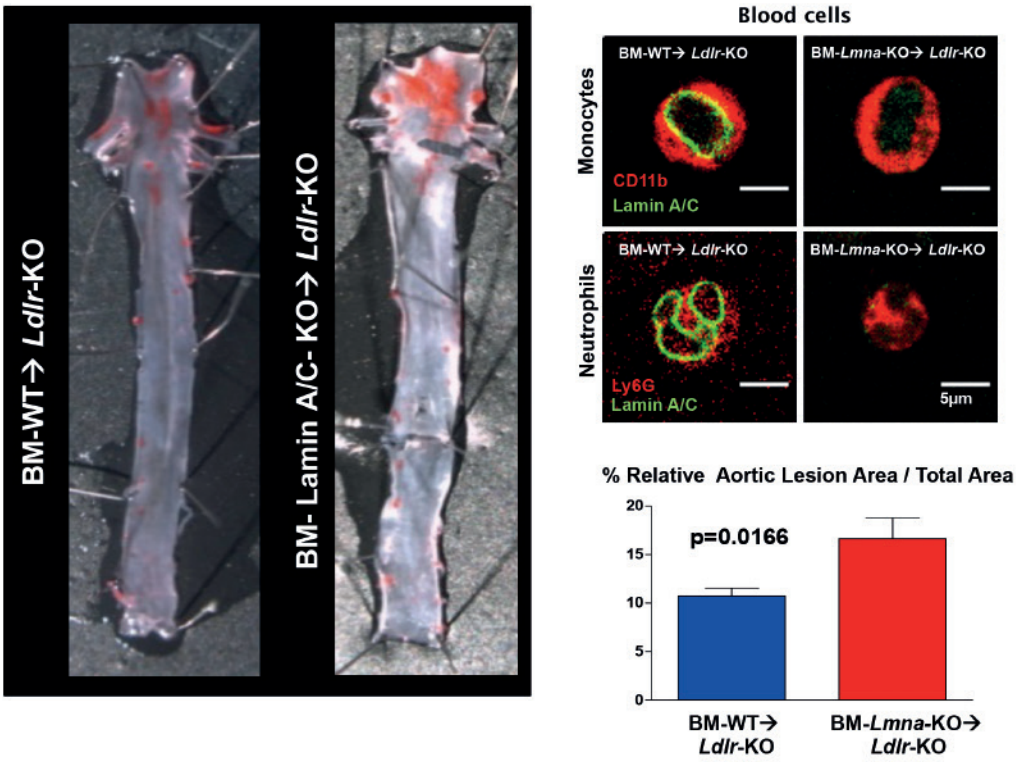
Predoctoral Researchers:
Alberto del Monte Monge
Victor Fanjul Hevia
Pedro Molina Sánchez
Magda Rita Hamczyk
Amanda Sánchez López
Raquel Toribio Fernández

Visiting Students:
María del Carmen Ortuño Costela
Dunia Asensio Cobo
Javier Gómez Ortega

Lab Manager:
Beatriz Julia Dorado de la Corte

Technicians:
María Jesús Andrés Manzano
Marta Blanco Berrocal
Cristina González Gómez

Visiting Scientists:
Immacolata Giordano
Giovanna Granata
Claudia Gucciardi
Andreu Llobera Adán
Ricardo Villa Bellosta



Lamin A/C deficiency in immune cells aggravates atherosclerosis. Ldlr-KO mice transplanted with wild-type bone marrow have perinuclear lamin A/C (green) in circulating monocytes and neutrophils (red), which is absent in mice reconstituted with Lmna-KO bone marrow. Oil-red-O staining reveals significantly increased atherosclerosis in aortas of BM-Lmna-KO->Ldlr-KO mice.

MAJOR GRANTS

- *European Commission FP7-ICT-2011-8 (LIPHOS-317916)*
- *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. FIS (CP11/00145) PI: J.M. González Granado*
- *Fundación Ramón Areces (XVII Concurso Nacional para la Adjudicación de Ayudas a la Investigación en Ciencias de la Vida y de la Materia). PI: J.M. González Granado*

SELECTED PUBLICATIONS

Arroyo, AG, Andrés, V. **ADAMTS7 in cardiovascular disease: From bedside to bench and back again?** *Circulation* (2015) 131:1156-9

Rivera-Torres, J, Guzmán-Martínez, G, Villa-Bellosta, R, Orbe, J, González-Gómez, C, Serrano, M, Díez, J, Andrés, V*, Maraver, A*. **Targeting γ-secretases protects against angiotensin II-induced cardiac hypertrophy**. *J Hypertension* (2015) 33:843–50
* Co-corresponding authors

Molina-Sánchez P, Chèvre R, Rius C, Fuster JJ, Andrés V. **Loss of p27 phosphorylation at Ser10 accelerates early atherogenesis by promoting leukocyte recruitment via RhoA/ROCK**. *J Mol Cell Cardiol* (2015) 84:84-94

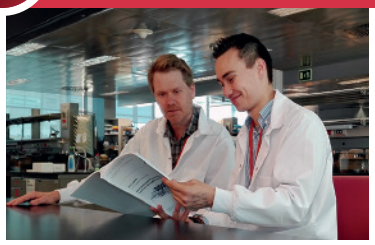
Chèvre R, González-Granado JM, Megens RTA, Sreeramkumar V, Silvestre-Roig C, Molina-Sánchez P, Weber C, Soehnlein O, Hidalgo A*, Andrés V*. **High-resolution imaging of intravascular atherogenic inflammation in live mice**. *Circ Res* (2014) 114:770-9 *(issue cover)*

González-Granado JM*, Silvestre-Roig C, Rocha-Perugini V, Trigueros-Motos L, Cibrian D, Morlino G, Blanco-Berrocal M, Osorio FG, Freije JMP, López-Otín C, Sánchez-Madrid F*, Andrés V*. **Nuclear envelope lamin-A couples actin dynamics with immunological synapse architecture and T cell activation**. *Science Signal* (2014) 7:ra37 *(issue cover)*
* Co-corresponding authors



Experimental pathology of atherosclerosis

Head of Laboratory
Jacob Fog Bentzon



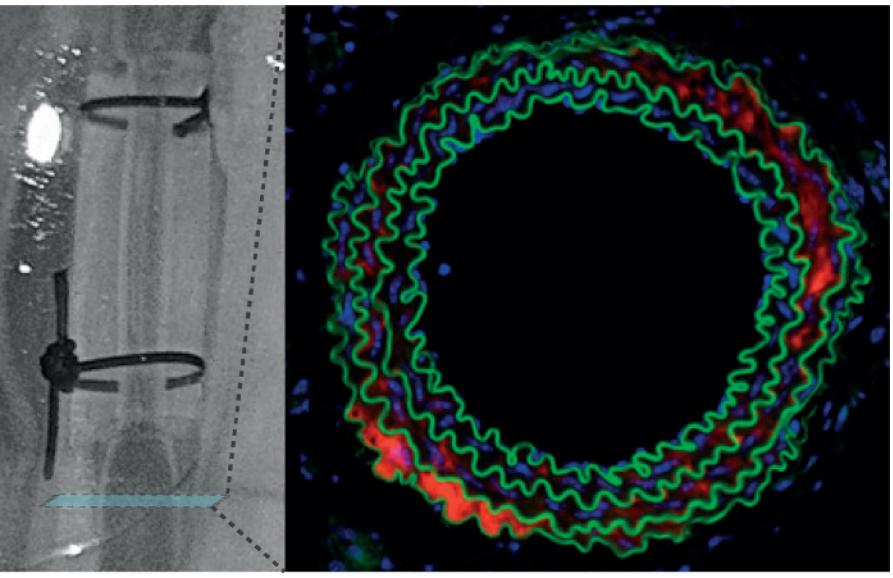
Visiting Scientist:
Kevin Jacobsen

RESEARCH INTEREST

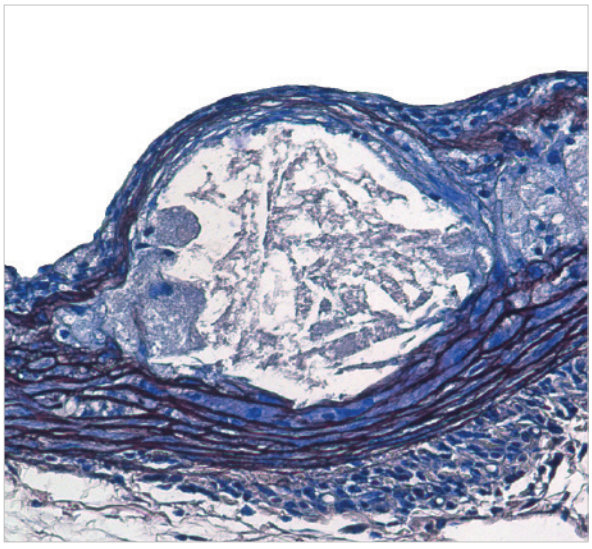
The development of atherosclerosis and its complications heart attack and stroke is a major constraint to living long and heatlhy lives around the world. Our mission is to explore the mechanisms leading to atherosclerosis and finding ways to prevent its progression. The group joined CNIC from Aarhus Universty in September 2015 and the work involves extensive collaboration between Spain and Denmark.

An important element of our strategy has been the development of new tools for atherosclerosis research. Gene modified minipigs with atherosclerosis, created by animal cloning and now established at CNIC, offer human-like dimensions and pathology for studies with a direct translational outlook. As a complementary method for basic research, we have devised a virus-mediated gene transfer technique to induce atherosclerosis in mice, circumventing the need for complicated breeding programmes and offering greater flexibility in the design of experiments. This method is currently being implemented in many research groups around the world.

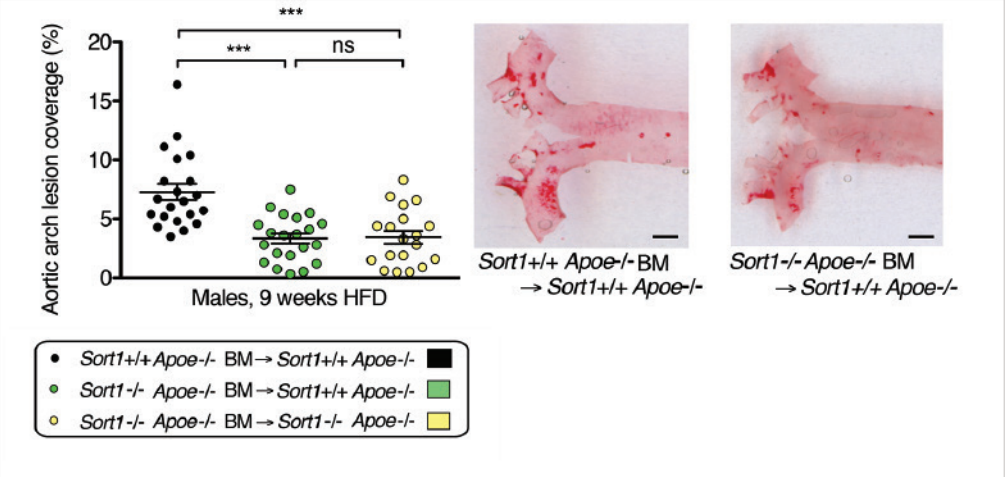
Applying these and other techniques, our group has recently described how blood flow controls atherosclerosis susceptibility of arteries by regulating the composition of the arterial matrix and its ability to sequester cholesterol-rich lipoproteins. Furthermore, we have identied a sorting receptor for proinflammatory cytokines that facilitates the development of atherosclerosis in mice.



Retention of red fluorescencent lipoproteins in an area exposed to experimentally induced atherogenic blood flow. The plane (left) indicates the location of the section shown (right).



Fibroatheromatous atherosclerosis induced by injecting a PCSK9-encoding recombinant virus in a mouse.



Lack of sortilin in circulating immune cells impairs the development of atherosclerosis in hypercholesterolemic Apoe-deficient mice.

MAJOR GRANTS

- Det Frie Forskningsråd, Sapere Aude Level II grant (DFF – 4004-00459). Funds held at Aarhus University.
- Novo Nordisk Fonden, Interdisciplinary Synergy grant (PI: Søren Moestrup). Funds held at University of Southern Denmark.

SELECTED PUBLICATIONS

Al-Mashhadi RH, Bjørklund MM, Mortensen MB, Christoffersen Christina, Larsen T, Falk E, Bentzon JF. **Diabetes with poor glycemic control does not promote atherosclerosis in genetically modified hypercholesterolemic minipigs**. *Diabetologia* (2015) 58: 1926-36

Steffensen LB, Mortensen MB, Kjolby M, Hagensen MK, Oxvig C, Bentzon JF. **Disturbed laminar blood flow vastly augments lipoprotein retention in the artery wall: A key mechanism distinguishing susceptible from resistant sites.** *Arterioscler Thromb Vasc Biol* (2015) 35: 1928-35

Mortensen MB, Kjolby M, Gunnersen S, Larsen JV, Palmfeldt J, Falk E, Nykjaer A, Bentzon JF. **Targeting sortilin in immune cells reduces proinflammatory cytokines and atherosclerosis.** *J Clin Invest* (2014) 24: 5317-22

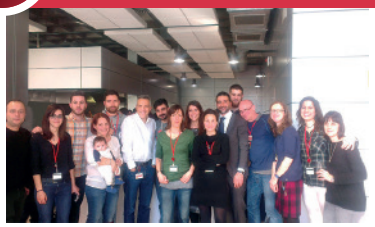
Bjørklund MM, Hollensen AK, Hagensen MK, Dagnæs-Hansen F, Christoffersen C, Giehm Mikkelsen J, Bentzon JF. **Induction of atherosclerosis in mice and hamsters without germline genetic engineering.** *Circ Res* (2014) 114: 1684-9

Bentzon JF, Otsuka F, Virmani R, Falk E. **Compendium review: Mechanisms of plaque formation and rupture.** *Circ Res* (2014) 114: 1852-66



Intercellular signalling in cardiac development & disease

Head of Laboratory
José Luis de la Pompa



RESEARCH INTEREST

We study the molecular mechanisms that regulate heart development, as we believe that this is an essential step toward understanding congenital heart disease and the eventual design of therapies to treat it. In the last year we have studied the role of various intercellular signals in chamber and valve development and cardiac regeneration, with the ultimate goal of identifying new molecular markers of cardiac disease or processes eventually amenable to therapeutic intervention.

During ventricular chamber development, the Notch signaling pathway first connects chamber endocardium and myocardium to sustain trabeculation. Notch signalinng later coordinates ventricular patterning and compaction with coronary vessel development to generate the mature chamber, via a temporal sequence of ligand signaling determined by the glycosyltransferase Manic Fringe (MFng). Early endocardial expression of MFng promotes Dll4-Notch1 signaling, which induces trabeculation in the developing ventricle. Ventricular maturation and compaction require MFng and Dll4 downregulation in the endocardium, which allows myocardial Jag1 and Jag2 signaling to Notch1 in this tissue. Perturbation of this signaling equilibrium severely disrupts heart chamber formation (Figure 1).

During cardiac valve development, endocardial Dll4-Notch1 signaling leads to epithelial-mesenchyme transition (EMT) and formation of the valve primordia. Later, Jag1-Notch1 signaling restrains Bmp-mediated valve mesenchyme proliferation by sustaining Hbegf-EGF receptor signaling. Our studies identify a mechanism of signaling crosstalk during valve morphogenesis implicated in the origin of congenital heart defects associated with reduced NOTCH function (Figure 2).

We also investigate the behavior, morphology and role of the endocardium during zebrafish cardiac regeneration. Time-lapse 3D-whole mount imaging in adult zebrafish hearts reveals a highly dynamic endocardium: spared endocardial cells remain after cryoinjury and proliferate shortly after cryoinjury, showing strongly upregulated Notch signaling (Figure 3). Endocardial cells expand within the injury site and form a structure that persists throughout regeneration. Examination of cardiomyocyte dynamics in Notch gain- and loss-of-function models reveals that Notch promotes cardiomyocyte proliferation. Notch signaling is thus a key regulator of endocardial gene expression and morphology.

Research Scientist:
Donal MacGrogan

Postdoctoral Researchers:
Beatriz Martínez Poveda
Belén Prados
Tania Papoutsi

Predoctoral Researchers:
Juliane Münch
Gaetano D'Amato
Dimitrios Grivas
Stanislao Igor Travisano
Paula Gómez Apiñaniz
Marcos Siguero Álvarez
Rebeca Torregrosa Carrión

Masters Student:
Alejandro Salguero

Technicians:
Patricia Martínez Martín
Vanessa Bou Pérez
Abel Galicia Martín
Sara Perruca Magro

Visiting Scientists:
José María Pérez-Pomares

Res@CNIC Fellow:
Adolfo Sobrino Balandrón

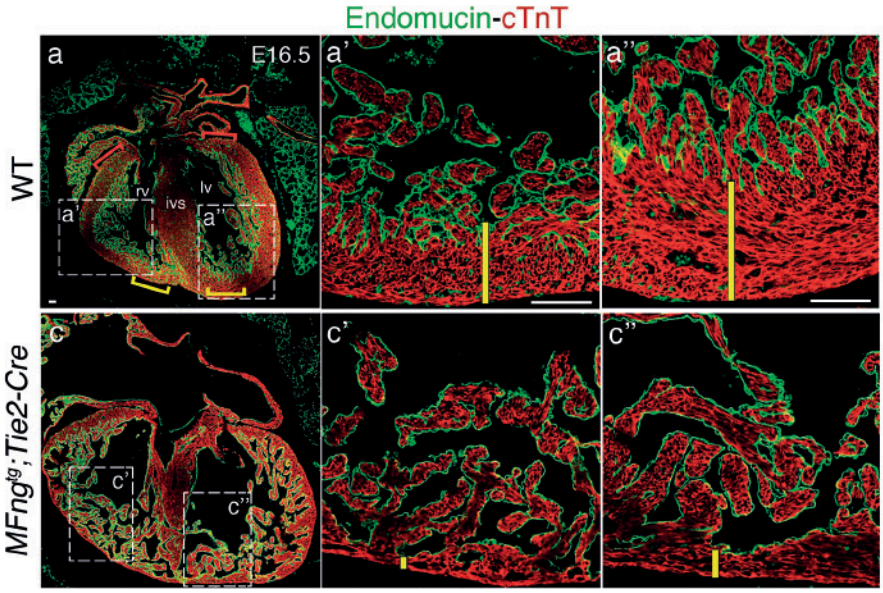


Figure 1.
Notch signaling abrogation disrupts compaction. E16.5 heart sections stained with endomucin (green) and cTnT antibodies (red) to delineate chamber endocardium and myocardium. The WT heart (**a-a''**) has a thick, cTnT-positive compact myocardium in both ventricles, with compacting trabeculae covered by endomucin-positive endocardium. The *MFng^{tg};Tie2-Cre* heart (**c-c''**) has a very thin compact myocardium, uncompacted trabeculae and a disrupted ventricular septum. The yellow bar indicates the thickness of compact myocardium. Scale bar=100µm.

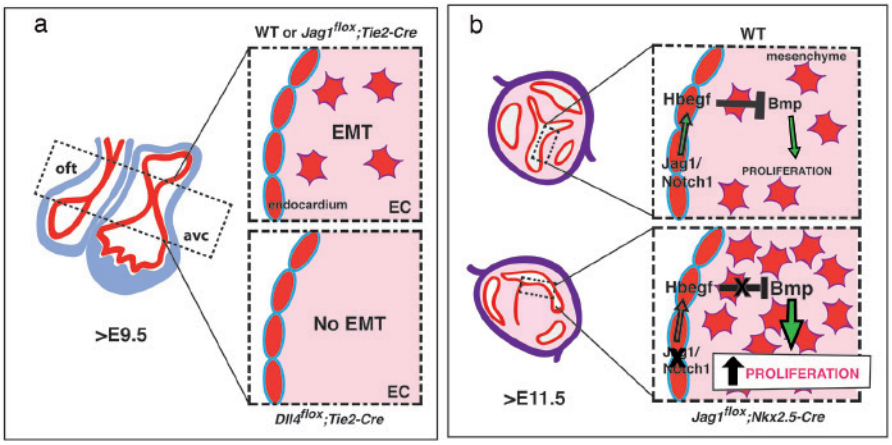


Figure 2.
Regulation of valve primordium formation and morphogenesis by sequential ligand-dependent Notch activation. **(a)** Endocardial Dll4, but not Jag1, is required for EMT. **(b)** Endocardial Jag1 is required for post-EMT valve morphogenesis. In WT valves, Jag1-Notch1 signaling restricts mesenchyme cell proliferation by downregulating Bmp signaling via Hbegf. *Jag1^{flox};Nkx2.5-Cre* mutants have reduced *Hbegf* expression, resulting in increased Bmp signaling and uncontrolled proliferation.

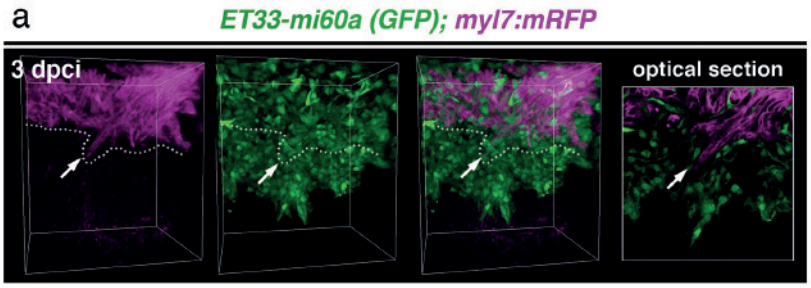


Figure 3.
In the regenerating zebrafish heart the endocardium expands within the injury site and precedes regeneration of the myocardium. **(a)** Volume rendering and an optical section of a region of the inner injury border in an injured *ET33mi-60A; myl7mRFP* ventricle at 3 dpci, with endocardium labeled green and myocardium magenta. Dense endocardial cells (green) surround migrating cardiomyocytes (magenta, white arrowhead) and precede them into the injury site. The dotted line demarcates the regenerating myocardium.

MAJOR GRANTS

- *European Commission. Marie Curie Action Initial Training Network (ITN) (FP7-PEOPLE-2011-ITN, "CardioNeT" 289600) (Coordinador E. Lara)*
- *Ministerio de Economía y Competitividad. Red de excelencia Temática (SAF2015-71863-REDT)*
- *Ministerio de Economía y Competitividad. FIS RETICS (TERCEL: RD12/0019/0003 and RIC: RD12/0042/0005)*
- *Ministerio de Economía y Competitividad (SAF2013-45543-R)*
- *Fundación BBVA (2015-2017)*
- *Fundación La Marató (2016-2018)*

SELECTED PUBLICATIONS

D'Amato G, Luxán G, Del Monte-Nieto G, Martínez-Poveda B, Torroja C, Walter W, Bochter MS, Benedito R, Cole S, Martinez F, Hadjantonakis AK, Uemura A, Jiménez-Borreguero LJ, de la Pompa JL **Sequential Notch activation regulates ventricular chamber development**. *Nat Cell Biol* (doi: 10.1038/ncb3280. Epub 2015 Dec 7)

de Luxán G, D'Amato G, MacGrogan D, de la Pompa JL. **Endocardial Notch Signaling in Cardiac Development and Disease**. *Circ Res* (doi: 10.1161/CIRCRESAHA.115.305350. Epub 2015 Dec 3)

Papoutsi T, Odelin G, Moore-Morris T, Pucéat M, de la Pompa JL, Robert B, Zaffran S **Msx1CreERT2 knock-In allele: A useful tool to target embryonic and adult cardiac valves**. *Genesis* (2015) 53:337-45

VanDusen NJ, Casanovas J, Vincentz JW, Firulli BA, Osterwalder M, Lopez-Rios J, Zeller R, Zhou B, Grego-Bessa J, **de La Pompa JL**, Shou W, Firulli AB. Hand2 is an essential regulator for two Notch-dependent functions within the embryonic endocardium. Cell Rep. 2014 Dec 24;9(6):2071-83.

MacGrogan D, Luxán G, Driessen-Mol A, Bouten C, Baaijens F, de La Pompa JL **How to Make a Heart Valve: From Embryonic Development to Bioengineering of Living Valve Substitutes**. *Cold Spring Harb Perspect Med* (2014) 4: a013912



Matrix metalloproteinases in angiogenesis and inflammation

Head of Laboratory
Alicia G. Arroyo



RESEARCH INTEREST

In order for the vasculature to optimally deliver nutrients and oxygen throughout the body, endothelial cells must adapt to varying tissue needs, and this results in a high degree of vascular heterogeneity. However, we still know relatively little about the mechanisms that govern capillary patterning in homeostasis and upon injury. Our group is dedicated to elucidating how the microvascular network responds to inflammation and contributes to tissue repair. Our research focuses on membrane-type matrix metalloproteinases, endopeptidases able to perform proteolytic and also non-proteolytic actions. Our previous work showed that the protease MT1-MMP plays a key role in angiogenesis and inflammation by processing extracellular matrix components or regulating intracellular signals, such as Rac1 pathway components. We have also shown that the protease MT4-MMP is essential for proper arterial vascular function through its cleavage of osteopontin. Our recent in vivo data suggest that these proteases have different actions in endothelial cells and macrophages and that targeting MT1-MMP versus MT4-MMP has distinct tissue- and context-dependent impacts on the microvasculature during inflammation. Our laboratory is currently investigating: i) MT1-MMP and MT4-MMP actions in the cardiac microvasculature; and ii) the role of macrophage-secreted MT1-MMP or MT4-MMP substrates in the vascular response during cardiac repair.

We are pursuing these goals using 2D and 3D angiogenic models, high- and super-resolution microscopy, 3D image analysis, proteomics, bioinformatics, protein modeling, lentiviral strategies and genetically modified mouse lines. We ultimately intend to apply this knowledge to develop novel angiotherapies aimed at enhancing capillary perfusion and tissue performance in several pathophysiological contexts.

Research Scientist:
Pilar Gonzalo

Postdoctoral Researchers:
Vanessa Moreno
Susana Rocha
Lorenzo Veschini
(until March 2015)

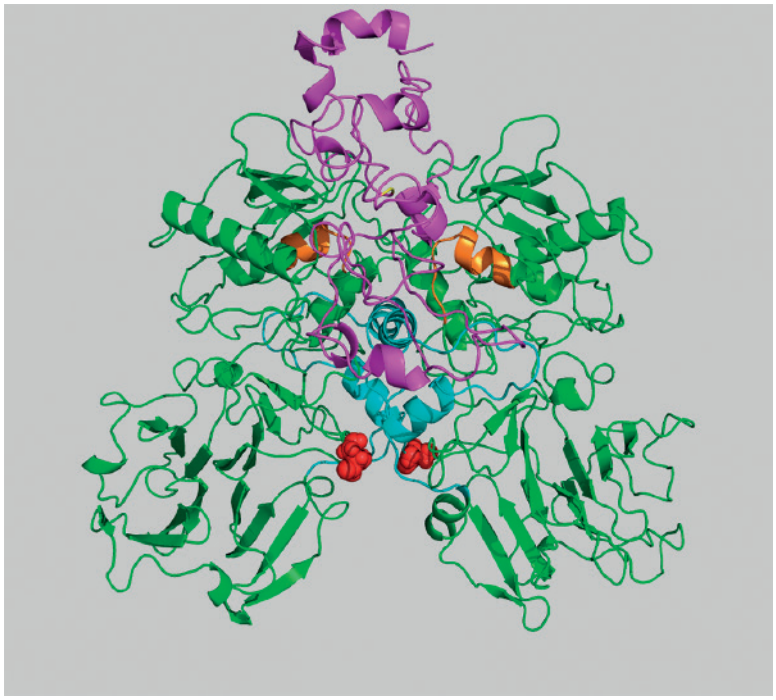
Predoctoral Researchers:
Cristina Clemente
Sergio Esteban
Polyxeni Gkontra
Jesús Gómez Escudero
Mara Martín Alonso
Magdalena Maria Zak
Álvaro Sahún

Technicians:
Laura Balonga
Ángel Colmenar

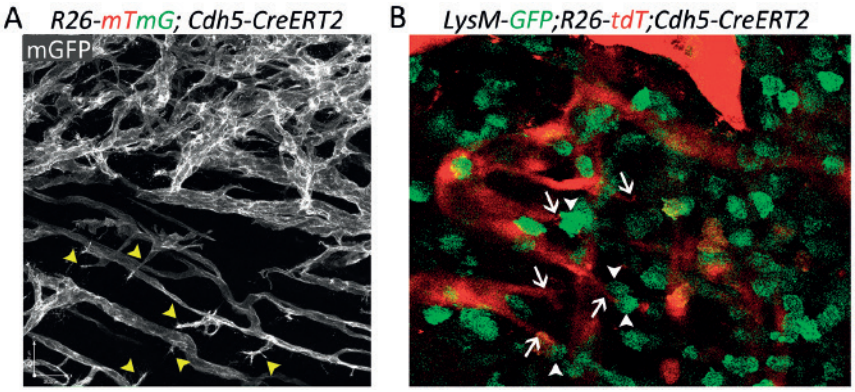
Visiting Scientist:
Cristina Sánchez-Camacho

Masters Student:
Ricardo Santamaría

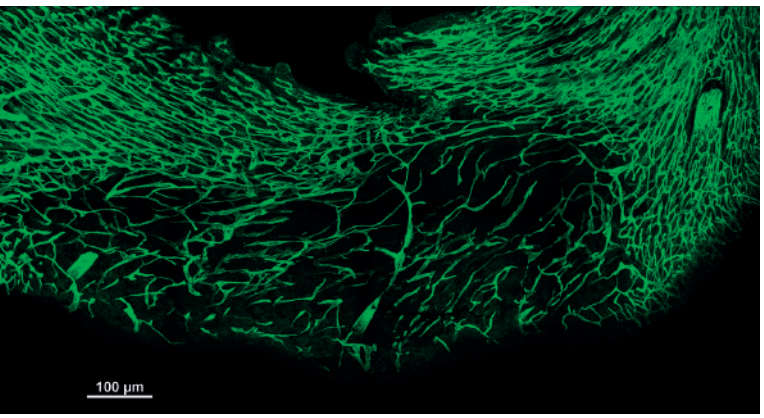
Project Manager:
Lilit Manukyan
(CardioNext ITN)



In silico modeling of substrate/protease docking. In collaboration with the Bioinformatics Unit, we use in silico modeling to explore the accessibility of potential novel substrates to the catalytic sites of membrane type-matrix metalloproteinases. The image shows the docking model of osteopontin (purple) with the human protease MT4-MMP homodimer (green); note that osteopontin is located close to the MT4-MMP catalytic center (orange; see Martín et al., 2015, for more details). In silico modeled cleavage sites are then experimentally validated.



Exploring macrophage/vascular communication in vivo. The dorsal skinfold chamber model allows direct visualization of macrophages and endothelial cells during inflammatory angiogenesis. Confocal imaging of genetically-labeled mice shows in vivo capillary sprouting (arrowheads in A), and macrophages labeled with GFP (arrowheads in B) interacting with Tomato-expressing endothelial sprouts (arrows in B) in response to TNFα in the skin vasculature.



3D confocal microscopy image analysis of the cardiac microvasculature. 3D-volumetric composition of confocal microscopy images from thick heart sections allows the visualization and analysis of the cardiac microvasculature with unprecedented resolution. The image shows the maximal projection of multiple images acquired from thick heart sections (60 µm) and stained for the endothelial cell marker ICAM-2 (green). The heart in the image is from a newborn mouse 5 days after cryoinjury; note that the affected area has a reduced vascular density.

MAJOR GRANTS

- *Ministerio de Economía y Competitividad (SAF2014-52050-R)*
- *Ministerio de Economía y Competitividad. FIS RETICS (RIC: RD12/0042/0023)*
- *Comunidad Autónoma de Madrid. Redes de Excelencia. ANGIOBODIES 2.0 (S2010/BMD-2312)*
- *Fundació La Marató TV3 (165/C/2012)*
- *European Union (PITN-GA-2013-608027) (CardioNext) (Coordinator)*

SELECTED PUBLICATIONS

Gkontra P, Żak MM, Norton K-A, Santos A, Popel AS, Arroyo AG. **A 3D Fractal-Based Approach towards Understanding Changes in the Infarcted Heart Microvasculature**. Medical Image Computing and Computer-Assisted Intervention-MICCAI 2015. *Lecture Notes in Computer Science* (2015) 9351: 173-80

Oller J, Alfranca A, Méndez-Barbero N, Villahoz S, Lozano-Vidal N, Martín-Alonso M, Arroyo AG, Escolano A, Armesilla AL, Campanero MR, Redondo JM. **C/EBPβ and Nuclear Factor of Activated T Cells Differentially Regulate Adamts-1 Induction by Stimuli Associated with Vascular Remodeling**. *Mol Cell Biol* (2015) 35 :3409-22

Martín-Alonso M, García-Redondo AB, Guo D, Camafeita E, Martínez F, Alfranca A, Méndez-Barbero N, Pollán Á, Sánchez-Camacho C, Denhardt DT, Seiki M, Vázquez J, Salaices M, Redondo JM, Milewicz D, Arroyo AG. **Deficiency of MMP17/MT4-MMP proteolytic activity predisposes to aortic aneurysm in mice**. *Circ Res* (2015) 117: e13-26

Arroyo AG, Andrés V. **ADAMTS7 in cardiovascular disease: from bedside to bench and back again?** *Circulation* (2015) 131: 1156-9

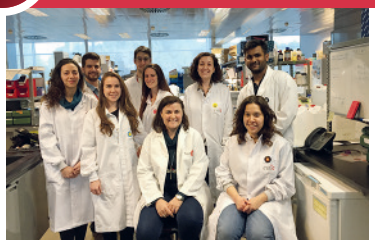
Udi Y, Grossman M, Solomonov I, Dym O, Rozenberg H, Moreno V, Cuniasse P, Dive V, Arroyo AG, Sagi I. **Inhibition mechanism of membrane metalloprotease by an exosite-swiveling conformational antibody**. *Structure* (2015) 23: 104-15

Moreno V, Gonzalo P, Gómez-Escudero J, Pollán Á, Acín-Pérez R, Breckenridge M, Yáñez-Mó M, Barreiro O, Orsenigo F, Kadomatsu K, Chen CS, Enríquez JA, Dejana E, Sánchez-Madrid F, Arroyo AG. **An EMMPRIN-γ-catenin-Nm23 complex drives ATP production and actomyosin contractility at endothelial junctions**. *J Cell Sci* (2014) 127: 3768-81



Regulatory molecules of inflammatory processes

Head of Laboratory
Pilar Martín Fernández



RESEARCH INTEREST

Our group studies the control of inflammation in autoimmune and cardiovascular diseases. Recently, our attention has focused on the potential of miRNAs derived from Th17 or regulatory T (Treg) cells in the design of strategies to combat and diagnose these diseases. Much of our work is conducted in mouse models of myocarditis and peripheral post-ischemic neovascularization, a model of peripheral artery disease (PAD). The true incidence of myocarditis is unknown because it is frequently first diagnosed as non-ischemic dilated cardiomyopathy; PAD affects 1 in 3 people aged 70 years or above. These diseases can easily become chronic and life-threatening, and are associated with devastating long-term side effects and high medical costs. Inadequate understanding of Th17 and Treg biology is an obstacle to the development of immunotherapy protocols for these diseases. Our recent work characterized the role of Th17 cells and Tregs in heart, skin and lung inflammatory diseases. Our current work focuses on these antagonistic T cell subsets, expressing Rorγt^{+} and Foxp3^{+}, and their central role in the control of inflammation. Defects in the development or function of these cells exacerbates autoimmune and cardiovascular disorders.

We are also interested in the role of Th17 cells and Tregs in the rejection of allogenic grafts and heart transplants. Several clinical trials are currently using Tregs to ameliorate the effects of graft-versus-host disease (GvHD) in hematologic cancer and leukemia patients. However, the therapeutic use of Tregs in transplant recipients is still under development, and new markers and protocols are needed for their correct identification, purification and expansion.

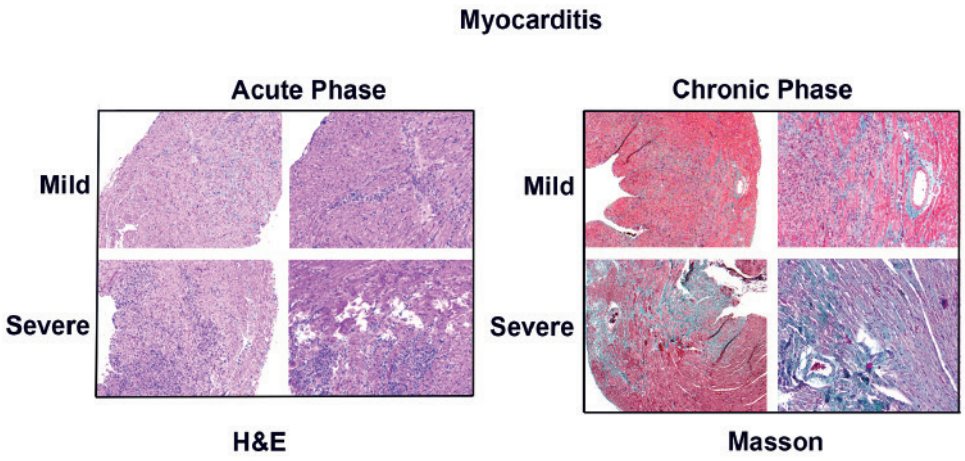
Postdoctoral Researcher:
Aikaterini Tsilingiri

Predoctoral Researchers:
Raquel Sánchez Díaz
Marta Relaño Orasio

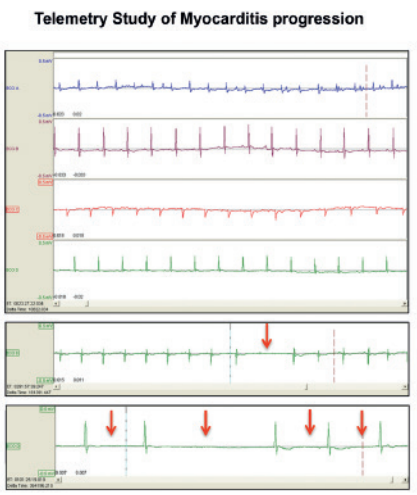
Masters Students:
Rafael Blanco Domínguez
Avinash Londhe

Technicians:
Juan José Lazcano Duque
Sandra Lasarte Ramiro
Irene García Fernández

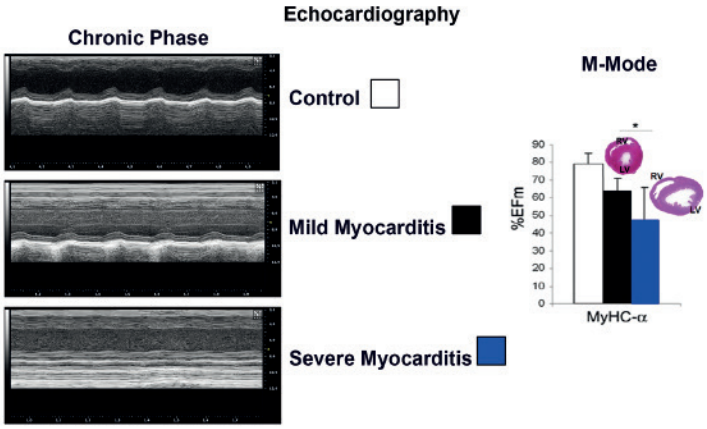
Visiting Scientists:
Beatriz Linillos
Miguel Fernández de la Torre
Daniel García Rivas



Histological analysis of myocarditis. *Left*. Hematoxylin and Eosin staining of heart sections from mice in the acute phase of experimental autoimmune myocarditis (EAM), 21 days after MyHC-α peptide immunization. Small and large infiltrates are shown in mice with mild or severe EAM. *Right*. Masson's trichrome staining reveals enhanced fibrosis in mice with severe manifestation of myocarditis in the chronic phase (56 days after MyHC-α peptide immunization). Right panels; high-power views of infiltrates and collagen deposition in the left ventricle (magnification x 100).



Electrophysiological study with implantable telemetry. Representative telemetrically recorded ECGs from mice during the progression of myocarditis, analogous to Holter monitoring in humans. The ECGs were obtained from mice fitted with implanted wireless radiofrequency transmitters implanted into the abdominal cavity. Recordings were performed before inoculation with MyHC-α peptide and during the acute and chronic phases of EAM. Telemetry reveals a progressive increase in the number of sinus pauses (indicated by red arrows) in parallel with disease progression.



Cardiac function in the chronic phase of myocarditis. M-mode transthoracic echocardiography of left ventricular function reveals a reduction in cardiac contractility in mice with mild or acute myocarditis. Left ventricular fractional shortening (not shown) and ejection fraction (EF) were both significantly smaller than in controls (nonimmunized mice). RV; right ventricle, LV; left ventricle.

MAJOR GRANTS

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

SELECTED PUBLICATIONS

Fabbiano S, Menacho-Márquez M, Robles-Valero J, Pericacho M, Matesanz-Marín A, García-Macías C, Sevilla MA, Montero MJ, Alarcón B, López-Novoa JM, Martín P, Bustelo XR. **Immunosuppression-Independent Role of Regulatory T Cells against Hypertension-Driven Renal Dysfunctions.** *Mol Cell Biol* (2015) 35 :3528-46

Liappas G, Gónzalez-Mateo GT, Majano P, Sánchez-Tomero JA, Ruiz-Ortega M, Rodrigues Díez R, Martín P, Sanchez-Díaz R, Selgas R, López-Cabrera M, Aguilera Peralta A. **T Helper 17/Regulatory T Cell Balance and Experimental Models of Peritoneal Dialysis-Induced Damage.** *Biomed Res Int* (2015) 2015:416480

Cortés JR; Sánchez-Díaz R; Bovolenta ER; Barreiro O; Lasarte S; Matesanz-Marin A; Toribio ML; Sanchez-Madrid F; Martin P. **Maintenance of immune tolerance by Foxp3(+) regulatory T cells requires CD69 expression.** *J Autoimmun* (2014) 55: 51-62

De la Fuente H; Cruz-Adalia A; Martínez del Hoyo G; Cebrian D; Bonay P; Pérez-Hernández D; Vázquez J; Navarro P; Gutierrez-Gallego R; Ramirez-Huesca M; Martín P; Sánchez-Madrid F. **The leukocyte activation receptor CD69 controls T cell differentiation through its interaction with galectin-1.** *Mol Cell Biol* (2014) 34: 2479-87



B lymphocyte biology

Head of Laboratory
Almudena R Ramiro



RESEARCH INTEREST

B cells lead the humoral immune response thanks to the generation of a vast collection of antibodies that can specifically recognize pathogens and tag them for removal. The very same mechanisms responsible for antibody diversity have profound biomedical implications in autoimmunity, immunodeficiency, and cancer.

Our lab is focused on the molecular and cellular events that take place in germinal centers—microstructures generated by B cells during immune responses. Our interests cover basic aspects of B cell biology, including the DNA remodeling associated with antibody diversification by the enzyme AID in germinal centers, the regulatory programs driven by microRNAs in germinal centers, and the generation of animal models to explore the impact of these events on the etiology of disease, most notably in inflammation and cancer.

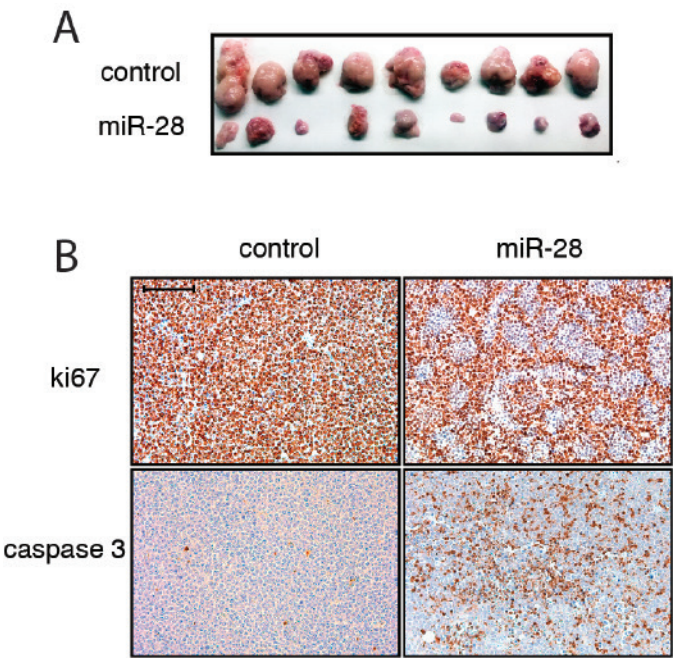
Our recent work has shown that microRNAs contribute to immune tolerance, and that individual microRNAs play critical roles in the regulation of germinal centers and can act as oncogenes (miR217) or tumor suppressors (miR28). In addition, we have developed mouse models to study different regulatory aspects of AID activity in vivo. Finally, we are characterizing the functional contribution of antibodies and their diversification to atherogenesis.

Research Scientists:
Virginia G de Yébenes
María Pilar Delgado

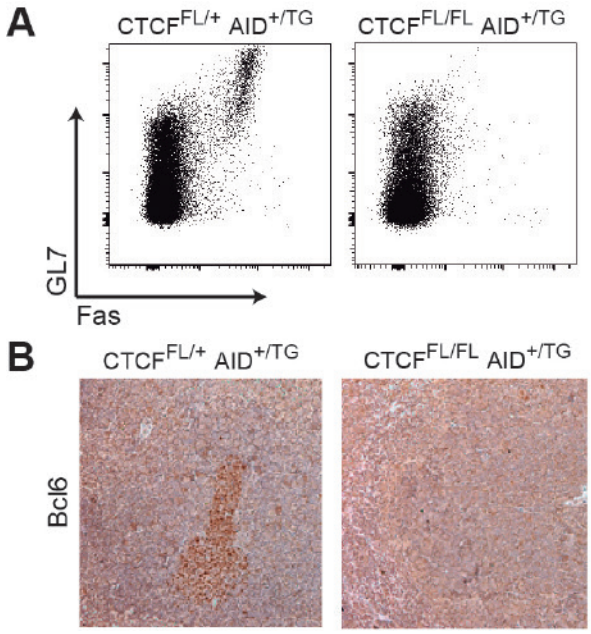
Predoctoral Researchers:
Nahikari Bartolomé
Arantxa Pérez-García
Ángel F. Álvarez
Cristina Lorenzo
Ester Marina

Technician:
Sonia Mur
Dobromira Veselinova

Student:
Pablo Garzón



miR28 impairs B cell lymphoma growth. A) Xenograft tumors were generated with Burkitt lymphoma cells lentivirally transduced with either miR-28 or a scramble construct; cells were injected in the flank of immunodeficient recipient mice. The picture shows representative images of tumors after 21 days of growth. B) miR-28 impairs proliferation and survival of lymphoma cells. Xenograft tumors were stained with Ki67 to monitor proliferation (upper panels) and caspase-3 (lower panels) to monitor apoptosis.



CTCF is required for germinal center generation. A) CTCF-deficient germinal center B cells were generated by crossing mice bearing CTCF-floxed alleles ($CTCF^{FL/FL}$) with mice expressing an AID-driven Cre recombinase ($AIDCre^{+/TG}$). Germinal centers were generated by promoting a T dependent response in mice and analyzed by flow cytometry of Fas+GL7+ cells from spleen. Representative plots are shown of control mice ($CTCF^{FL/FL}$ $AIDCre^{+/TG}$, left) and CTCF-deficient mice ($CTCF^{FL/FL}$ $AIDCre^{+/TG}$, right). B) Representative immunoghistochemical staining with the germinal center marker BCL6 in the same spleens as in A.

MAJOR GRANTS

- Ministerio de Economía y Competitividad (SAF2013-42767-R)

SELECTED PUBLICATIONS

Pérez-García A, Pérez-Durán P, Wossning T, Sernandez IV, Mur SM, Cañamero M, Real FX, Ramiro AR. **AID-expressing epithelium is protected from oncogenic transformation by an NKG2D surveillance pathway**. *EMBO Mol Med* (2015) 7: 1327-36.

Ramiro AR, Barreto VM. **Activation-induced cytidine deaminase and active cytidine demethylation**. *Trends Biochem Sci*. (2015) 40: 172-81

de Yébenes VG, Bartolomé-Izquierdo N, Nogales-Cadenas R, Pérez-Durán P, Mur SM, Martínez N, Di Lisio L, Robbiani DF, Pascual-Montano A, Cañamero M, Piris MA, Ramiro AR. **miR-217 is an oncogene that enhances the germinal center reaction.** *Blood* (2014) 124: 229-39



Gene regulation in cardiovascular remodelling and inflammation

Head of Laboratory
Juan Miguel Redondo Moya



RESEARCH INTEREST

Much of our work centers on the regulation of calcineurin (CN) signaling in angiogenesis and inflammation. In recent years, we have characterized the mechanisms and sequences involved in the interactions of CN with NFAT and other substrates and with immunosuppressive drugs, and we have characterized how specific CN targeting modulates inflammatory responses. More recently, we have studied mediators in vascular and cardiac remodeling related to Angiotensin II and CN pathways. We are currently elucidating the mechanisms that mediate this remodeling and have generated conditional mice deficient for CN and Rcan1 isoforms in the endothelial, vascular smooth muscle, and cardiomyocyte compartments. We have already identified genes regulated by CN in different mouse models of cardiac hypertrophy (CH), and are characterizing their roles in CH using conditional cardiac CN and Rcan1mice. We are also elucidating the role of Chd4/NuRD in cardiac homeostasis and have found that the NuRD complex determines skeletal muscle identity by silencing the skeletal muscle program in cardiomyocytes and the cardiac program in skeletal muscle. Another major area of interest is the mechanisms that mediate aortic diseases such as familial forms of thoracic aortic aneurysm and dissection (TAAD), including Marfan syndrome. We have identified a number of mediators that play a major role in the pathogenesis of these diseases, and we are now characterizing the underlying mechanisms.

Research Scientists:
Sara Martínez Martínez
Pablo Gómez del Arco

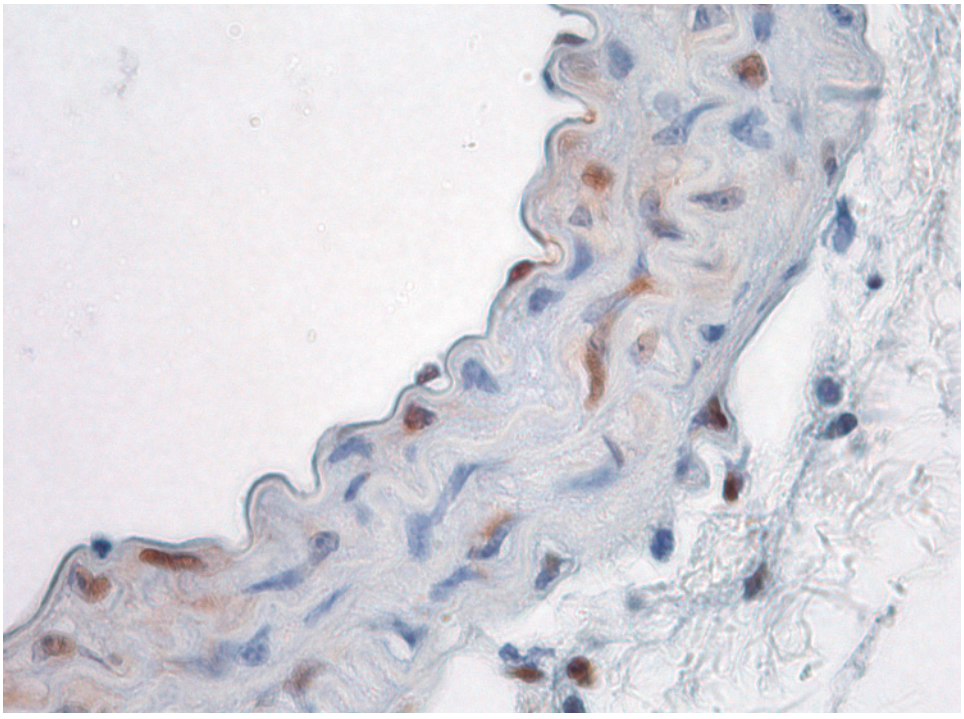
Postdoctoral Researchers:
Nerea Méndez Barbero

Predoctoral Researchers:
Yuri Chiodo
Jorge Oller Pedrosa
Silvia Villahoz
Paula Sofía Yunes Leites

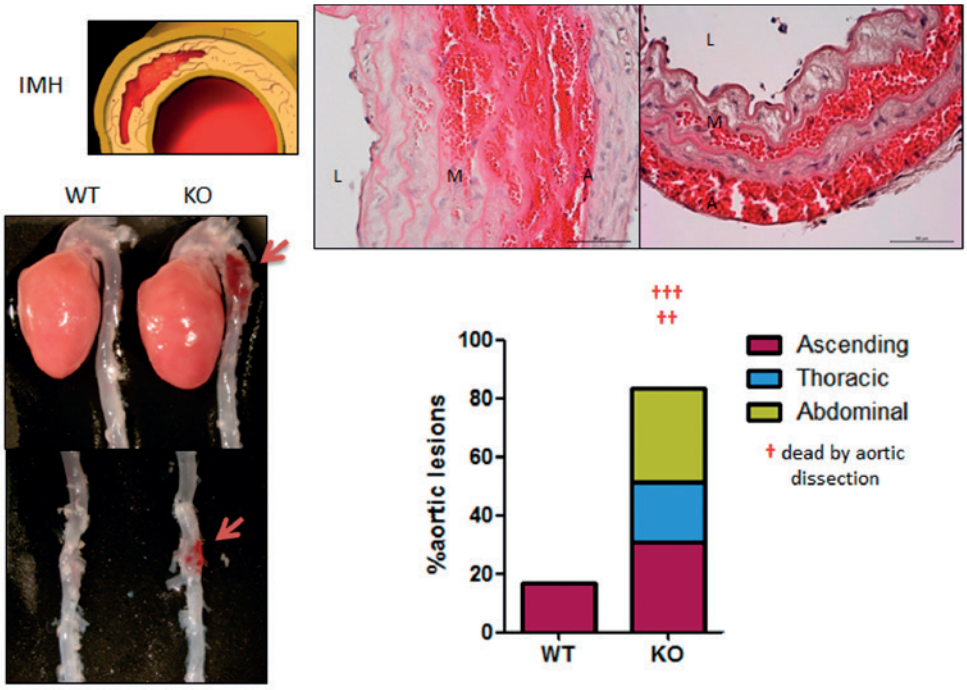
Student:
Lizet Sandra Iturri Canelas

Technicians:
Dolores López Maderuelo
Rut Alberca Rodríguez
Beatriz Carolina Ornés Poleo
Alicia Peral Rodríguez

Visiting Scientists:
Ángel Luis Armesilla Arpa
Miguel Ramón Campanero García



Ang-II induces C/EBPβ activation in the aorta. Inmunostaining of phosphorylated C/EBPβ (brown) in aortic tissue of a mouse infused with Ang-II. Nuclei are counterstained in blue.



Mouse model of aortic intramural hematoma (IMH) induced by vascular pathological stimuli. This phenotype has been identified in several mouse models of deficiency for recently identified targets in vascular wall remodeling. Intramural hematomas can develop into life-threatening aortic dissections.

MAJOR GRANTS

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

SELECTED PUBLICATIONS

Oller J, Alfranca A, Méndez-Barbero N, Villahoz S, Lozano-Vidal N, Martín-Alonso M, Arroyo AG, Escolano A, Armesilla AL, Campanero MR*, Redondo JM*. **C/EBPβ and Nuclear Factor of Activated T Cells Differentially Regulate Adamts-1 Induction by Stimuli Associated with Vascular Remodeling.** *Mol Cell Biol* (2015) 35:3409-22
* Co-corresponding authors

Martín-Alonso M, García-Redondo AB, Guo D, Camafeita E, Martínez F, Alfranca A, Méndez-Barbero N, Pollán Á, Sánchez-Camacho C, Denhardt DT, Seiki M, Vázquez J, Salaices M, Redondo JM, Milewicz D, Arroyo AG. **Deficiency of MMP17/MT4-MMP proteolytic activity predisposes to aortic aneurysm in mice**. *Circ Res* (2015) 117:e13-26

Baggott RR, Alfranca A, López-Maderuelo D, Mohamed TM, Escolano A, Oller J, Ornes BC, Kurusamy S, Rowther FB, Brown JE, Oceandy D, Cartwright EJ, Wang W, Gómez-del Arco P, Martínez-Martínez S, Neyses L, Redondo JM*, Armesilla AL*. **Plasma membrane calcium ATPase isoform 4 inhibits vascular endothelial growth factor-mediated angiogenesis through interaction with calcineurin.** *Arterioscler Thromb Vasc Biol* (2014) 34: 2310-20

Sánchez SA, Méndez-Barbero N, Santos-Beneit AM, Esteban V, Jiménez-Borreguero LJ, Campanero MR*, Redondo JM*. **Nonlinear optical 3-dimensional method for quantifying atherosclerosis burden.** *Circ Cardiovasc Imaging* (2014) 7: 566-9
* Co-corresponding authors

Escolano A, Martínez-Martínez S, Alfranca A, Urso K, Izquierdo HM, Delgado M, Martín F, Sabio G, Sancho D, Gómez-del Arco P and Redondo JM. **Specific calcineurin-targeting in macrophages confers resistance to inflammation via MKP-1 and p38**. *EMBO J* (2014) 33:1117-33



CNIC-UAM COLLABORATIVE PROGRAM Intercellular communication in the inflammatory response

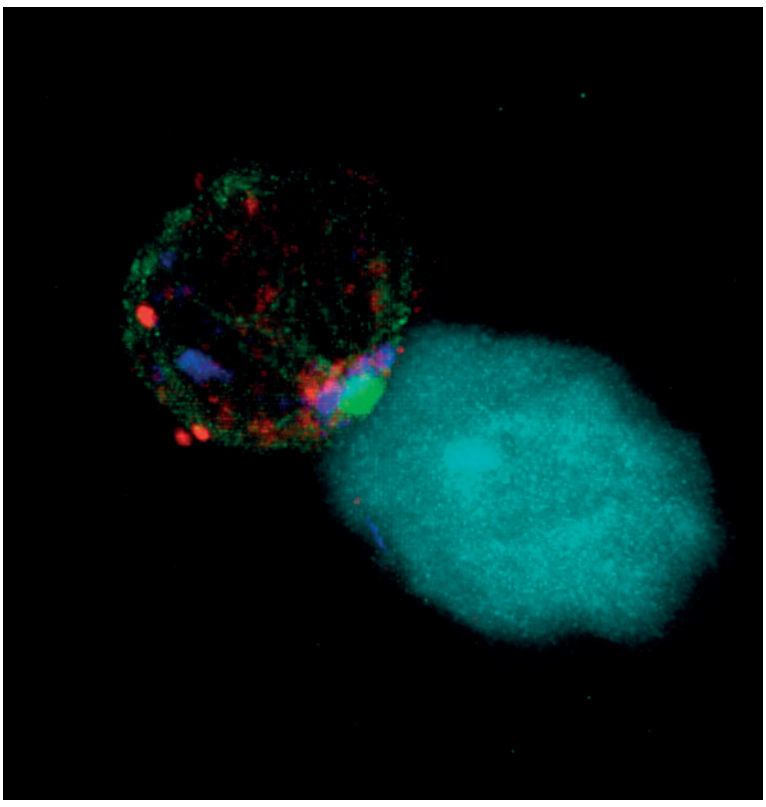
RESEARCH INTEREST

The group pursues three main lines of research.

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 and 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 immunoregulatory molecules such as CD69, galectins, aminoacid transporters and HDAC6 in animal models of atherosclerosis and psoriasis in humans in order to identify the molecular basis of these inflammatory diseases.



HDAC6 regulates lytic granule localization at the immune synapse.

Head of Laboratory

Francisco Sánchez Madrid



Research Scientists:
Gloria Martínez del Hoyo
María Mittelbrunn

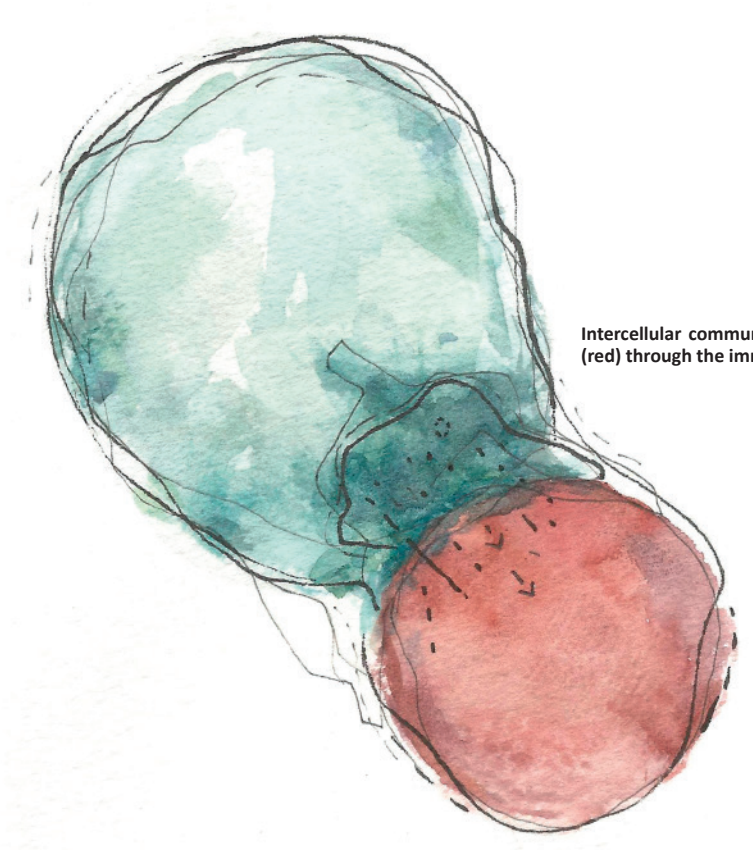
Postdoctoral Researchers:
Hortensia de la Fuente
Noa B. Martín
Vera Rocha
Danay Cibrián
Lola Fernández Messina

Predoctoral Researchers:
Francesc Baixauli
Cristina Gutiérrez
Giulia Morlino
Norman Núñez
Mª Laura Saiz
Carolina Villarroya
Olga Moreno
Noelia Blas
Eugenio Bustos
Daniel Torralba
José Pintor

Technicians:
Marta Esther Ramírez
María José López

Visiting Scientists:
María Navarro
Laura Martínez Muñoz
Aránzazu Cruz

Student:
Irene Fernández Delgado



Intercellular communication between a T cell (blue) and an antigen-presenting cell (red) through the immune synapse. Illustration by Lucía Gutiérrez Vázquez.

MAJOR GRANTS

- *European Commission. ERC Advanced Investigators Grant (ERC-2011-AdG 20110310) (GENTRIS)*
- *Ministerio de Economía y Competitividad (SAF2014-55579-R)*
- *Ministerio de Economía y Competitividad. FIS RETICS (RIC: RD12/0042/0056)*
- *Comunidad de Madrid. Redes de Excelencia. INDISNET (P2010/BMD-2332)*
- *Ministerio de Economía y Competitividad. FIS (PI11/00939) PI: Gloria Martínez del Hoyo*

SELECTED PUBLICATIONS

Mittelbrunn M, Vicente-Manzanares M, Sánchez-Madrid F. **Organizing polarized delivery of exosomes at synapses.** *Traffic* (2015) 16: 327-37

Baixauli F, Acín-Pérez R, Villarroya-Beltrí C, Mazzeo C, Nuñez-Andrade N, Gabandé-Rodriguez E, Ledesma MD, Blázquez A, Martin MA, Falcón-Pérez JM, Redondo JM, Enríquez JA, Mittelbrunn M. **Mitochondrial Respiration Controls Lysosomal Function during Inflammatory T Cell Responses**. *Cell Metab* (2015) 22:485-98

Martínez del Hoyo G, Ramírez-Huesca M, Levy S, Boucheix C, Rubinstein E, Minguito de la Escalera M, González-Cintado L, Ardavín C, Veiga E, Yáñez-Mó M, Sánchez-Madrid F. **CD81 controls immunity to Listeria infection through rac-dependent inhibition of proinflammatory mediator release and activation of cytotoxic T cells.** *J Immunol* (2015) 194:6090-101

Morlino G, Barreiro O, Baixauli F, Robles-Valero J, González-Granado JM, Villa-Bellosta R, Cuenca J, Sánchez-Sorzano CO, Veiga E, Martín-Cófreces NB, Sánchez-Madrid F. **Miro-1 links mitochondria and microtubule dynein motors to control lymphocyte migration and polarity**. *Mol Cell Biol* (2014) 34:1412-26

de la Fuente H, Cruz-Adalia A, Martinez Del Hoyo G, Cibrian-Vera D, Bonay P, Perez-Hernandez D, Vazquez J, Navarro P, Gutierrez-Gallego R, Ramirez-Huesca M, Martin P, Sanchez-Madrid F. **The Leukocyte Activation Receptor CD69 Controls T Cell Differentiation through Its Interaction with Galectin-1.** *Mol Cell Biol* (2014) 34: 2479-87



Cardiovascular proteomics

Head of Laboratory
Jesús María Vázquez Cobos



RESEARCH INTEREST

Our group works on the development of high-throughput quantitative approaches for the dynamic analysis of the deep proteome, which are being applied to basic and translational projects in the cardiovascular field. We are developing novel bioinformatics algorithms for the analysis of very large numbers of samples, including protein identification and systems biology interpretation of quantitative data, and for the study of posttranslational modifications (PTM).

Among other projects, we are using these approaches to explore the molecular mechanisms underlying the byphasic pattern of edema in the pig heart after infarction, and the preconditioning effect of treatments that ameliorate the heart damage produced by ischemia/reperfusion.

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 PTMs. 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 novel factors and PTMs that modulate complex and supercomplex assembly.

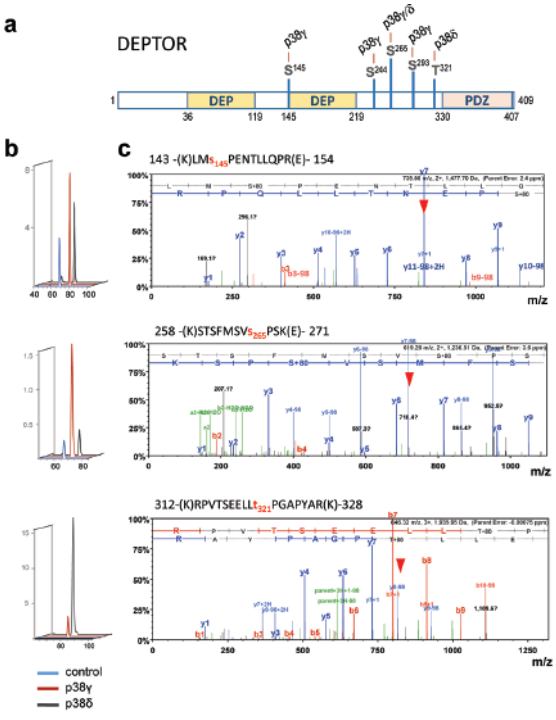
We are also performing translational studies in large cohorts of human samples to uncover molecular mechanisms and biomarkers of cardiovascular disease. We are currently undertaking a high-throughput proteomics analysis of plasma from participants in the PESA study, in the search for factors that correlate with the extent of subclinical atherosclerotic events such as calcium deposition and plaque formation. We are also setting up a mass spectrometry-based platform for targeted and hypothesis-free analysis of lipids and small metabolites.

Postdoctoral Researchers:
Estefanía Núñez Sánchez
Elena Bonzón Kulichenko
Inmaculada Jorge Cerrudo
Alessia Ferranini
Spyridon Michalakopoulos

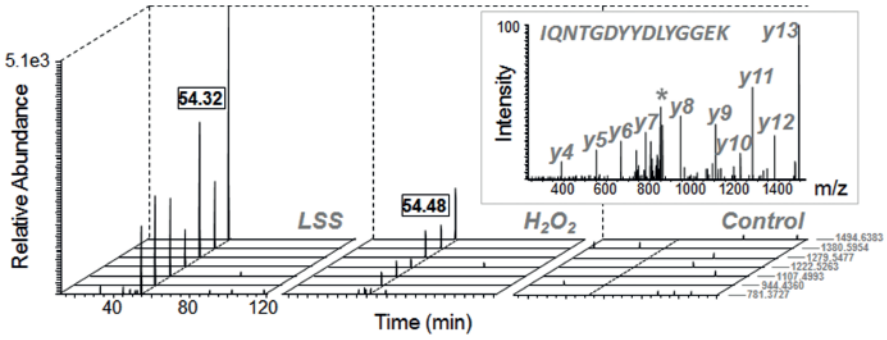
Predoctoral Researchers:
Fernando García Marqués
Marco Trevisan Herraz
Marta Loureiro
Navratan Bagwan
Aleksandra Ronja

Masters Students:
Celia Castañs García
Jesús Lavado García

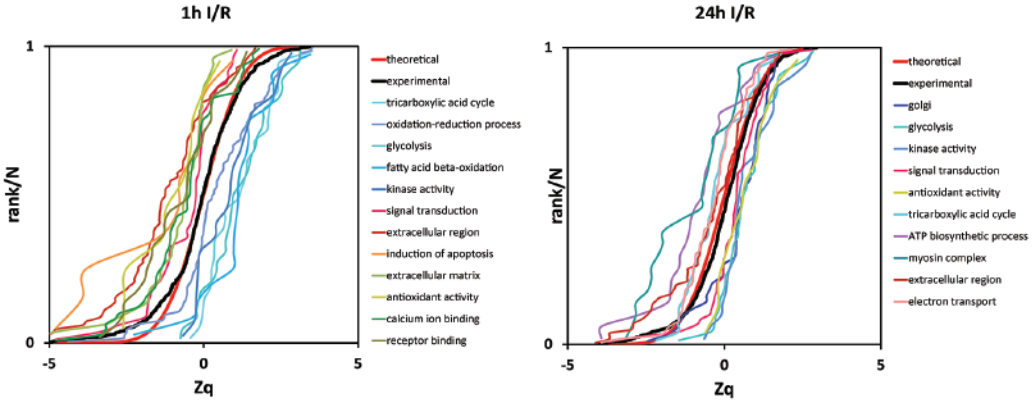
Visiting Scientists:
Elena Burillo
Diego Martínez López
Montserrat Baldán



Analysis of DEPTOR phosphorylation by p38γ and p38δ kinases *in vivo* by mass spectrometry. (a) Scheme of DEPTOR phosphorylation sites. (b) Quantitative analysis of phosphorylation. (c) MS/MS spectra of each phosphopeptide, showing its sequence and assignation of the modified site.



Phosphatase SHP2 is sulfenylated in conditions of laminar shear stress (LSS). Sulfenylated SHP2 was immunoprecipitated using a specific antibody and the amount of SHP2 was quantified by mass spectrometry. Seven of the fragments of the SHP2 peptide indicated in the inset were quantified, showing that sulfenylation of SHP2 takes place when the samples were treated with H_2O_2 or subjected to LSS.



Systems biology analysis of protein abundance changes in a pig infarct model. A high-throughput quantitative proteomics analysis was performed to compare heart tissue from infarcted and remote areas after 1h ischemia and 1h or 24 hreperfusion. The analysis revealed a clearly coordinated behavior of proteins belonging to the indicated functional categories.

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)*
- *Fundació La Marató de TV3*

SELECTED PUBLICATIONS

González-Terán B, López JA, Rodríguez E, Leiva L, Martínez-Martínez S, Bernal JA, Jiménez-Borreguero LJ, Redondo JM, Vazquez J, Sabio G. **p38γ and δ promote heart hypertrophy by targeting the mTOR-inhibitory protein DEPTOR for degradation.** *Nat Commun* (accepted)

Osorio FG, Soria-Valles C, Santiago-Fernández O, Bernal T, Mittelbrunn M, Colado E, Rodríguez F, Bonzon-Kulichenko E, Vázquez J, Porta-de-la-Riva M, Cerón J, Fueyo A, Li J, Green AR, Freije JMP, López-Otín C. **Loss of the proteostasis modulator AIRAPL causes myeloid transformation by deregulating IGF-1 signaling.** *Nat Med* (doi:10.1038/nm.4013. Epub 2015 Dec 21)

Mara Martín-Alonso M, García-Redondo AB, Guo D, Camafeita E, Martínez F, Sánchez-Camacho C, Pollán Á, Alfranca A, Seiki M, Redondo JM, Vázquez J, Salaices M, Milewicz D, Arroyo AG. **Deficiency of MT4-MMP proteolytic activity causes a dilative arterial disorder.** *Circ Res* (2015) 117: e13-26

Bonzon-Kulichenko E, Garcia-Marques F, Trevisan-Herraz M, Vázquez J. **Revisiting peptide identification by high-accuracy mass spectrometry: problems associated to the use of narrow mass precursor windows.** *J Proteome Res* (2015) 14: 700-10

Navarro P, Trevisan-Herraz M, Bonzon-Kulichenko E, Núñez E, Martínez-Acedo P, Pérez-Hernández D, Jorge I, Mesa R, Calvo E, Carrascal M, Hernáez ML, García F, Bárcena JA, Ashman K, Abian J, Gil C, Redondo JM, Vázquez J. **General statistical framework for quantitative proteomics by stable isotope labeling.** *J Proteome Res* (2014) 13: 1234-47