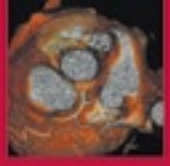




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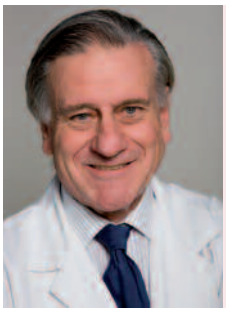

Dear friends,

With the publication of this CNIC Scientific Report for 2008, it seems appropriate first to reflect on the progress made at the Centre in the three years of my association with it. The CNIC was created to be the nexus of an expanded Spanish contribution to cardiovascular research and to play a leading role in the application of research results in clinical practice, both nationally and internationally. This prioritization of translational research is reflected in the Centre's organization, defined in 2006, in which three basic research departments—Vascular Biology and Inflammation, Cardiovascular Developmental Biology and Regenerative Cardiology—are complemented by three with a more directly clinical orientation—Atherothrombosis and Imaging, Epidemiology and Translational Research. The creation of these departments saw the recruitment of new staff, including at the most senior level, doubling the number of researchers and laying the foundation for the future growth of the Centre.

This growth continued in 2008 with the incorporation of José Luis de la Pompa (Developmental Biology), José Antonio Enríquez (Regenerative Cardiology) and Vicente Andrés (Atherothrombosis and Imaging), whose research groups are scheduled to move to the CNIC in 2009. Last year also saw the creation of new core facilities in Cellomics and Microscopy Imaging, further consolidating the CNIC's infrastructure for advanced biomedical research; and the restaffing of the Comparative Medicine Unit instigated a major project to create the capacity for large animal work, essential for preclinical evaluation of basic research findings. Another important advance was the definition of the formulation for the CNIC Polypill, which is now set to go into clinical development. 2008 was also a landmark year for science communication, with the inaugural CNIC symposium in July and the first meeting on translational research in November. Most important of all, all of this activity has been accompanied by the increasing scientific production evident in the pages of this report.

Another key strategic objective at the CNIC is the discovery and professional training of new researchers, and we have devised tailored programmes for young investigators and more experienced research staff at each stage of their careers. New programmes launched in 2008 mostly focused on translational research (CardioJoven and Cardio-Image), but the CNIC also cemented its relationship with the university sector through its participation in the Masters-PhD programme.

The CNIC continues to benefit tremendously from the support of the Pro-CNIC Foundation, a not-for-profit consortium of some of the major players in Spanish civil society that injects significant private money and also introduces welcome managerial and business expertise. This innovative public-private partnership is fundamental to the CNIC's agility and dynamism, and I would particularly like to acknowledge the Pro-CNIC members' steadfast commitment to our project.

Looking back on 2008, it is very satisfactory to see the progress made across the full range of the CNIC's activities. Nonetheless, last year was also marked by great sadness, with the tragic death of Elisa Bello, Head of the Library and Information Service. Elisa was a loved respected colleague and her loss is keenly felt by everyone at the CNIC.

I look forward to seeing the CNIC's progress continue in the coming years, and I am confident that the Centre will rapidly establish itself as a reference centre for basic and applied cardiovascular research.

Valentín Fuster.

Scientific President

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The CNIC is organized into six research departments, supported by an integrated infrastructure of core technical units.

Vascular Biology and Inflammation

The groups in the VBI department use a variety of molecular, cellular, tissue and animal models to investigate the functioning of the vascular system in physiological settings and in the pathological inflammatory situations that lie at the root of atherothrombotic disease.

Regenerative Cardiology

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

Cardiovascular Developmental Biology

The CDB department investigates the origin, differentiation and integration of cardiovascular cell lineages in chick, mouse, and zebrafish models, using complementary experimental embryology, genetic and mass screening approaches. This focus is complemented by studies in classical models such as the limb primordium and mammalian blastocyst.

Atherothrombosis and Cardiovascular Imaging

The ACI department is dedicated to developing non-invasive technologies for molecular-resolution imaging. Through these technologies, vulnerable plaques can be identified and characterized, providing invaluable information on the underlying molecular mechanisms of disease and leading to tools for accurate diagnosis and targeted drug delivery.

Cardiovascular Epidemiology and Population Genetics

This multidisciplinary department integrates population studies with the latest advances in basic and clinical research to identify environmental and genetic risk factors underlying the incidence, development and prognosis of cardiovascular disease. The aim is to devise effective strategies for disease prevention and improved healthcare delivery.

Translational Cardiovascular Research

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

Technical Units

The CNIC's technical units and other service facilities provide advanced support with a range of core methodologies. The following units are currently established: Comparative Medicine, Transgenesis, Gene Targeting and Viral Vectors, Microscopy and Dynamic Imaging, Cellomics, Proteomics, and Genomics.

Intercellular Communication in the Inflammatory Response

Head of Laboratory: ***Francisco Sánchez-Madrid***

Research Scientists: *Pilar Martín*
Juan Manuel Serrador
María Yáñez-Mó

Postdoctoral Researchers: *Olga Barreiro*
Hortensia de la Fuente
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Predoctoral Researchers: *Arantxa Cruz*
Almudena García
Cristina Gutiérrez
Mónica Sala-Valdés
María Sales Ibiza
Emilio Tejera

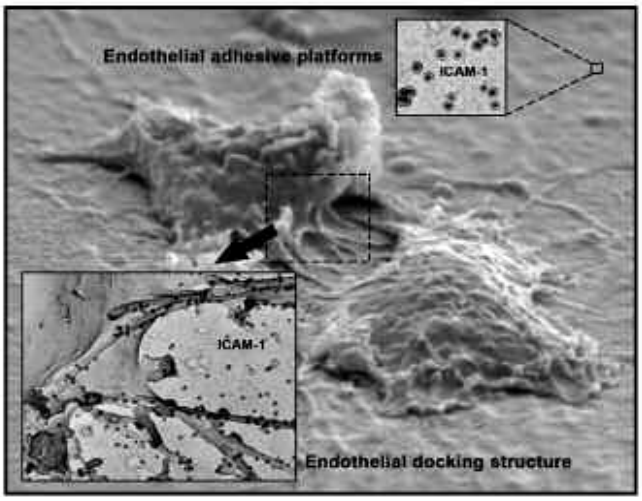
Technician: *Marta Ramírez*

RESEARCH INTEREST

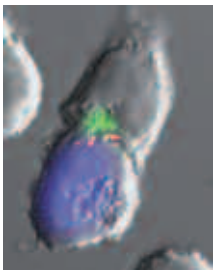
An understanding of the mechanisms through which immune-inflammatory responses are activated and amplified requires detailed knowledge of the molecular and cellular interactions that control the extravasation, orientation and directed migration of leukocytes, as well as the steps in the formation of the immunological synapse. Initiation of the immune response requires controlled recruitment of membrane receptors to specific locations and local activation of kinases, other regulatory molecules and cytoskeletal components.

Our research addresses the supra-molecular organization of membrane microdomains that combine to form adhesive platforms which integrate arrays of adhesion receptors (integrins- and IgSF member-tetraspanin complexes). These platforms act in a spatio-temporally coordinated manner during leukocyte–endothelial and cognate T lymphocyte–antigen presenting cell (APC) interactions. We also study the connections of these platforms to the cytoskeletal network and the signalling pathways that coordinate proper adhesion and migration. Our experimental strategy involves novel molecular dynamic studies using advanced and innovative analytical microscopy techniques in primary living cells. Much of our work is done in mice deficient for adhesion or regulatory receptors, to establish the roles of these molecules in chronic inflammatory processes and autoimmune diseases. We are applying these approaches in models of allergic asthma and experimental autoimmune myocarditis, using genetically modified mice (CD69 and PSGL-1).

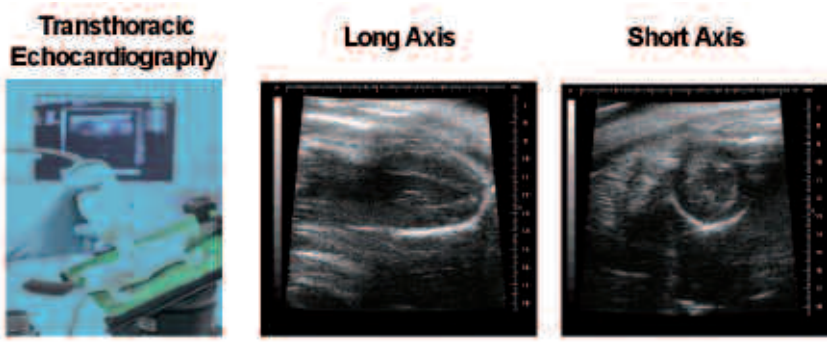
We are also interested in T lymphocyte synthesis of nitric oxide (NO) and other soluble mediators that regulate the production of pro-inflammatory cytokines. Our interest here centres on the molecular mechanisms regulating polarized secretion during antigen-dependent T cell–APC interactions and the regulatory role of T-cell derived NO in the development of the chronic inflammation associated with cardiovascular disease.



Endothelial adhesive platforms, stained for ICAM-1 in the image, spatio-temporally organize molecules with similar characteristics and functions at the plasma membrane to facilitate their coordinated action in the formation of docking structures during extravasation, a crucial process for the inflammatory response that requires rapid kinetics.



Localization of eNOS (green) on the Golgi apparatus and CD3 (red) in the immune synapse of a T lymphocyte in contact with a superantigen-E–pulsed antigen presenting B cell (blue).



Echocardiogram analysis of heart dysfunction in a CD69-deficient mouse model of experimental autoimmune myositis (EAM).

MAJOR GRANTS

Plan Nacional de I+D+I. MICINN (SAF2008-02635 to FSM)

FUNDACIÓN GENOMA ESPAÑA. MEICA Project (coordinator, FSM).

ISCIII (Red RECAVA to FSM)

Plan Nacional de I+D+I. MICINN (SAF2008-02719 to PM)

ISCIII (PI070356 to JMS)

SELECTED PUBLICATIONS

Olazabal IM, Martin-Cofreces NB, Mittelbrunn M, Martinez Del Hoyo G, Alarcon B and Sanchez-Madrid F. **Activation outcomes induced in naive CD8 T-cells by macrophages primed via "phagocytic" and nonphagocytic pathways**. *Mol Biol Cell* (2008) 19: 701-710

Valenzuela-Fernandez A, Cabrero JR, Serrador JM and Sanchez-Madrid F. **HDAC6: a key regulator of cytoskeleton, cell migration and cell-cell interactions**. *Trends Cell Biol* (2008) 18: 291-297

Ibiza S, Perez-Rodriguez A, Ortega A, Martinez-Ruiz A, Barreiro O, Garcia-Dominguez CA, Victor VM, Esplugues JV, Rojas JM, Sanchez-Madrid F and Serrador JM. **Endothelial nitric oxide synthase regulates N-Ras activation on the Golgi complex of antigen-stimulated T cells**. *Proc Natl Acad Sci USA* (2008) 105: 10507-10512

Yanez-Mo M, Barreiro O, Gonzalo P, Batista A, Megias D, Genis L, Sachs N, Sala-Valdes M, Alonso MA, Montoya MC, Sonnenberg A, Arroyo AG and Sanchez-Madrid F. **MT1-MMP collagenolytic activity is regulated through association with tetraspanin CD151 in primary endothelial cells**. *Blood* (2008) 112: 3217-3226

Martin-Cofreces NB, Robles-Valero J, Cabrero JR, Mittelbrunn M, Gordon-Alonso M, Sung CH, Alarcon B, Vazquez J and Sanchez-Madrid F. **MTOC translocation modulates IS formation and controls sustained T cell signaling**. *J Cell Biol* (2008) 182: 951-962

Barreiro O, Zamai M, Yanez-Mo M, Tejera E, Lopez-Romero P, Monk PN, Gratton E, Caiolfa VR and Sanchez-Madrid F. **Endothelial adhesion receptors are recruited to adherent leukocytes by inclusion in preformed tetraspanin nanoplatforms**. *J Cell Biol* (2008) 183: 527-542

Regulation of Gene Expression in Vascular Endothelium

Head of Laboratory: ***Juan Miguel Redondo***

Research Scientist: *Eva Cano*

Postdoctoral Researchers: *Arántzazu Alfranca*
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Perla Niño Moreno

Predoctoral Researchers: *Amelia Escolano*
Katia Urso

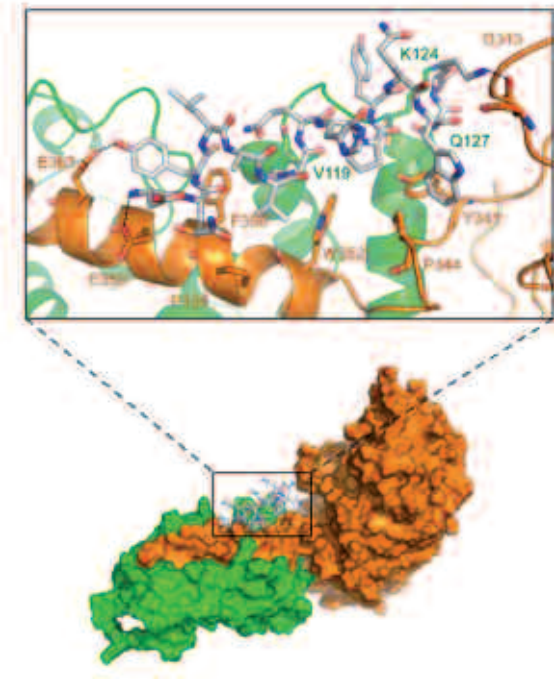
Technicians: *Ana Guio*
Dolores López
Isabel Mirones
Belen Ramirez
Raquel Sánchez
Felipe Were

RESEARCH INTEREST

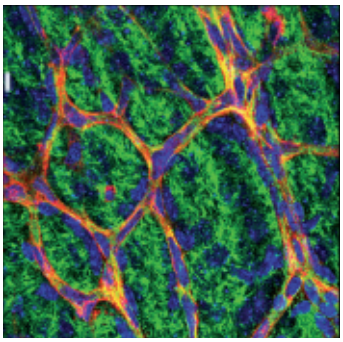
The calcium-calcineurin-NFAT (CN-NFAT) pathway regulates important biological processes, including development of the immune, vascular and nervous systems, heart-valve morphogenesis and pancreatic beta-cell function, and is implicated in many related pathological processes. We study the regulation and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

Regarding angiogenesis, we have addressed the regulation of NFAT in endothelial cells by VEGF and the profile and actions of prostanoids released by activated endothelium. Our results suggest that endothelium-derived PGH_2 can serve as the substrate for PGE_2 synthesis by neighbouring tumour cells. Related studies show that PGE_2-induced angiogenesis is mediated by Alk5-Smad3 signalling, via MT1-MMP-mediated cleavage of TGF-ß. We are currently exploring the gene expression programme triggered by Ang-II in cardiomyocytes and vascular smooth muscle and the role of CN-NFAT signalling in this process.

A separate area of interest relates to the inflammatory reaction initiated by stroke. Cerebral ischemia triggers local production of inflammatory mediators, of which glial cells are an efficient source. This production sustains immune-inflammatory signalling if not halted by endogenous or exogenous anti-inflammatory agents. We are interested in the signalling pathways that contribute to lesion expansion, or conversely have a role in lesion containment and repair of the injured brain. In this context, we have been studying the role of calcium-dependent pathways in astroglial cells.



Docking and molecular dynamic simulations of binding between calcineurin and the NFAT-derived LxVP peptide. LxVP (sticks) is positioned parallel to the ß-subunit-binding helix of CnA (orange) and forms additional contacts with residues in CnB (green).



Section of retina from the eye of a 4-day old mouse, immunostained for isolectin B4 (red) and NFATc3 (green). Blue shows Hoechst staining.

MAJOR GRANTS

MICINN (SAF2006-08348 to JMR)

ISCIII (Red RECAVA to JMR)

S-BIO-0194-2006 (CARDIOVREP-Comunidad de Madridto JMR)

ISC III (PI06/0491 to EC).

SELECTED PUBLICATIONS

Canellada A, Ramirez BG, Minami T, Redondo JM and Cano E. **Calcium/calcineurin signaling in primary cortical astrocyte cultures Rcan1-4 and cyclooxygenase-2 as NFAT target genes**. *Glia* (2008) 56: 709-722

Alfranca A, Lopez-Oliva JM, Genis L, Lopez-Maderuelo D, Mirones I, Salvado D, Quesada AJ, Arroyo AG and Redondo JM. **PGE_2 induces angiogenesis via MT1-MMP-mediated activation of the TGFbeta/Alk5 signaling pathway**. *Blood* (2008) 112: 1120-1128

Armand AS, Bourajjaj M, Martinez-Martinez S, Azzouzi HE, da Costa Martins PA, Hatzis P, Seidler T, Redondo JM and De Windt LJ. **Cooperative synergy between NFAT and MyoD regulates myogenin expression and myogenesis**. *J Biol Chem* (2008) 283: 29004-29010

Rodríguez A, Roy J, Martínez-Martínez S, López-Maderuelo MD, Niño-Moreno P, Ortí L, Pantoja-Uceda D, Pineda-Lucena A, Cyert MS, Redondo JM. **A conserved docking surface on calcineurin mediates interaction with substrates and immunosuppressants**. *Mol Cell* (accepted)

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

Integrin Signalling

Head of Laboratory: ***Miguel Ángel del Pozo***

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Predoctoral Researchers:
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Marta C. Guadamillas
Olivia Muriel
Miguel Foronda

Technicians:
Dácil M. Pavón
Sara Sánchez

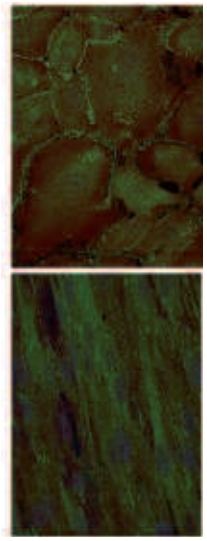
RESEARCH INTEREST

Cell function relies on integrated signal transduction by complex networks of proteins. We are investigating the mechanisms through which integrins (the main ECM receptors), Rho/Rac GTPases and caveolin-1 together regulate key processes in the pathogenesis of inflammatory and cardiovascular diseases: gene expression, cell cycle progression, migration, polarization, membrane trafficking and epithelial-mesenchymal transition (EMT). Our earlier work showed that integrins regulate membrane targeting of Rac/Rho family GTPases via cholesterol-enriched membrane microdomains (CEMM), and that internalization of Rac binding sites in CEMM is mediated by caveolin.

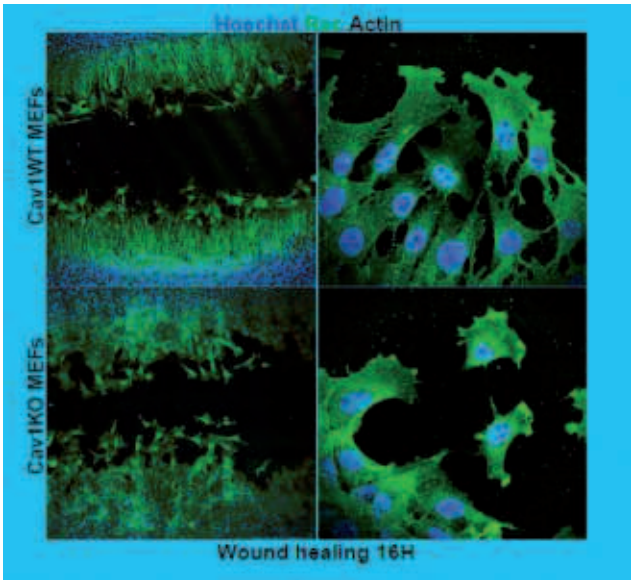
More recently we showed that Cav-1 regulates cell polarity and directional migration through coordination of Src kinase and Rho GTPase signalling. Cells from Cav-1 knockout mice lose normal cell polarity, exhibit impaired wound healing, and have decreased Rho activity and increased Rac and Cdc42 GTPase activities. These *ex vivo* findings support the altered phenotype observed *in vivo* in processes such as angiogenesis and dermal wound repair, which is defective in Cav-1 deficient mice. We are currently pursuing this question in a 3D setting.

We propose that by modulating vesicular trafficking, caveolin connects integrin-dependent adhesion to the membrane targeting of signalling molecules and their subsequent coupling to downstream effectors. In collaboration with the Cellomics Unit we are testing this through genome-wide RNAi-mediated knock-down combined with state-of-the-art fluorescence and live-imaging microscopy.

We have also reported a role for ERK/NF-κB/Snail1 signalling in the establishment of the EMT induced by inflammatory cytokines in primary human peritoneal mesothelial cells (MCs), in which epithelial cells are transformed into more migratory, fibroblast-like cells.



EMT of primary human peritoneal mesothelial cells. *Confocal immunofluorescence of untreated MCs (top) or MCs treated with TGFß1 (0.5 ng/ml) in combination with IL-1ß (0.5 ng/ml) for 56 hours (bottom). Images show immunostaining for the epithelial markers cytokeratin (red) and the tight junction component ZO-1 (green). Downregulation and remodelling of epithelial markers is characteristic of EMT.*



Absence of caveolin-1 expression in mouse embryonic fibroblasts (MEFs) correlates with decreased directionality of cell migration, decreased polarization and increased plasma membrane targeting of the small GTPase Rac

MAJOR GRANTS

EURYI Award (funded by ESF and EUROHORCS to MDP)

EMBO Young Investigator Programme Award (to MDP)

MICINN (SAF2008-02100 to MDP)

ISCIII (Redes Temáticas de Investigacion Cooperativa RD06/0020/1033 to MDP)

SELECTED PUBLICATIONS

Strippoli R, Benedicto I, Pérez Lozano ML, Cerezo A, López-Cabrera M and del Pozo MA. E**pithelial-to-mesenchymal transition of peritoneal mesothelial cells is regulated by an ERK/NF-kappaB/Snail1 pathway**. *Dis Model Mech* (accepted)

Grande-Garcia A and del Pozo MA. **Caveolin-1 in cell polarization and directional migration**. *Eur J Cell Biol* (2008) 87: 641-647

Santibanez JF, Blanco FJ, Garrido-Martin EM, Sanz-Rodriguez F, del Pozo MA and Bernabeu C. **Caveolin-1 interacts and cooperates with the transforming growth factor-beta type I receptor ALK1 in endothelial caveolae**. *Cardiovasc Res* (2008) 77: 791-799

Grande-García A, Echarri A, de Rooij J, Alderson NB, Waterman-Storer CM, Valdivielso JM and del Pozo MA. **Caveolin-1 regulates cell polarization and directional migration through Src kinase and Rho GTPases**. *J Cell Biol* (2007) 177: 683-694

del Pozo MA and Schwartz MA. **Rac, membrane heterogeneity, caveolin and regulation of growth by integrins**. *Trends Cell Biol* (2007) 17: 246-50

Matrix Metalloproteinases in Angiogenesis and Inflammation

Head of Laboratory: ***Alicia G. Arroyo***

Postdoctoral Researchers: *Pilar Gonzalo*
Rubén A. Mota
Fernando Neria

Predoctoral Researchers: *Victoria Hernández de Riquer*
Agnieszka Koziol
Vanessa Moreno
Emilio Tejera

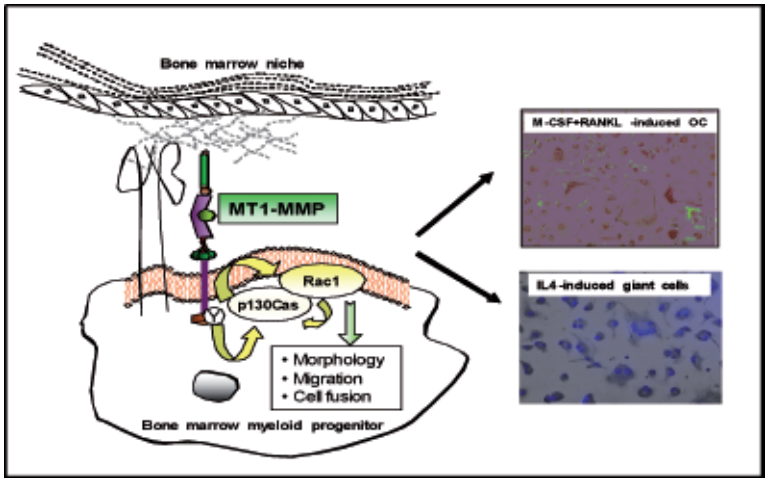
Technician: *Ángela Pollán*

RESEARCH INTEREST

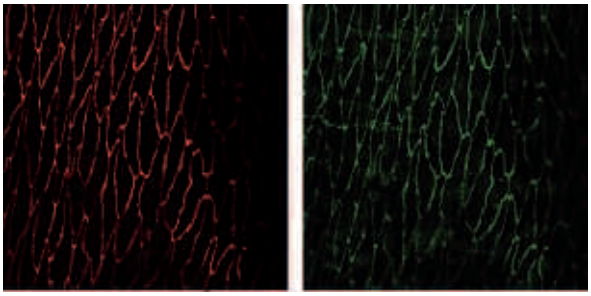
Our general area of interest is the role of matrix metalloproteinases (MMPs)—particularly membrane-type (MT) MMPs—in angiogenesis and inflammation. Our work has characterized the function and regulation of MT1-MMP in chemokine and nitric oxide-induced angiogenesis, myeloid cell fusion, and monocyte migration and transmigration. More recently we have focused on MT4-MMP, a leukocyte-expressed GPI-anchored protease of unknown activity and function.

We are currently conducting proteomic studies to identify the collection of cellular substrates (degradome) distinctly processed by MT1-MMP and MT4-MMP in endothelial cells and leukocytes, and further efforts are directed at defining the molecular networks in which these proteases participate in these cell types. The functional impact of MT1-MMP and MT4-MMP is being explored through studies in cell-based systems and in genetically modified mouse models of angiogenesis, leukocyte recruitment and inflammatory disorders such as atherosclerosis. We also have an interest in the characterization of new molecules of potential relevance to vascular integrity and angiogenesis, such as extracellular matrix metalloproteinase inducer (EMMPRIN).

By developing these projects we will extend our knowledge of where, when and how MT-MMPs and their regulators modulate endothelial and leukocyte behaviour during the establishment and progression of chronic inflammatory disorders.



MT1-MMP contributes to macrophage cell fusion during osteoclast development and giant cell formation through the regulation of Rac1 signalling in myeloid cells.



VE-cadherin immunostaining (green) reveals the integrity of the mouse aortic wall. The red staining shows the distribution of the tight junction protein ZO-1.

MAJOR GRANTS

ISCIII (Redes Temáticas de Investigación Cooperativa RETIC RECAVA RD06/0014/1016 to AA)

MICINN (SAF2008-02104 to AA).

FUNDACIÓN GENOMA ESPAÑA. MEICA Project (to AA).

SELECTED PUBLICATIONS

Alfranca A, Lopez-Oliva JM, Genis L, Lopez-Maderuelo D, Mirones I, Salvado D, Quesada AJ, Arroyo AG and Redondo JM. **PGE_2 induces angiogenesis via MT1-MMP-mediated activation of the TGFbeta/Alk5 signaling pathway**. *Blood* (2008) 112: 1120-1128

Yanez-Mo M, Barreiro O, Gonzalo P, Batista A, Megias D, Genis L, Sachs N, Sala-Valdes M, Alonso MA, Montoya MC, Sonnenberg A, Arroyo AG and Sanchez-Madrid F. **MT1-MMP collagenolytic activity is regulated through association with tetraspanin CD151 in primary endothelial cells**. *Blood* (2008) 112: 3217-3226

Genís L, Gonzalo P, Tutor AS, Gálvez BG, Martínez-Ruiz A, Zaragoza C, Lamas S, Tryggvason K, Apte SS, Arroyo AG. **Functional interplay between endothelial nitric oxide synthase and membrane type 1 matrix metalloproteinase in migrating endothelial cells**. *Blood* (2007) 110: 2916-2923

Bravo-Cordero JJ, Marrero-Diaz R, Megías D, Genís L, García-Grande A, García MA, Arroyo AG, Montoya MC. **MT1-MMP proinvasive activity is regulated by a novel Rab8-dependent exocytic pathway**. *EMBO J* (2007) 26:1499-1510

Gene Expression & Genetic Stability in Somatic Stem Cells

Head of Laboratory: ***Antonio Bernad***

Research Scientists: *Enrique Samper*
Manuel A. González
Antonio Díez

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Kausalia Vijayaragavan
Isabel Moscoso

Predoctoral Researchers: *Silvia García*
Alberto Izarra
Íñigo Valiente
María Tomé
David Lara
Beatriz Escudero

Support Scientists: *Candelas Carreiro*
Carmen Albo

Technician: *Vanessa Blanca*

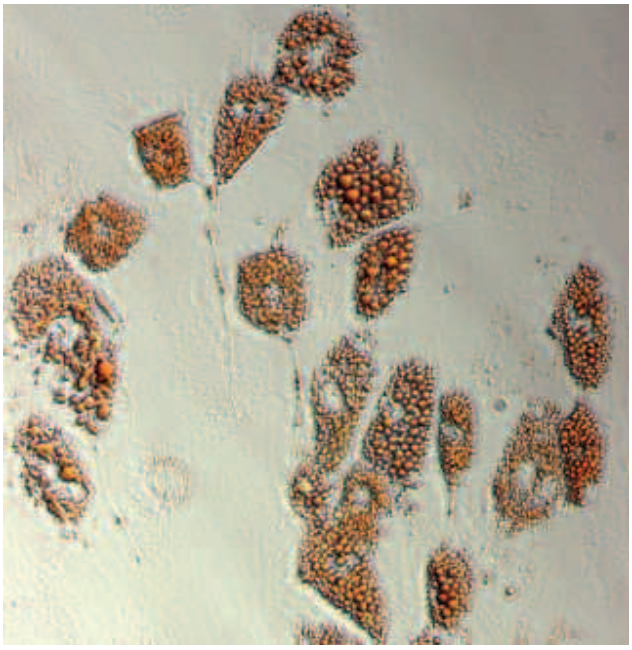
RESEARCH INTEREST

Adult stem cells (aSC) are crucial for the maintenance of organ homeostasis throughout life, and the genetic health of aSC is thus required to prevent disease, delay aging and counteract tissue damage. As they accumulate damage, somatic stem cells initiate senescence or apoptosis or can become genetically unstable, impacting negatively on the health of the organism. To understand how stem cells control the processes of auto-renewal and differentiation, we focus on several related areas, working mainly with murine and human mesenchymal cells (MSC) and cardiac stem cells (CSC).

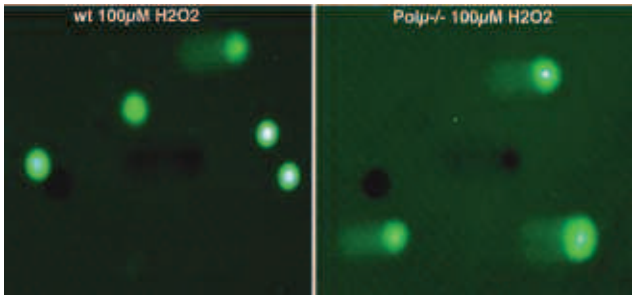
Our studies on stem cell genetic stability currently centre on the maintenance of haematopoietic stem cells, aging, tumour suppression, and the role of polymerase μ in DNA repair. Additionally, we are investigating the role of cell culture associated oxidative stress in the generation of genetic instability and senescence in MSCs. In the near future we plan to study the role of mitochondrial DNA repair in the maintenance of cardiovascular health by creating novel animal models of dilated cardiomyopathy.

Concerning the regulation of stem cell gene expression programmes, we have identified several microRNAs (miRNAs) as potential regulators of human MSC self-renewal or differentiation. We are now engaged in the functional characterization of these miRNAs and the identification of their target genes in MSCs.

Finally, we are investigating the role in post-injury cardiac healing played by the complement factors C3a and C5a expressed in different cell populations (macrophages, multipotent cells and cardiomyocytes).



Adipogenic differentiation of human mesenchymal stem cells isolated from bone marrow.



Comet assay to measure DNA breaks in bone marrow cells from wt or polμ$^{-/-}$ mice after exposure to oxidative stress (H_2O_2 treatment).

MAJOR GRANTS

FUNDACIÓN GENOMA ESPAÑA. MEICA Project (to AB)

ISCIII (Red de Investigación Cooperativa Terapia Celular TerCel to AB)

MICINN (SAF2005-08064-C04-01 to AB)

SELECTED PUBLICATIONS

Eswaran J, Bernad A, Ligos JM, Guinea B, Debreczeni JE, Sobott F, Parker SA, Najmanovich R, Turk BE and Knapp S. **Structure of the human protein kinase MPSK1 reveals an atypical activation loop architecture**. *Structure* (2008) 16: 115-124

Servert P, Diaz V, Lucas D, de la Cueva T, Rodriguez M, Garcia-Castro J, Alonso J, Martinez-A C, Gonzalez M and Bernad A. **In vivo site-specific recombination using the beta-rec/six system**. *BioTechniques* (2008) 45: 69-78

Samper E, Morgado L, Estrada JC, Bernad A, Hubbard A, Cadenas S and Melov S. **Increase in mitochondrial biogenesis, oxidative stress, and glycolysis in murine lymphomas**. *Free Radic Biol Med* (accepted)

Li X, Tjwa M, Van Hove I, Enholm B, Neven E, Paavonen K, Jeltsch M, Juan TD, Sievers RE, Chorianopoulos E, Wada H, Vanwildemeersch M, Noel A, Foidart JM, Springer ML, von Degenfeld G, Dewerchin M, Blau HM, Alitalo K, Eriksson U, Carmeliet P and Moons L. **Reevaluation of the role of VEGF-B suggests a restricted role in the revascularization of the ischemic myocardium**. *Arterioscler Thromb Vasc Biol* (2008) 28: 1614-1620

Gozalbo-Lopez B, Andrade P, Terrados G, de Andres B, Serrano N, Cortegano I, Palacios B, Bernad A, Blanco L, Marcos MA and Gaspar ML. **A role for DNA polymerase Ì in the emerging DJH rearrangements of the post-gastrulation mouse embryo**. *Mol Cell Biol* (accepted)

Stem Cell Signalling

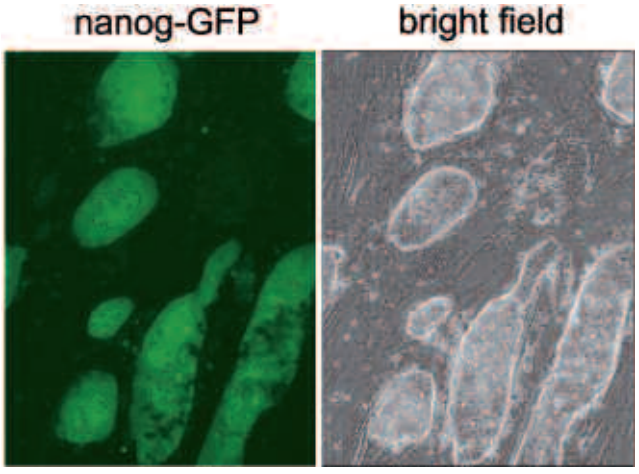
Head of Laboratory: ***Kenneth McCreath***

Research Scientist: *Ana Cervera*

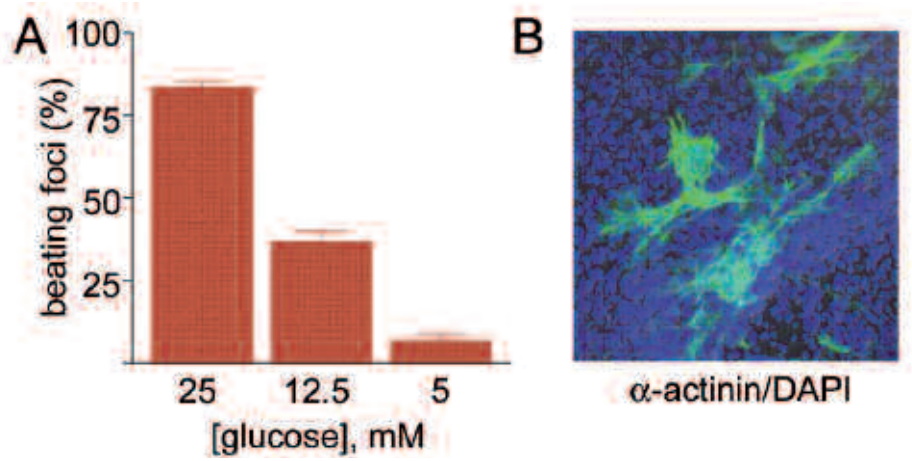
Predoctoral Researchers: *Verónica Sobrado*
Laura Gómez Cabañas
Francisco Luna-Crespo

RESEARCH INTEREST

The classical definition of mitochondria as the powerhouse of the cell has recently been overhauled by the fact that, besides directing oxidative phosphorylation and consequent generation of ATP, mitochondria integrate a plethora of intrinsic and extrinsic pathways. Using embryonic stem (ES) and induced pluripotential stem (iPS) cells as *in vitro* models, we are investigating the participation of mitochondria in the maintenance of stem cell pluripotency and their capacity for differentiation. In particular we are examining the role of mitochondrial-generated reactive oxygen species as signalling molecules during differentiation to the mesodermal/cardiovascular lineage. Furthermore, given that the inability of postnatal cardiac muscle cells to proliferate presents a major barrier to the functional restoration of the diseased heart, we are devising protocols for the directed differentiation of human iPS cells to cardiac progenitor populations, in the hope that these cells can be used in a therapeutic context. We are also examining the possibility that patient-derived stem cells with congenital defects might provide valuable models of cardiovascular disease. We have recently shown that dysfunction of succinate dehydrogenase (complex II of the mitochondrial respiratory chain) leads to a cellular phenotype characteristic of tumour progression. We are currently testing the hypothesis that succinate dehydrogenase deficiency leads to dysregulated histone modification, which most probably affects chromatin structure and gene expression.



Murine iPS cells were grown in the undifferentiated state as feeder-dependent cells with mitomycin-inactivated MEFs. GFP expression is driven by a knock-in of GFP at the NANOG locus.



Left: Numbers of foci of spontaneously beating cells in ES cultures differentiated for 10 days in the presence of serum and differing concentrations of glucose. Right: Differentiated ES cells (25 mM glucose) at day 10, immunostained for the cardiac structural protein ∝-actinin.

MAJOR GRANTS

ISCIII (PI06/0299 to KM).

SELECTED PUBLICATIONS

Cervera AM, Apostolova N, Crespo FL, Mata M and McCreath KJ. **Cells silenced for SDHB expression display characteristic features of the tumor phenotype**. *Cancer Res* (2008) 68: 4058-4067

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

Transcriptional Regulation of Oxidative Stress Protection Systems

Head of Laboratory: ***María Monsalve***

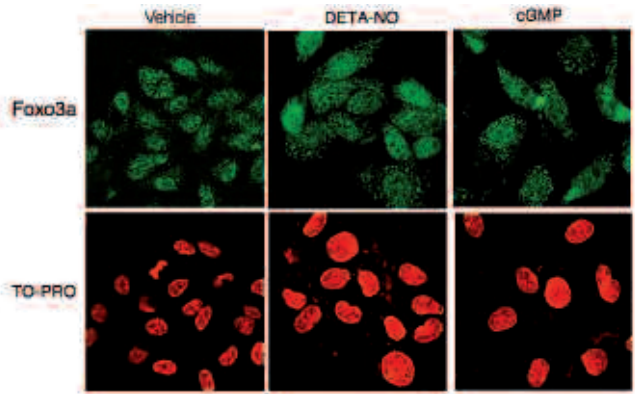
Postdoctoral Researchers: *Nieves García Quintans*
Alberto Tierrez

Predoctoral Researchers: *Yolanda Olmos*
Cristina Sánchez

Technician: *Brigitte Wilde*

RESEARCH INTEREST

Metabolic dysfunction and associated mitochondrial oxidative stress are emerging as primary causes and risk factors in many important human diseases. We propose that common metabolic dysfunctions that impair mitochondrial function, such as metabolic syndrome, diabetes, and nonalcoholic steatohepatitis, involve the inactivation of a set of transcription factors that protect against oxidative stress. Understanding the mechanisms that control the ROS detoxification system will be crucial for the development of new treatments. Our research is directed at identifying the key transcription factors involved in the regulation of this system, with special focus on the transcriptional coactivator PGC-1∝, a central modulator of oxidative metabolism and ROS detoxification. We are interested in how the activity and expression of this and related factors are regulated and how this regulation is altered in human disease, particularly diseases affecting the cardiovascular system. We believe that pharmacological activation of at least two of these transcriptional regulators is feasible and would impact positively on the metabolic and oxidative stress dysfunction of patients. A key aim of our work is therefore to identify molecules that modulate the activity of these factors and could hence serve as the starting point for the development of new treatment strategies.



NO inactivates the transcription factor Foxo3a.
Immunofluorescence analysis of the cellular localization of Foxo3a (green) in serum-starved bovine aortic endothelial cells incubated with the NO donor DETA-NO or cGMP. Nuclei are stained with TO-PRO (red).

MAJOR GRANTS

MEC (SAF2006-01619 to MM).

MEC (CSD2007-00020 to MM).

SELECTED PUBLICATIONS

Monsalve M, Borniquel S, Valle I and Lamas S. **Mitochondrial dysfunction in human pathologies**. *Front Biosci* (2007) 12: 1131-1153

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

Stem Cell Aging

Head of Laboratory: ***Susana González***

Postdoctoral Researcher: *Celia Cerrato*

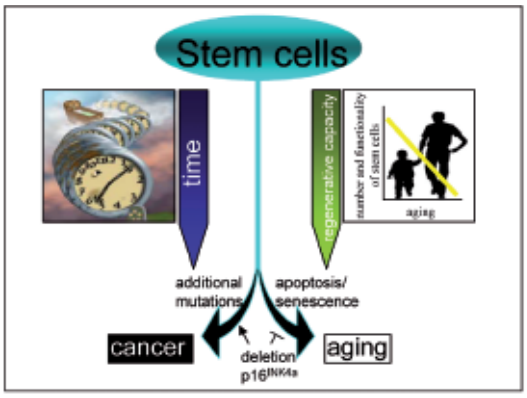
Predoctoral Researchers: *Cristiana Oliveira*
Pilar Mendoza
Antonio Herrera
Antonio Montes

Technician: *Christian Torrenteras*

RESEARCH INTEREST

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

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



Our initial stem-cell pool decreases in number and functionality through our lives, contributing to age-related diseases. Mutations, such as loss of the tumour suppressor p16INK4a, increase the risk of cancer, but also expand the stem-cell pool and thus delay age-related pathologies.

MAJOR GRANTS

Human Frontier Science Program Organization (Career Development Award to SG)

ISCIII (PI06/0627 to SG).

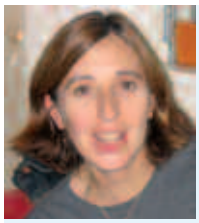
SELECTED PUBLICATIONS

Gonzalez S, Pisano D, and Serrano M. **Mechanistic principles of chromatin remodeling guided by siRNAs and miRNAs**. *Cell Cycle* (2008) 7: 2601-2608

Benetti R, Gonzalo S, Jaco I, Muñoz P, Gonzalez S, Schoeftner S, Murchison E, Andl T, Chen T, Klatt P, Li E, Serrano M, Millar S, Hannon G and Blasco MA. **A mammalian microRNA cluster controls DNA methylation and telomere recombination via Rbl2-dependent regulation of DNA methyltransferases**. *Nat Struct Mol Biol.* (2008) 15: 268-279.

de Yebenes VG, Belver L, Pisano DG, Gonzalez S, Villasante A, Croce C, He L and Ramiro AR. **miR-181b negatively regulates activation-induced cytidine deaminase in B cells**. *J Exp Med* (2008) 205: 2199-2206

Nuclear Receptor Signalling



Head of Laboratory: ***Mercedes Ricote***

Postdoctoral Researcher: *Piedad Menéndez*

Predoctoral Researchers: *Daniel Alameda*
Marta Cedenilla

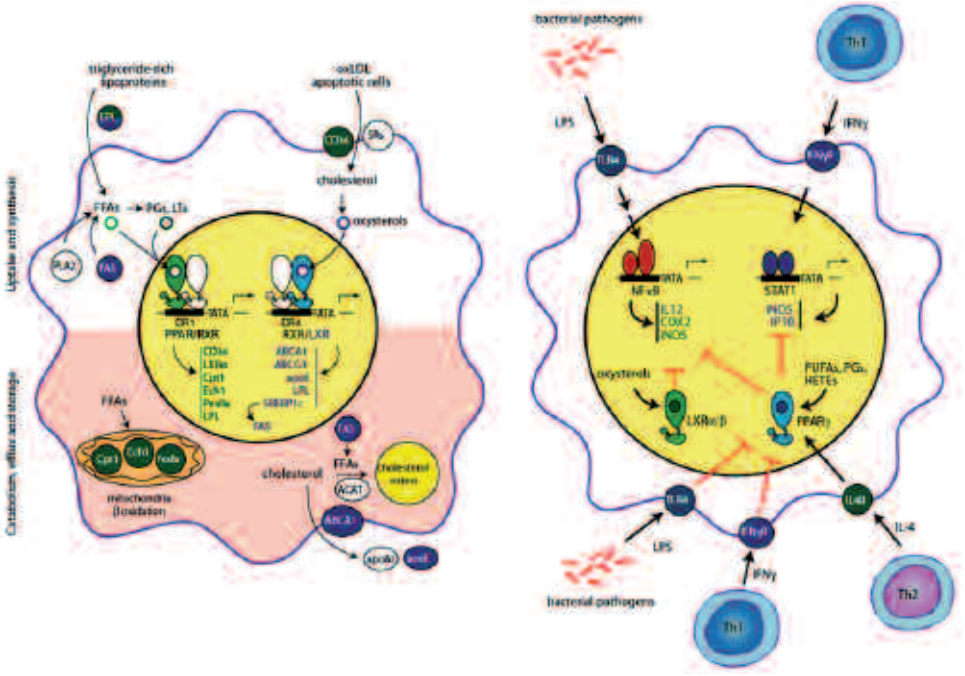
Technician: *Vanessa Núñez*

RESEARCH INTEREST

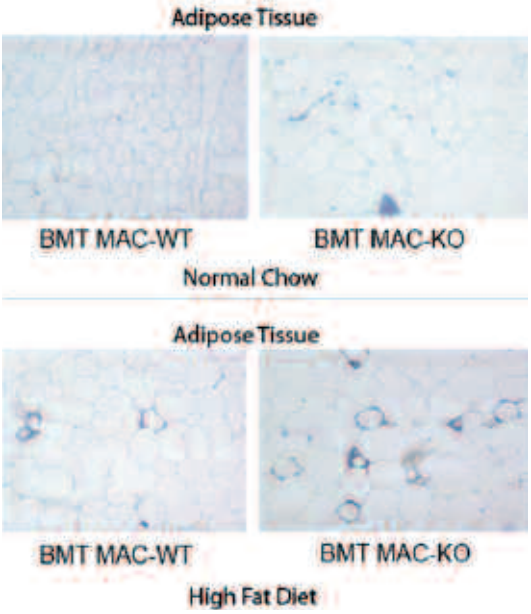
Nuclear hormone receptors constitute a superfamily of ligand-activated transcription factors with diverse roles in mammalian physiology. While these proteins have long been recognized as central to development and homeostasis, recent work has begun to define an unexpected role for specific receptors in chronic human diseases such as obesity, diabetes and cardiovascular disease—the leading causes of morbidity and mortality in industrialized societies. The common threads linking these disorders are lipid dysregulation and inflammation, and nuclear receptors, including PPARs (peroxisome proliferators-activated receptors) and RXRs (retinoic X receptors), have emerged as key regulators of inflammation and lipid homeostasis in macrophages.

Our hypothesis is that chronic activation of inflammatory pathways plays a key role in the pathogenesis of insulin resistance and atherosclerosis, and that macrophages are a key target of the anti-diabetic and anti-atherosclerotic actions of PPAR and RXR ligands. Recent evidence from our group and others demonstrates that deletion of PPARγ prevents macrophage polarization to the alternative activated phenotype, impairs insulin action and exacerbates atherosclerosis. We are currently investigating the molecular basis of insulin-resistance and atherosclerosis in mice selectively lacking PPARγ expression in macrophages.

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



Regulation of lipid metabolism and inflammation in macrophages by nuclear receptors in macrophages.



Immunohistochemical detection of macrophage-specific antigen F4/80 in adipose tissue. Increased F4/80 staining was observed in adipose tissue from bone-marrow-transplant macrophage-PPARγ knockout mice fed a normal or high-fat diet.

MAJOR GRANTS

MEC (SAF 2006-01010 to MR).

CDTI (Programa CENIT to MR).

FUNDACIÓN GENOMA ESPAÑA. MEICA Project (to MR).

SELECTED PUBLICATIONS

Hevener AL, Olefsky JM, Reichart D, Nguyen MTA, Bandyopadyhay G, Leung H-Y, Watt MJ, Benner C, Febraio MA, Nguyen A-K, Folian B, Subramaniam S, Gonzalez FJ, Glass CK and Ricote M. **Macrophage specific disruption of PPARγ leads to skeletal muscle and hepatic insulin resistance and diminished TZD-action**. *J Clin Invest* (2007) 117: 1658-1669

Pascual G, Ricote M and Hevener AL. **Macrophage peroxisome proliferators activated receptor γ as a therapeutic target to combat Type 2 diabetes**. *Expert Opin Ther Targets* (2007) 11: 1-18

Ricote M and Glass CK. **PPARs and mechanisms of transrepression**. *Biochim Biophys Acta* (2007) 1771: 926-935

Toll-like Receptors and Innate Immunity in Cardiovascular Disease and Regeneration

Head of Laboratory: ***Sonsoles Hortelano***

Invited Scientist: *Francisca Través*

Predoctoral Researcher: *Raquel López*

Technician: *Gemma Benito*

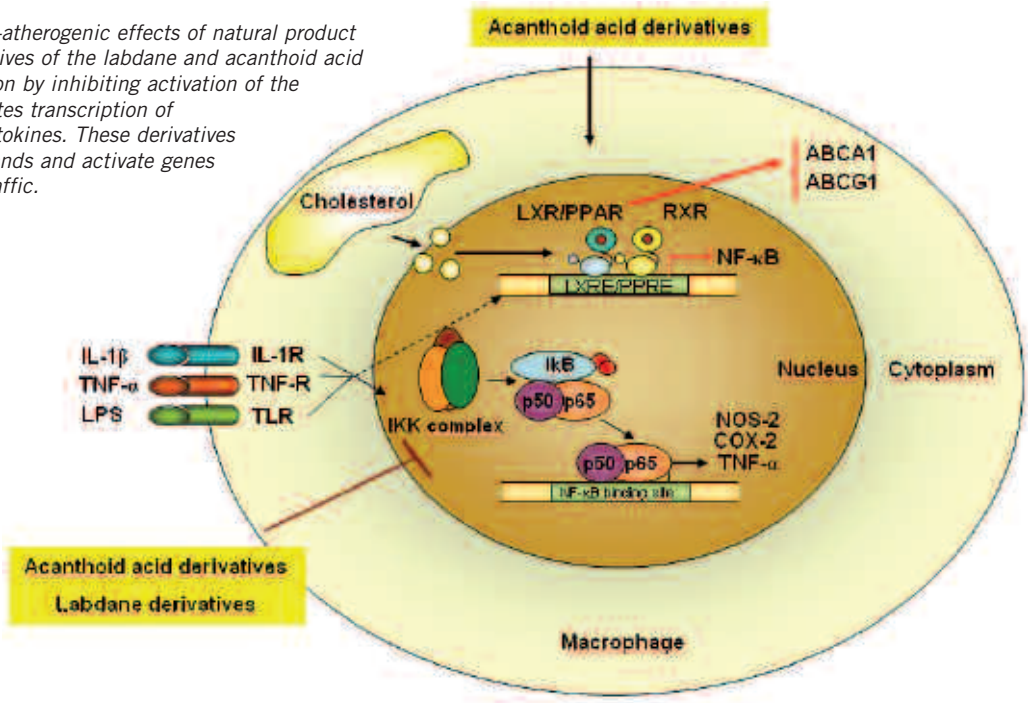
RESEARCH INTEREST

Our group is interested in the molecular basis of the inflammatory response, with a focus on the resolution of this process as a means of avoiding the establishment of chronic inflammatory diseases, including several cardiovascular pathologies.

One of the key cell types in the inflammatory process is the macrophage. Macrophages are actively involved in the initiation of inflammation and the activation of the immune system, leading to the release of cytokines that amplify the initial inflammatory stimulus. Activation of monocytes/macrophages is an important initial step in the cascade of events leading to many inflammatory diseases, including atherosclerosis, sepsis and also cardiac injury after myocardial infarction.

Our group studies the antiatherogenic and antiinflammatory potential of several natural product derivatives that act as specific ligands of LXR nuclear receptors (liver X receptors). These receptors play a key role in the regression of the atherosclerotic lesion, and can also prevent its formation or facilitate recovery of the damaged tissue.

We are also investigating the importance of the tumour suppressor ARF in the development of cardiovascular lesions. This gene plays a key role in the regulation of tumour proliferation, but we postulate that it might also be a key modulator of inflammation. We recently identified a decreased inflammatory response in ARF-deficient animals, and our results suggest an involvement of the activation of Toll-like receptors, particularly TLR4. Based on these results, we are analysing the responses of ARF-deficient animals in the context of cardiovascular pathologies including myocardial infarction.



Anti-inflammatory and anti-atherogenic effects of natural product derivatives. Terpene derivatives of the labdane and acanthoid acid families inhibit inflammation by inhibiting activation of the IKK complex, which regulates transcription of inflammatory genes and cytokines. These derivatives additionally act as LXR ligands and activate genes that regulate cholesterol traffic.

MAJOR GRANTS

ISCIII (PI050050 to SH).

ISCIII (PI080070 to SH).

SELECTED PUBLICATIONS

Hortelano S, Zeini M, Casado M, Martín-Sanz P, and Boscá L. **Animal models for the study of liver regeneration: role of nitric oxide and prostaglandins**. *Front Biosci*. (2007) 12: 13-21

Través PG, Hortelano S, Zeini M, Chao T-H, Lam T, Theodorakis EA, Palladino MA, Castrillo A, and Boscá L. **Selective activation of Liver X Receptors by acanthoic acid-related diterpenes: Contribution to protective mechanisms against atherogenesis**. *Mol Pharmacol*. 71:1545-53 (2007)

Zeini M, López-Fontal R, Través PG, Benito G and Hortelano S. **Differential sensitivity to apoptosis among the cells that contribute to the atherosclerotic disease**. *Biochem Biophys Res Commun* (2007) 363: 444-450

Giron N, Través PG, Rodriguez B, Lopez-Fontal R, Bosca L, Hortelano S and de las Heras B. **Suppression of inflammatory responses by labdane-type diterpenoids**. *Toxicol Appl Pharmacol* (2008) 228: 179-189

Díaz-Viciedo R, Hortelano S, Girón N, Massó JM, Rodríguez B, Villar A and de las Heras B. **Modulation of inflammatory responses by diterpene acids from Helianthus annuus L**. *Biochem Biophys Res Commun* (2008) 369: 761-766

Organogenesis and Tissue Homeostasis

Head of Laboratory: ***Miguel Torres***

Research Scientists:
Laura Carramolino
Nadia Mercader
Juan José Sanz-Ezquerro

Postdoctoral Researchers:
Cristina Clavería
Adrian C. Grimes
Laura Padrón

Predoctoral Researchers:
Jesús Chamorro
Clara García
Juan Manuel González
Daniel Martín
Alberto Roselló
Catalina-Ana Rosselló
Verónica Uribe

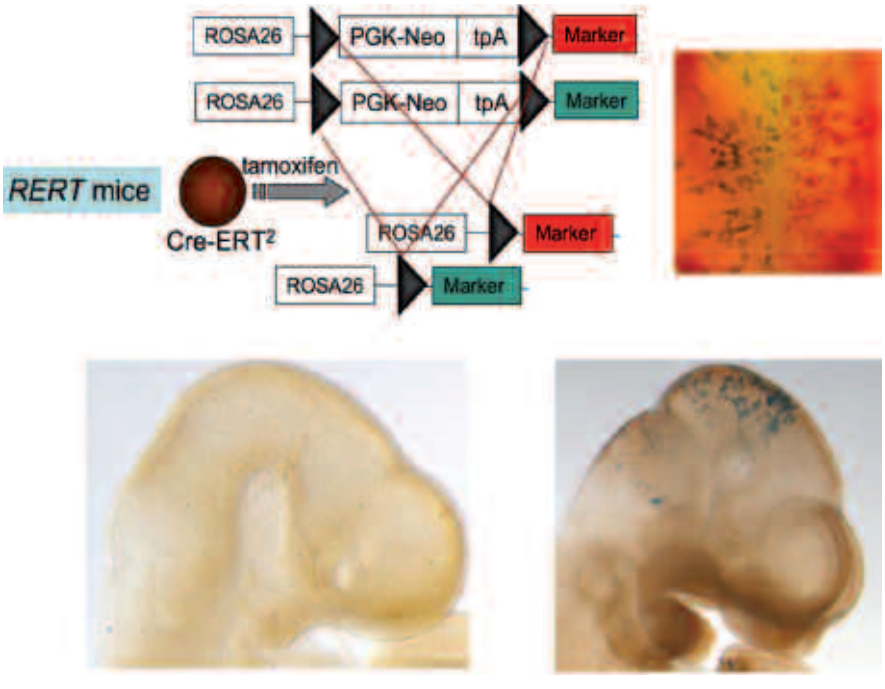
Technicians:
Claudio Badía
Joana Fuentes
Rocío Sierra
Silvia Vela

RESEARCH INTEREST

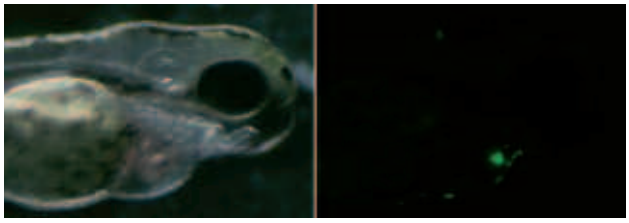
Recent studies in our laboratory have focused on two topics—the role of transcription factors in cardiovascular and limb development and the development of new genetic approaches in the mouse for cell clonal analysis and cell mosaic analysis.

In the first area, we have developed mouse models that allow controlled gain- and loss-of-function of the homeodomain transcription factors Meis and Pbx, revealing new roles for these factors in heart and limb patterning. Combined multigene mutation of this family yields cardiac phenotypes similar to congenital defects seen in human populations, suggesting involvement of this gene family in the generation of congenital heart defects. We have also begun an analysis of TBX transcription factors during heart development in the zebrafish, isolating a battery of putative target genes. Also in zebrafish, we are developing transgenic tools for the study of the epicardial heart layer, a tissue layer important in heart development and regeneration but whose biology remains poorly understood. We have also isolated the ARID family member Arid3b, a new transcription factor involved in cardiac development and expressed in cardiac precursors of the heart field. Mouse lacking Arid3b show defective heart tube formation and other severe cardiovascular phenotypes. We are studying the cellular and molecular targets of Arid3b and its roles in embryogenesis.

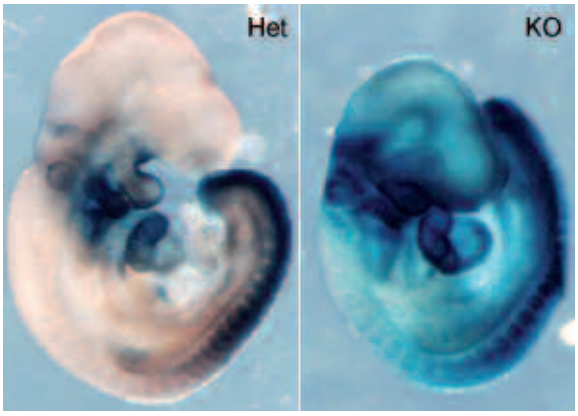
In the second area, we have developed a new *in vivo* clonal analysis strategy capable of determining the lineage relationships among the cells of any developing organ. This strategy can be used both for fixed and live specimen analysis. Recently we have shown that coupling 3D imaging techniques with clonal analysis makes it possible to reconstruct the behaviour of cells during the patterning of complex organs.



A universal clonal analysis system in the mouse
Clones randomly labelled with two independent cell markers are induced independently and at a very low frequency at the desired time, allowing study of the progeny of single labelled cells.
Top: Strategy for conditional genetic modification (left) and an example of cellular clones induced in the same specimen (right).
Bottom: Examples of clones induced independently in two specimens.



Generation of an epicardium-specific reporter line in zebrafish. *Conserved regulatory elements of the gene WT1 were tested for their ability to drive GFP expression in the epicardium of transgenic zebrafish larvae. (Left panel, bright field; right panel, GFP-positive cells around the heart.)*



Arid3b expression and mutant phenotype. *Arid3b mutant heterozygotes (Het) develop normally, while homozygotes (KO) die at E10.5, presenting a severe cardiovascular phenotype consisting of pericardial oedema and abnormal heart looping. X-gal staining at E9.5 shows a clear Arid3b expression domain in developing heart.*

MAJOR GRANTS

ISCIII (RETICS TerCel. RD06/0010 to MT).

MEC (BFU2006-10978 to MT).

Human Frontier Science Program Organization (GP0008/2004-C to MT).

MEC (BFU2006-12859 to JJSE).

MEC (BFU2008-00212 to NM).

SELECTED PUBLICATIONS

Mercader N, Selleri L, Criado LM, Pallares P, Parras C, Cleary ML and Torres M. **Ectopic Meis1 expression in the mouse limb bud alters P-D patterning in a Pbx1-independent manner**. *Int J Dev Biol* (accepted)

Burn SF, Boot MJ, de Angelis C, Doohan R, Arques CG, Torres M and Hill RE. **The dynamics of spleen morphogenesis**. *Dev Biol* (2008) 318: 303-311

Boot MJ, Westerberg CH, Sanz-Ezquerro J, Cotterell J, Schweitzer R, Torres M and Sharpe J. **In vitro whole-organ imaging 4D quantification of growing mouse limb buds**. *Nat Methods* (2008) 5: 609-612

Functional Genomics of Embryonic Pluripotency and Heart Development

Head of Laboratory: ***Miguel Manzanares***

Postdoctoral Researchers: *Eva Alonso*
Cristina Arias
Susana Cañó

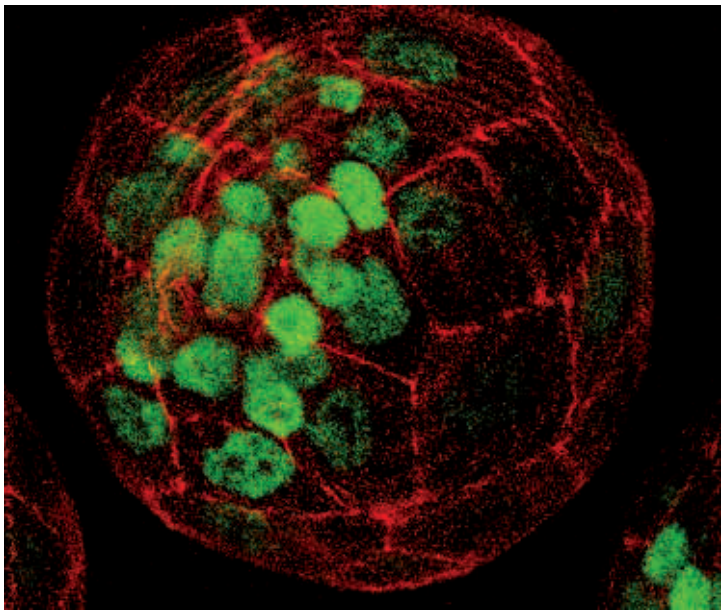
Predoctoral Researchers: *Beatriz Fdez.-Tresguerres*
Barbara Pernaute
Teresa Rayón

RESEARCH INTEREST

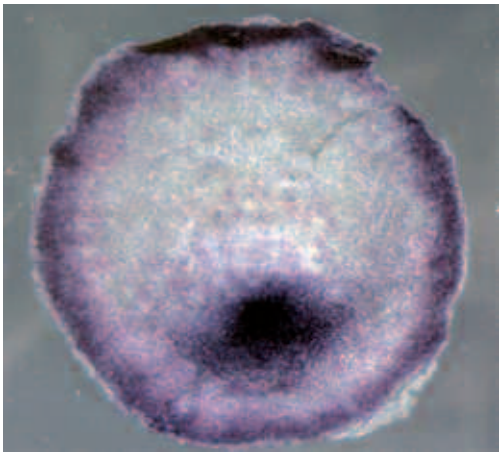
How the genome is co-ordinately regulated during development remains one of the major unanswered questions in modern biology. We are exploring this issue by means of a comparative and functional approach, with the aim of understanding how gene regulatory networks were assembled during evolution and how this origin determines their function.

We are particularly interested in understanding the function of the gene regulatory network that controls embryonic pluripotency in the mouse embryo, and have examined the degree of conservation of genes, and interactions between them, with other vertebrates. We find that the core transcriptional factors involved in pluripotency (Oct4, Sox2 and Nanog) are conserved, but that their assembly into a network occurred only in the mammalian lineage. Downstream target genes of this core set have been recruited mainly through the appearance of enhancer elements not conserved between mammals and other amniotes. Furthermore, we find that genes involved in the specification of the trophoblast, a cell population specific to mammals, are present in extraembryonic tissues in chick embryos, but that their regulatory associations are different from what is found in mouse.

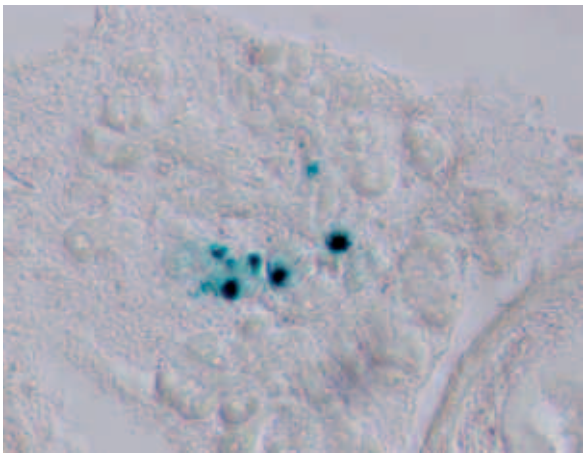
Among other studies related to genome evolution and function, we are using genome-wide association studies to address the potential regulatory function during development of intergenic genomic regions that have been found to increase the risk of common human diseases. We have found that regions in the vicinity of HHEX can drive pancreas expression in transgenic mouse embryos, correlating with the association of this region with type 2 diabetes and obesity. We are carrying out similar analyses of regions associated with increased risk of atrial fibrillation, and are also dissecting the regulatory elements of the Irx genes (involved in heart development) and ion channel subunit genes that show differential expression during heart development.



Distribution of the Oct4 pluripotency factor in the mouse blastocyst. Oct4 (green) is expressed specifically in the inner cell mass, while the trophoectoderm cells (outlined in red) express other transcription factors such as Cdx2 and Eomes



Expression of the T-box factor Eomes in extraembryonic tissues (periphery) and the nascent primitive streak of the early chick embryo. In the mouse blastocyst, Eomes is involved in specification of the trophoblast, the precursor of the extraembryonic placenta.



An evolutionarily conserved genomic region from the human HHEX gene, associated with an increased risk of type 2 diabetes, acts as a cis-regulatory element with enhancer activity in the developing mouse pancreas

MAJOR GRANTS

MICINN (BFU2008-00838 to MM)

MEC (CONSOLIDER CSD2007-0008 to MM)

CAM (S-SAL-0190-2006 CELDEV-CM to MM)

SELECTED PUBLICATIONS

Quijano C, Tomancak P, Lopez-Marti J, Suyama M, Bork P, Milan M, Torrents D and Manzanares M. **Selective maintenance of Drosophila tandemly-arranged duplicated genes during evolution**. *Genome Biol* (2008) 9: R176

Alonso ME, Pernaute B, Crespo M, Gómez-Skarmeta JL and Manzanares M. **Understanding the regulatory genome**. *Intl. J Dev Biol* (accepted)

Cardiovascular Imaging

The Cardiovascular Imaging Lab is a multi-centre group formed through national and international collaborations.

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

Research Scientists: *Luis Jesús Jiménez Borreguero* (CNIC, Hospital de la Princesa, Madrid)
Juan José Badimón (Mt.Sinai Medical Center)
Zahi Fayad (Mt.Sinai Medical Center)
Juan Carlos Murciano (CNIC)
Jesús Mateo (CNIC)

Predoctoral Researchers: *Oscar Marcos* (CNIC)
Patricia Garcia (CNIC)

Technician: *Fátima Esquivel* (CNIC)

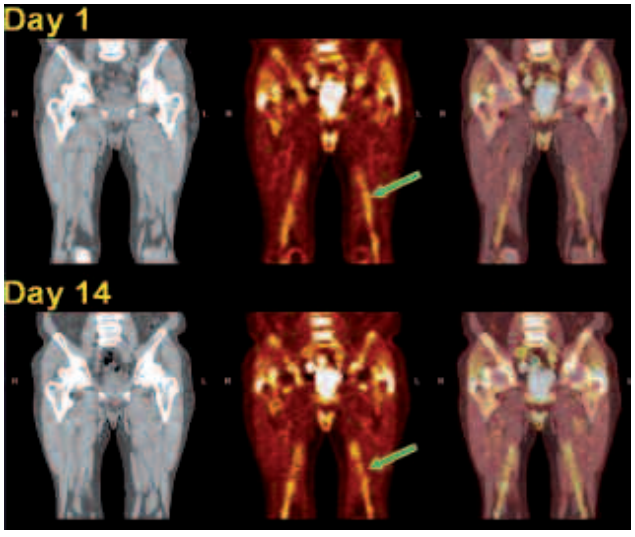
RESEARCH INTEREST

The laboratory is dedicated to developing and advancing non-invasive molecular-resolution technologies for precise diagnosis and treatment of cardiovascular disease.

Animal studies address four key areas: the aortic territory (especially in relation to aneurysm), the brain and aging, vascular regeneration ("vasa vasorum"), and atherothrombosis as an inflammatory disease. Projects include preclinical validation of PET/MRI hybrid systems and magnetic particle imaging (MPI) in small animals, MRI of macrophages to detect medium-risk plaques, and imaging of plaque neovascularization using ultrasensitive perfusion techniques. We also plan to run preclinical drugs tests in a model of coronary disease in pigs with collateral coronary circulation, and to test the viability of ultrasound rupture of drug-loaded microbubbles for targeted drug delivery.

Advanced imaging in humans will be conducted at the Human Cardiovascular Imaging Laboratory, established in 2008 at the Hospital Carlos III, Madrid. There are three priority areas. Improved detection of people with high cardiovascular risk will be addressed by testing the PET/MRI hybrid system as part of the HYPERImage consortium. A study of specific cardiovascular risks and complications associated with HIV infection is planned. Finally, we are planning studies on heart failure. A multicentre trail will study the effect of β-blockers in the early stages of acute myocardial infarction. Other studies will focus on detecting patients at risk of developing ventricular dysfunction; these multimodality studies will combine ultrasound for evaluating diastolic function, MRI for systolic function, flow, elasticity and cavity geometry, and PET for detecting inflammatory markers. In addition, MRI sequences will be developed to detect diffuse fibrosis in hypertensive cardiopathy.

Human Cardiovascular Imaging equipment currently consists of apparatus for PET/3 Tesla MRI, a CT multidetector and ultrasound apparatus.



Coronal CT (left), FDG-PET (middle) and fused PET/CT (right) images of the femoral artery territory at scan 1 (top) and scan 2 (bottom), separated by two weeks. Note little change in the amount of FDG uptake in the femoral artery between the two scans. Green arrows on the FDG-PET images highlight accumulation of FDG in the femoral artery.

MAJOR GRANTS

EC (201651 HyperImage to VF).

SELECTED PUBLICATIONS

Beller GA, Bonow RO, Fuster V, American College of Cardiology Foundation, American Heart Association and American College of Physicians Task Force on Clinical Competence and Training. **ACCF 2008 Recommendations for Training in Adult Cardiovascular Medicine Core Cardiology Training (COCATS 3) (revision of the 2002 COCATS Training Statement)**. *J Am Coll Cardiol* (2008) 51: 335-338

Briley-Saebo KC, Shaw PX, Mulder WJ, Choi SH, Vucic E, Aguinaldo JG, Witztum JL, Fuster V, Tsimikas S and Fayad ZA. **Targeted molecular probes for imaging atherosclerotic lesions with magnetic resonance using antibodies that recognize oxidation-specific epitopes**. *Circulation* (2008) 117: 3206-3215

Elmariah S, Smith SC,Jr and Fuster V. **Late medical versus interventional therapy for stable ST-segment elevation myocardial infarction**. *Nat Clin Pract Cardiovasc Med* (2008) 5: 42-52

Ibanez B, Vilahur G, Cimmino G, Speidl WS, Pinero A, Choi BG, Zafar MU, Santos-Gallego CG, Krause B, Badimon L, Fuster V and Badimon JJ. **Rapid change in plaque size, composition, and molecular footprint after recombinant apolipoprotein A-I Milano (ETC-216) administration: magnetic resonance imaging study in an experimental model of atherosclerosis**. *J Am Coll Cardiol* (2008) 51: 1104-1109

Rudd JH, Myers KS, Bansilal S, Machac J, Pinto CA, Tong C, Rafique A, Hargeaves R, Farkouh M, Fuster V and Fayad ZA. **Atherosclerosis inflammation imaging with 18F-FDG PET: carotid, iliac, and femoral uptake reproducibility, quantification methods, and recommendations**. *J Nucl Med* (2008) 49: 871-878

Sanz J, Moreno PR and Fuster V. **The year in atherothrombosis**. *J Am Coll Cardiol* (2008) 51: 944-955

Animal models of Cardiovascular Disease

Head of Laboratory: ***Carlos Zaragoza***

Postdoctoral Researcher: *Tania Rodríguez*

Predoctoral Researchers: *Carlos Antonio Tarín*
Begoña Lavín
Concepción García

Technician: *Mónica Gómez*

RESEARCH INTEREST

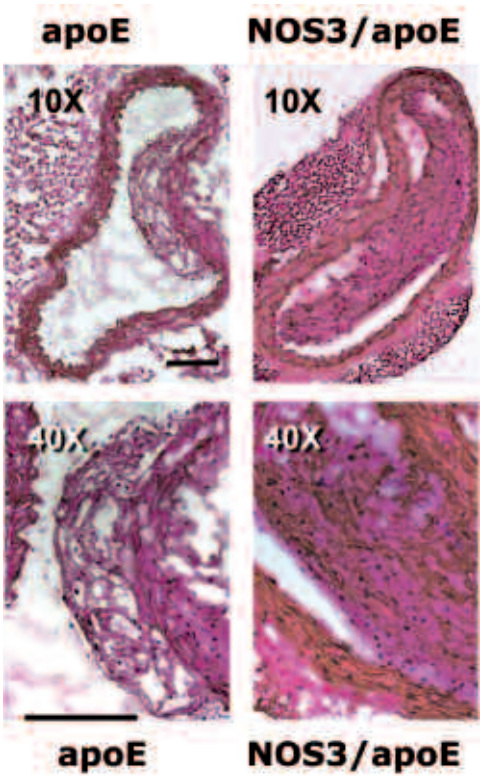
Our research concerns the contribution of vasoactive factors and proteolytic enzymes to the development and progression of atherosclerosis, aneurysm, and myocardial infarction, three of the most prevalent cardiovascular diseases. Our current projects are the following.

Contribution of nitric oxide (NO) to the activation of extracellular matrix metalloproteinases (MMPs) during aortic endothelial cell migration: Molecular mechanisms elicited by NO in endothelial cell migration are studied in *in vitro* and *in vivo* wound healing assays. **NO-mediated cardioprotection in late myocardial preconditioning:** We are developing a model of ischemia/reperfusion injury in NOS null mice to explore the role of extracellular matrix metalloproteinase inducer (EMMPRIN) and to identify ways to inhibit the release of damaging agents.

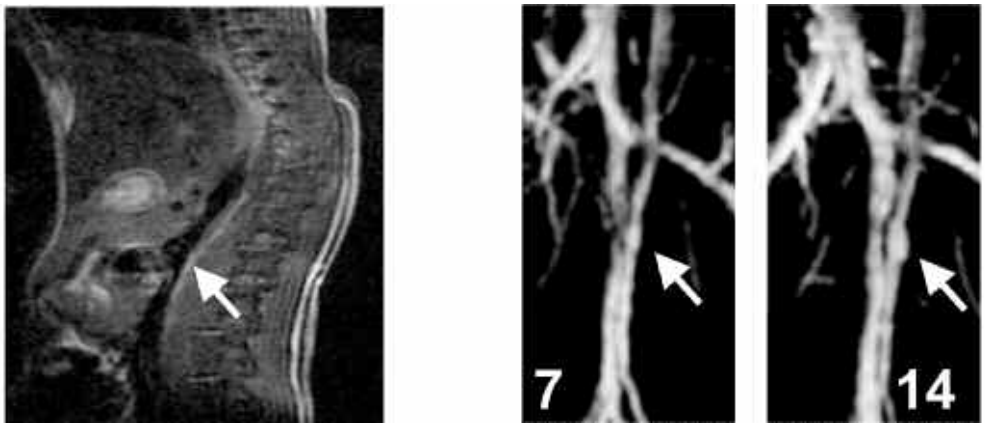
Contribution of MMPs to the migration and homing of endothelial progenitor cells during vascular wall repair: This project explores MMP and EPC functions through the use of high frequency molecular ultrasound to develop non-invasive tools for molecular monitoring of blood vessel wall repair.

New tools for non invasive detection of abdominal aortic aneurysms (AAA): We are using a mouse model of surgically-induced AAA to identify molecules involved in the development, progression, and rupture of the disease and to design tools for its non-invasive detection by molecular imaging. **Animal models of atherosclerosis:** We are generating a Tet-on based transgenic model of vulnerable plaques by inducing MMP-9 over expression in the vessels of Apo E-null mice. We are also exploring the inhibition by vasoactive factors of monocyte adhesion during early atherosclerosis.

Creation of a hybrid PET/MRI system. Our group works on preclinical validation of cardiovascular disease as a contributor to the EU **HYPERImage** Consortium.



Histological sections of aorta from mice deficient for apoE or doubly deficient for apoE and eNOS (NOS3). Haemotoxylin staining reveals atheromatous plaques, which almost completely occlude the vessel lumen in the doubly-deficient sample.



Imaging of abdominal aneurysm. The MRI scan (left) reveals an aneurysm (arrow) in mouse abdominal aorta 14 days after infusion with porcine elastase. The angiographs (right) show the growth of an aneurysm between 7 and 14 days after infusion.

MAJOR GRANTS

MEC (SAF2005-06025 to CZ)

MEC (SAF2008-04629 to CZ)

SELECTED PUBLICATIONS

Lizarbe TR, Garcia-Rama C, Tarin C, Saura M, Calvo E, Lopez JA, Lopez-Otin C, Folgueras AR, Lamas S and Zaragoza C. **Nitric oxide elicits functional MMP-13 protein-tyrosine nitration during wound repair**. *FASEB J* (2008) 22: 3207-3215

Tarín C, Gomez M, Calvo E, Lopez JA and Zaragoza C. **Endothelial Nitric Oxide deficiency reduces MMP-13-mediated cleavage of ICAM-1 in vascular endothelium. A role in atherosclerosis**. *Arterioscler Thromb Vasc Biol* (accepted)

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

Department of Cardiovascular Epidemiology and Population Genetics

Head of Department: ***Eliseo Guallar***

Research Scientists:	*Manuel Franco*
Postdoctoral Researchers:	*Martín Laclaustra* *José Luis Peñalvo*
Postresidency Fellow:	*María Téllez*
Biostatistician:	*Pedro López*

Cardiovascular Epidemiology is the study of the distribution of cardiovascular diseases and their genetic, environmental, lifestyle, and social determinants in human populations. Epidemiological studies integrate basic science, clinical data, and population-level factors to better understand the occurrence, natural history, and prognosis of cardiovascular disease. Epidemiologic data provide the quantitative foundation needed in clinical cardiovascular research, and constitute the basis for public health campaigns to control and eliminate cardiovascular diseases. The Department has identified the following specific goals.

- To improve our understanding of the aetiology of cardiovascular disease and its consequences in human populations.
- To improve and evaluate strategies for primary prevention, secondary prevention, and disease management.
- To translate epidemiologic discoveries into clinical practice and public health policy and to communicate this information to the general population.
- To train future leaders in cardiovascular clinical epidemiology, disease prevention, health promotion, and clinical research.

The Department began its activities in 2008, and during the year initiated several projects, including collaborations formalized through research and training agreements with the Johns Hopkins University School of Public Health and the Instituto Aragonés de Ciencias de la Salud.

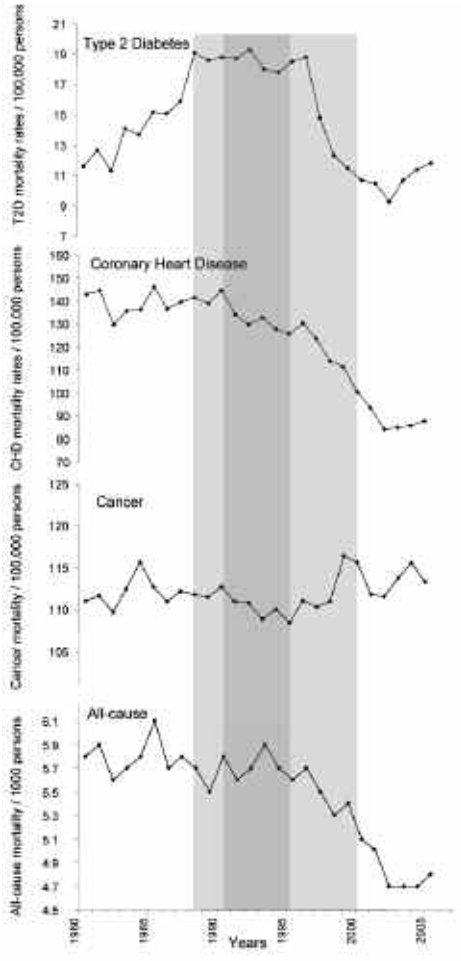
Aragon Workers' Health Study (with the Instituto Aragonés de Ciencias de la Salud). This project is a longitudinal study on the progress of subclinical cardiovascular disease quantified by ultrasound images. Traditional and novel risk factors will be evaluated among 4500 young and middle-aged men and women, using the presence of subclinical atherosclerosis and its progression for prognosis. The cohort consists of workers from the General Motors assembly plant in Figueruelas (Spain).

IM-JOVEN. The Department collaborates in the IM-JOVEN study, promoted by the Department of Translational Cardiovascular Research. IM-JOVEN is part of a large, multi-centre case-controlled study aimed at identifying the clinical, genetic and demographic characteristics that determine the occurrence of infarction in young women.

Collaboration in international projects. The Department collaborates in a variety of international epidemiological studies, including the Atherosclerosis Risk in Communities (ARIC) study, the Multiethnic Study of Atherosclerosis (MESA), and the Framingham Heart Study.

Members of the Department are also developing individual research lines on the nutritional and environmental determinants of cardiovascular disease. Department members contributed to the following research highlights during 2008.

- Establishment of an association between low-level exposure to inorganic arsenic and the prevalence of diabetes (EG).
- Evaluation of the cardiovascular health impact of caloric deprivation and increased energy expenditure during Cuba's "special period" (MF and EG).
- Establishment of novel associations between selenium (an antioxidant micronutrient) and a variety of cardiometabolic risk factors (ML and EG).
- Studies of the phyto-oestrogen composition of a variety of foodstuffs and of the health effects of phyto-oestrogens and other micronutrients (JLP).
- Documentation of wide variability in the availability of healthy foods across neighbourhoods, with a resulting high potential impact on preventive strategies (MF).
- Evaluation of the impact of cadmium exposure on the risk of hypertension (MT and EG).



Evaluation of the possible cardiovascular health impact of population-wide weight loss after Cuba's "special period".

MAJOR GRANTS

CNIC and Instituto Aragonés de Ciencias de la Salud (Aragón Workers Health Study)

MEC (SAF2008-01995 to JLP)

SELECTED PUBLICATIONS

Bleys J, Navas-Acien A and Guallar E. **Serum selenium levels and all-cause, cancer, and cardiovascular mortality among US adults**. *Arch Intern Med* (2008) 168, 404-410

Bleys J, Navas-Acien A, Stranges S, Menke A, Miller ER,3rd and Guallar E. **Serum selenium and serum lipids in US adults**. *Am J Clin Nutr* (2008) 88, 416-423

Navas-Acien A, Bleys J and Guallar E. **Selenium intake and cardiovascular risk what is new?** *Curr Opin Lipidol* (2008) 19, 43-49

Navas-Acien A, Schwartz BS, Rothenberg SJ, Hu H, Silbergeld EK and Guallar E. **Bone lead levels and blood pressure endpoints a meta-analysis**. *Epidemiology* (2008) 19, 496-504

Navas-Acien A, Silbergeld EK, Pastor-Barriuso R and Guallar E. **Arsenic exposure and prevalence of type 2 diabetes in US adults**. *JAMA* (2008) 300, 814-822

Tellez-Plaza M, Navas-Acien A, Crainiceanu CM and Guallar E. **Cadmium exposure and hypertension in the 1999-2004 National Health and Nutrition Examination Survey (NHANES)**. *Environ Health Perspect* (2008) 116, 51-56

Department of Translational Cardiovascular Research

Head of Department: ***Ginés Sanz***

Since the CNIC is not designed to have on-site facilities for patient recruitment or clinical research work-up, the Translational Cardiovascular Research department works in cooperation with hospitals and clinic's of the Spanish National Health system. Several lines of research have been defined during the last 3 years, and significant advances have been achieved during 2008.

Fixed-Dose combination therapy for cardiovascular prevention: The CNIC Polypill project. In 2008 we completed the galenic development of the CNIC Polypill in collaboration with the R+D department at Grupo Ferrer. We examined different polypill formulations to test the physical and chemical interactions of the three components and the pill's ability to withstand high temperature and humidity. The final formulation was selected on the basis of this analysis and its pharmacokinetics has been defined in preclinical studies.

Clinical development is now planned according to FDA and AEMPS recommendations. Protocols have been defined for studies of food interactions, pharmacokinetics, and pharmacodynamic comparisons between the polypill and the three components administered separately. These studies will start in May 2009. Further studies are planned to coordinate a comparison of the polypill concept in different socio-economic settings in South America and Europe.

IM-JOVEN. During 2008 we also developed the protocol for the IM-JOVEN study, the Spanish counterpart of the VIRGO study—an ambitious project aimed at identifying the clinical, social and genetic factors that determine the high mortality among young women with acute myocardial infarction. The VIRGO study is headed by Dr H. Krumholz at Yale, with funding from the NIH. IM-JOVEN is coordinated by the CNIC, the Spanish Society of Cardiology and the RECAVA and HERACLES networks.

Early detection of cardiac involvement in Chagas disease. This study will analyse the predictive value of echocardiography, MRI and biomarkers for early detection of cardiac involvement in 102 individuals (51 Chagas patients and 51 controls). Forty-one participants were recruited last year, including 21 recently diagnosed asymptomatic Chagas patients and 10 non-infected control individuals. All participants were assessed by conventional 2D-echocardiography with diastolic function analysis and image acquisition for applying Speckle Tracking" and 2D-strain. Preliminary results were presented at the 2008 American Heart Association annual meeting in Orlando (USA).

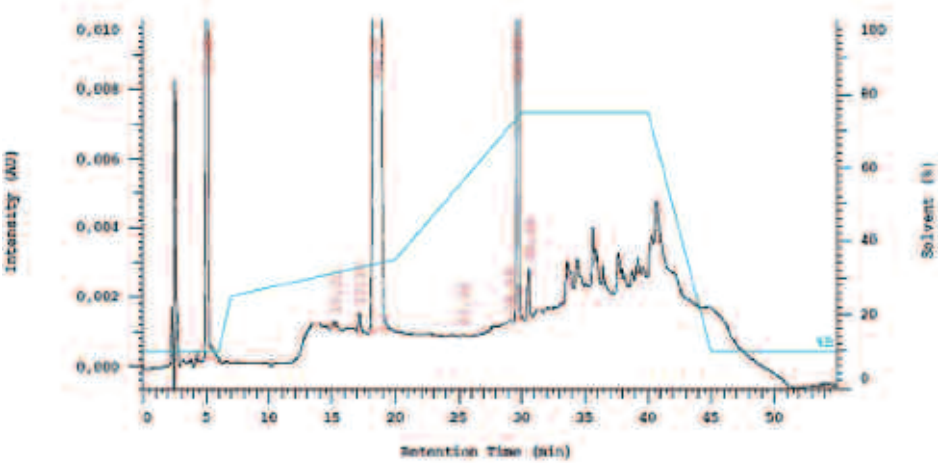
Translational Training Programmes. Training programmes aimed at bringing clinical and basic research closer are an integral part of our work. Last year saw the launch of the CardioJoven programme.

Translational Research Grants. In 2007, the Department launched a specific programme of grants to finance translational cardiovascular research projects. This programme supports projects that facilitate the conversion of knowledge generated through research into improvements in clinical practice, and promotes collaboration between the CNIC and leading cardiovascular research groups in Spain and abroad.

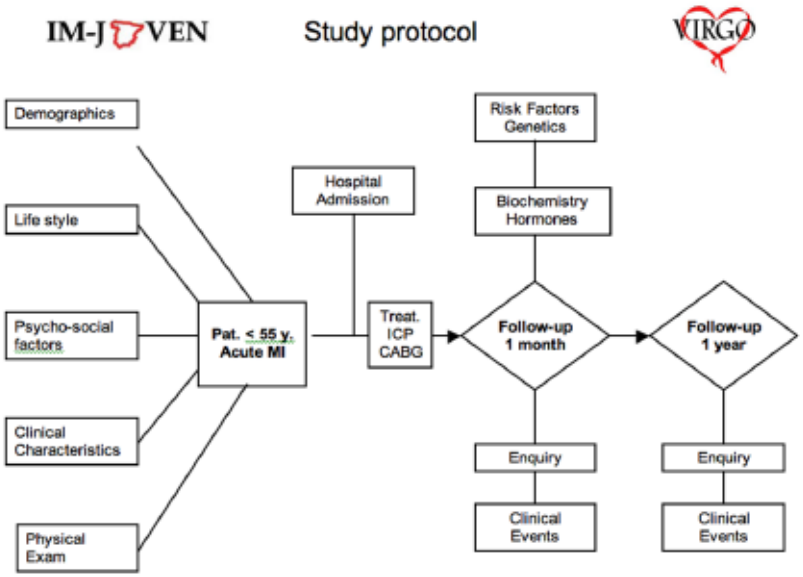
CNIC Translational Research Grants awarded in 2008:

Sudden cardiac death: Translating basic science into clinical care. Principal Investigator: Ramón Brugada (Universidad de Girona, Spain)

Prognostic factors of cardiovascular mortality and morbidity in a cohort of families with genetic diagnosis of familial hypercholesterolaemia. Principal Investigator: Pedro Mata (Fundación Jiménez Díaz, Madrid, Spain)



HPLC analysis of an early polypill formulation showing the presence of an impurity after maintenance for 8 weeks at 50 ºC.



The VIRGO and IM-JOVEN study protocol

MAJOR GRANTS

ISCIII (PI07/0773 to GS)

SELECTED PUBLICATIONS

Rigol M, Solanes N, Sionis A, Galvez C, Martorell J, Rojo I, Brunet M, Ramirez J, Roque M, Roig E, Perez-Villa F, Barquin L, Pomar JL, Sanz G and Heras M. **Effects of cyclosporine, tacrolimus and sirolimus on vascular changes related to immune response**. *J Heart Lung Transplant* (2008) 27: 416-422

Gascon J, Albajar P, Canas E, Flores M, Gomez i Prat J, Herrera RN, Lafuente CA, Luciardi HL, Moncayo A, Molina L, Munoz J, Puente S, Sanz G, Trevino B, Sergio-Salles X, Working Group of the second workshop on "Imported Chagas' Disease, a New Challenge in Public Health" and Spanish Society of Tropical Medicine and International Health. **Diagnosis, management and treatment of chronic Chagas' heart disease in areas where Trypanosoma cruzi infection is not endemic**. *Enferm Infecc Microbiol Clin* (2008) 26: 99-106

Rigol M, Solanes N, Roqué M, Farré J, Batlle M, Roura S, Bellera N, Prat-Vidal C, Sionis A, Ramírez J, Sitges M, Sanz G, Bayés-Genís A and Heras M. **Hemosiderin deposits confounds tracking of iron-oxide-labeled stem cells: an experimental study**. *Transplant Proc* (2008) 40: 3619-3622

Sanz G, Fuster V and Medscape. **Fixed-dose combination therapy and secondary cardiovascular prevention: rationale, selection of drugs and target population**. *Nat Clin Pract Cardiovasc Med* (accepted)

Comparative Medicine

The Comparative Medicine Unit supports *in vivo* work at the CNIC. This support covers animal housing and husbandry, experimental techniques and other services. The animal models housed at present are mouse, rat and zebrafish. Housing and husbandry conditions conform to EU Directive 86/609/EEC, enforced in Spanish law under Real Decreto 1201/2005, which introduces the Ethical Review Committee and mandatory training for those involved in animal experiments. The Unit also adheres to the updates of the Council of Europe Recommendation of 18 June 2007 on guidelines for the accommodation and care of animals used for experimental and other scientific purposes.

The Unit is organized into three core work areas:

- The *Pathology Core* (PC) is run by an on-site laboratory animal pathologist. The PC provides support and collaboration in clinical pathology, anatomic pathology, and histopathology. The PC has established collaborations with the Comparative Pathology Laboratory of the Weill Cornell Medical College and the Memorial Sloan-Kettering Center in New York.
- The *Experimental Surgery Core* (ESC) provides highly specialized expertise in surgical procedures, minimally invasive intervention, and life support.
- The *Quality Control Core* (QCC) is run by a senior microbiologist and monitors the health and the genetic status of the animals on site.

The Unit also provides a comprehensive phenotype evaluation service. This service combines *in vivo* evaluation, imaging strategies, and clinical and anatomic pathology to characterize complex phenotypes—including multisystemic phenotypes or syndromes—for the development and validation of genetically engineered mouse models.

Transgenesis

Head of Unit: ***Luís-Miguel Criado Rodríguez***

Support Scientist: *José Mª Fernández Toro*

Technician: *David Esteban Martínez*

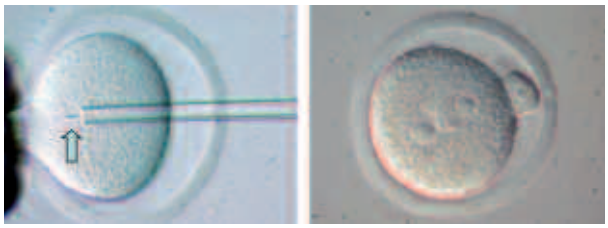
RESEARCH INTEREST

The Transgenesis Unit generates genetically-modified mice, so-called transgenic mice, to serve the needs of the CNIC research groups. Our goals fall into two areas: understanding how genic activity translates into the complexity of a whole live organism and generating mouse models of human cardiovascular illnesses.

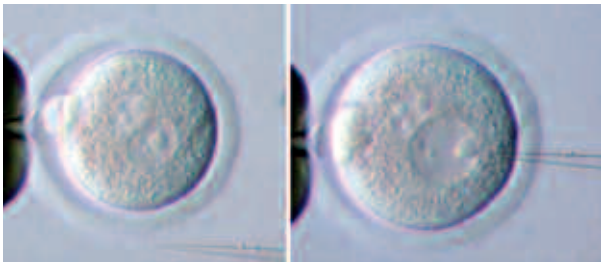
Transgenic mice are produced in the Unit by pronuclear microinjection of zygotes with DNA in solution or by subzonal microinjection with recombinant lentiviruses. Chimeric mice, for generating knock-out (KO) and knock-in (KI) mice, are produced either by aggregation of eight-cell embryos (E8C) with genetically-modified mouse embryonic stem cells (ES cells) or by microinjecting ES cells into E8C or blastocysts. Other key services and techniques include rederivation of mouse strains by embryo transfer, cyropreservation of mouse strains (frozen embryos or sperm), ***in vitro*** fertilization (IVF), and intracytoplasmic sperm injection (ICSI).

New equipment installed in 2008

- 2 high-performance vibration isolation tables (TMC, Model 63-500) to be used with the microinjection set-ups and associated instruments.
- 1 external light source for fluorescence and a set of filters (TXR, GFP2, and violet) for a LEICA MZ-10F stereomicroscope.
- 1 micro-osmometer (Advanced Instruments INC., Model 3320).
- 1 UltraSeal21 ultrasonic sealer from Penetrating Innovations.



Intracytoplasmic Sperm Injection (ICSI). The arrow indicates the mouse spermatozoid head released into the oocyte. The image on the right shows a resulting zygote, with the two pronuclei clearly visible inside the cytoplasm.



Pronuclear injection with DNA in solution for the production of transgenic mice. Left: pronuclear mouse zygote (strain B6CBAF2) anchored with a holding needle; the microinjection needle containing the DNA solution is visible at the bottom of the image. Right: Expanded pronucleus after microinjection.

Subzonal, or perivitelline, microinjection of genetically modified lentivirus into a mouse zygote for the production of transgenic mice. Note the expansion of the perivitelline space (between the zygote and the zona pellucida) after microinjection.

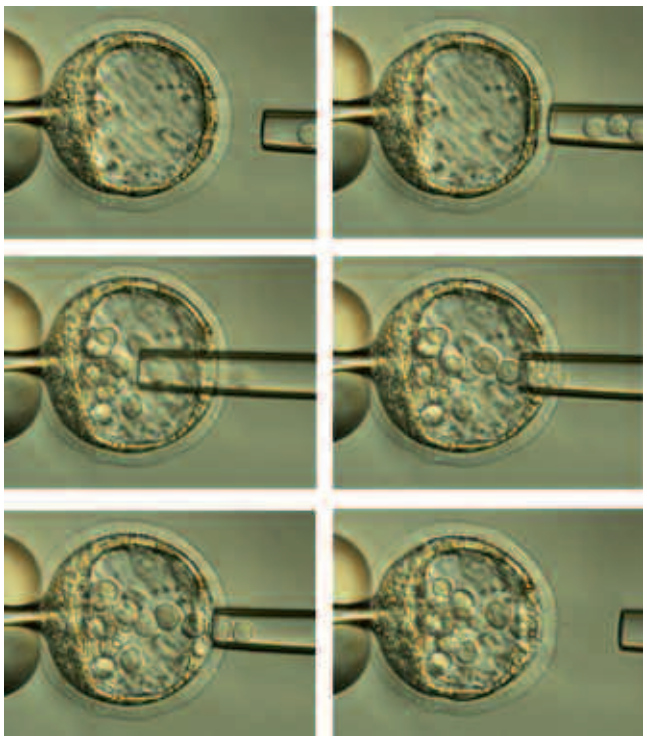


Photo sequence showing the microinjection of genetically-modified mouse embryonic stem cells (ES Cells) into a mouse blastocyst.

SELECTED PUBLICATIONS

Mercader N, Selleri L, Criado LM, Pallares P, Parras C, Cleary ML and Torres M. **Ectopic Meis1 expression in the mouse limb bud alters P-D patterning in a Pbx1-independent manner**. *Int J Dev Biol* (accepted)

Pallares P, Garcia-Fernandez RA, Criado LM, Letelier CA, Esteban D, Fernandez-Toro JM, Flores JM and Gonzalez-Bulnes A. **Disruption of the endothelial nitric oxide synthase gene affects ovulation, fertilization and early embryo survival in a knockout mouse model**. *Reproduction* (2008) 136: 573-579

Gene Targeting and Viral Vectors

Head of Gene Targeting Facility: ***Giovanna Giovinazzo***

Head of Viral Vector Facility: ***Juan Carlos Ramírez***

Support Scientist: *Piedad Fernández*

Technicians: *Maria Angeles Sanguino*
Raúl Torres
Aida García

GENE TARGETING FACILITY

Knock-out, knock-in and conditionally mutant mouse models are today essential tools in biomedical research. The Gene Targeting Facility was set up in 2008 to coordinate the design and production of genetically modified mice through homologous recombination and stem-cell manipulation procedures.

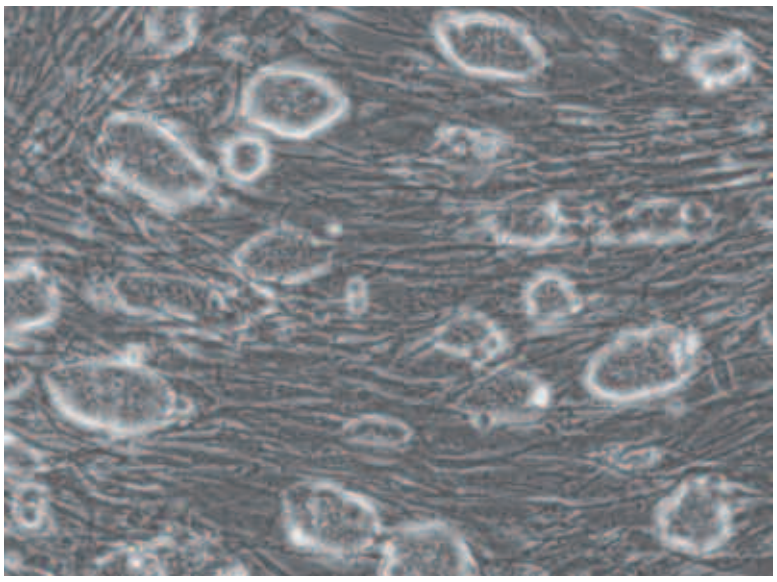
The Facility provides a comprehensive range of support services to CNIC researchers, starting with the definition of an appropriate targeting strategy in consultation with the researchers and including the following services:

- Sequencing and probe and primer design
- Purification of targeting vectors for ES cell electroporation
- Expansion of positive clones and their verification by Southern blot or PCR
- Karyotyping
- Culture and preparation of cells for blastocyst injection, breeding of chimeras to obtain germ line transmission of the targeted allele, and verification of germ line transmission by Southern blot or PCR

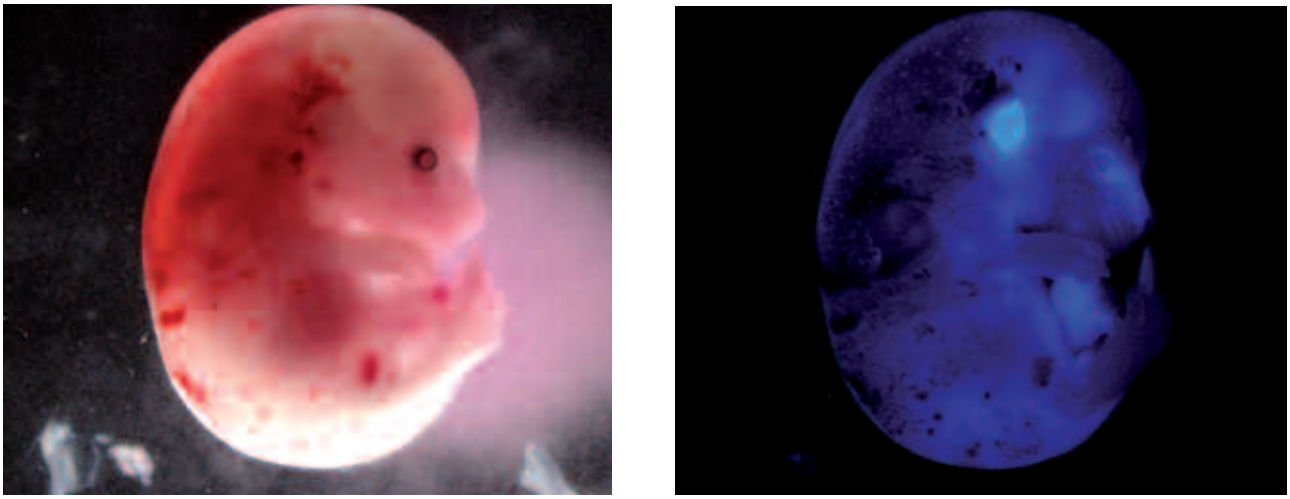
VIRAL VECTOR FACILITY

The Viral Vector Facility provides investigators with access to state-of-the-art viral vector technology for preclinical studies and other basic research applications. Assistance is provided with custom-designed or ready-made vectors, and other core services include plasmid design and cloning, advice on serotype or pseudotype selection, provision of cloning vectors, and design and development of vectors for homologous recombination in mouse and human stem cells.

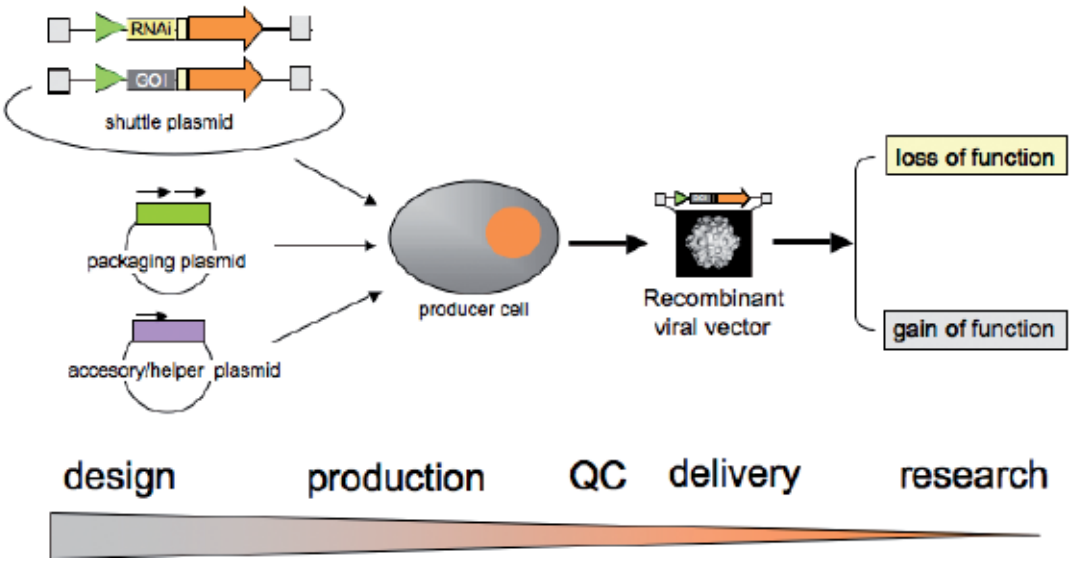
The Facility works with lentivirus, retrovirus, adenovirus and adeno-associated vectors. HIV-derived second and third generation VSV-pseudotyped lentiviruses are routinely available, and we can also provide viruses pseudotyped with other envelope glycoproteins. Retroviral vectors are derived from common MoMLV pseudotyped with amphotropic and ecotropic envelopes. Our adenoviral vectors include backbones derived from Ad5 with E1- and E3-deleted regions. Our AAV-based vectors are pseudotyped with a variety of serotype capsids and have transduction profiles superior to those of previous generations in a variety of target tissues. We currently offer AAV vectors based on serotypes 2 and, importantly, on serotypes 8 or 9, which have been proven to target cardiac tissue *in vivo*.



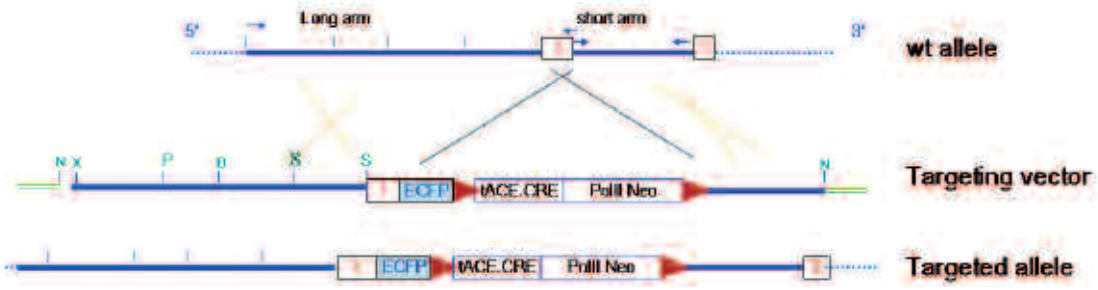
Embryonic stem cells



Genetically-modified mouse embryo expressing the reporter CFP (blue) from the meis1 locus.



Viral infection procedure for gain or loss of function



Homologous recombination gene targeting strategy

Microscopy and Dynamic Imaging

Head of Unit: ***Veleria R. Caiolfa***

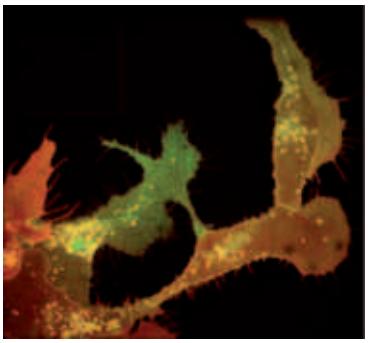
Support Scientists: *Moreno Zamai*
Christian Hellriegel
Elvira Arza

RESEARCH INTEREST

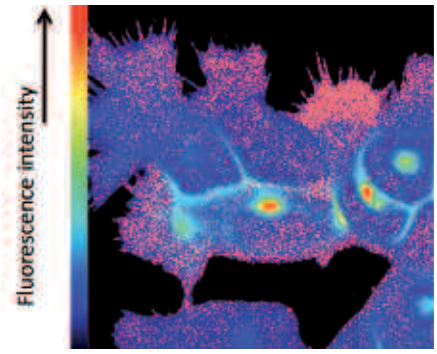
The Microscopy and Dynamic Imaging Unit provides state-of-the-art expertise and training in optical microscopy to scientists at the CNIC and beyond.

"Dynamic Imaging" refers to an array of technologies that employ the properties of light (particularly fluorescence and bioluminescence) to probe molecular and cellular behaviour and interactions. To multi-dimensional (multi-D) imaging, we add cutting-edge molecular spectromicroscopy imaging, which allows quantification of biological events through time and space. Imaging methodologies include immunolabelling, time-lapse, multi-colour TIRFM, FRET-FLIM and 3D cross sectioning. We also provide capabilities in the tracking of single molecules, intracellular vesicles and cells, and in fluctuation analysis techniques such as FCS (fluorescence correlation spectroscopy), RICS and N&B—to quantify diffusion of single proteins, monomer-dimer-oligomer equilibrium, stoichiometry of protein and ligand binding, etc. Resources are maintained for spectroscopy, microscopy, biochemistry, cell culture and data analysis.

The Unit pursues its own cell biophysical research programme, and is additionally involved in the development of new approaches in optical spectro-microscopy of live cells. The main interest is in the spatial targeting and assembly of receptors to cell membrane domains, molecular associations, translocations and intracellular trafficking, as they vary dynamically in living cells.



Detection of co-localized GPI-anchored uPAR receptors in the plasma membrane of live HEK293 cells by scanning confocal microscopy. The receptors are co-expressed as mEGFP (green) and mRFP (red) fluorescent chimeras. The green and red receptors co-localize (yellow) in the basal membrane and in intracellular vesicles of some cells.



FRET-FLIM analysis of equivalent GPI-anchored uPAR receptors in the plasma membrane of live HEK293 cells by 2-photon raster scanning microscopy. The figure shows the superposition of the 2-photon image (LUT scale) with the FRET efficiency (pink mask) determined by FLIM. FRET is observed at the edges of the basal membrane. Only a subset of co-localized green and red receptors also interact, forming true homophilic complexes.

SELECTED PUBLICATIONS

Malengo G, Andolfo A, Sidenius N, Gratton E, Zamai M and Caiolfa VR. **Fluorescence correlation spectroscopy and photon counting histogram on membrane proteins: functional dynamics of the glycosylphosphatidylinositol-anchored urokinase plasminogen activator receptor**. *J Biomed Opt.* (2008) 13: 031215

Digman MA, Caiolfa VR, Zamai M and Gratton E. **The phasor approach to fluorescence lifetime imaging analysis**. *Biophys J.* (2008) 94: L14-L16

Barreiro O, Zamai M, Yanez-Mo M, Tejera E, Lopez-Romero P, Monk PN, Gratton E, Caiolfa VR and Sanchez-Madrid F. **Endothelial adhesion receptors are recruited to adherent leukocytes by inclusion in preformed tetraspanin nanoplatforms**. *J Cell Biol* (2008) 183: 527-542

Caiolfa VR, Zamai M, Malengo G, Andolfo A, Madsen CD, Sutin J, Digman MA, Gratton E, Blasi F and Sidenius N. **Monomer dimer dynamics and distribution of GPI-anchored uPAR are determined by cell surface protein assemblies**. *J Cell Biol* (2007) 179: 1067-1082

Cellomics

Head of Unit: ***María Montoya***

Support Scientists: *José Manuel Ligos*

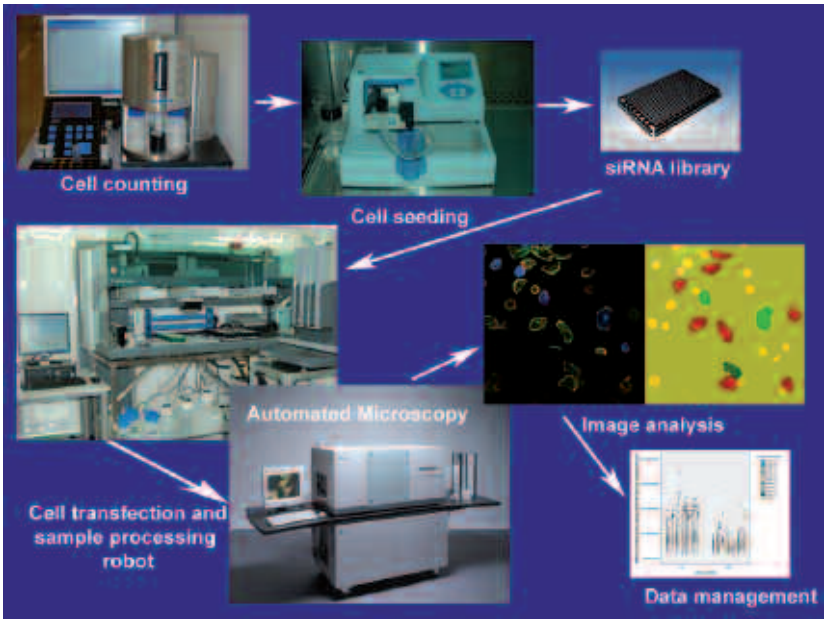
Technicians: *Mariano Vitón*
Raquel Nieto

RESEARCH INTEREST

The Cellomics Unit is dedicated to providing support and innovation in all aspects of cell analysis. The Unit was formed in December 2008 through the union of the Cytometry Unit and the newly-established High Content Screening (HCS) service. HCS is an emerging technology that originated in the drug discovery field, and extracts multiparametric data from cells by automating cell biology assays through a combination of liquid handling robotics, automated microscopy and image analysis. Application of HCS to RNA interference loss-of-function studies will enable CNIC researchers to perform genetic screens for cell-based systems-biology studies.

Installed equipment.

- Three latest-generation digital analytical flow cytometers. A FACSCanto II (Becton Dickenson) and a Cyan (Beckman Coulter) each incorporate 3 lasers capable of detecting 11 parameters simultaneously. An additional FACSCanto II is equipped with 2 lasers and a multiplate loader, capable of analysing 8 parameters.
- Two high speed flow sorters: a MoFlo (Beckman Coulter) equipped with three lasers for 10 parameters, and a custom-made FACSAria II equipped with 4 lasers and digital technology for 18 parameters.
- A liquid handling workstation with three robotic arms connected to a cell culture incubator with 110 plate throughput (Freedom EVO, Tecan).
- An automated confocal microscope for high-resolution, high-speed microplate reading (Opera QEHS, Perkin Elmer) equipped with 4 lasers, on-board liquid dispenser, and an incubator for live cell imaging.
- Dedicated cytometry and image analysis software packages (Modfit, FlowJo, Acapella).



High content screening workflow, showing dedicated equipment housed in the Unit

MAJOR GRANTS

- ISCIII (PI06/1839 to MM).

SELECTED PUBLICATIONS

Yanez-Mo M, Barreiro O, Gonzalo P, Batista A, Megias D, Genis L, Sachs N, Sala-Valdes M, Alonso MA, Montoya MC, Sonnenberg A, Arroyo AG and Sanchez-Madrid F. **MT1-MMP collagenolytic activity is regulated through association with tetraspanin CD151 in primary endothelial cells**. *Blood* (2008) 112: 3217-3226

Eswaran J, Bernad A, Ligos JM, Guinea B, Debreczeni JE, Sobott F, Parker SA, Najmanovich R, Turk BE and Knapp S. **Structure of the human protein kinase MPSK1 reveals an atypical activation loop architecture**. *Structure* (2008) 16: 115-124

Megías D, Marrero R, Del Peso BM, García MA, Bravo-Cordero JJ, García-Grande A, Santos A and Montoya MC. **Novel lambda FRET spectral confocal microscopy imaging method**. *Microsc Res Tech* (accepted)

Lucas D, Escudero B, Ligos JM, Segovia JC, Estrada JC, Terrados G, Blanco L, Samper E and Bernad A. **Altered hematopoiesis in mice lacking DNA polymerase mu is due to inefficient double-strand break repair**. *PLoS Genet* (accepted)

Marrero-Diaz R, Bravo-Cordero JJ, Megías D, García MA, Bartolomé RA, Teixido J and Montoya MC. **Polarized MT1-MMP-CD44 interaction and CD44 cleavage during cell retraction reveal an essential role for MT1-MMP in CD44 mediated invasion**. *Cell Motil Cytoskeleton* (accepted)

Proteomics

Head of Unit: ***Juan Antonio López del Olmo***

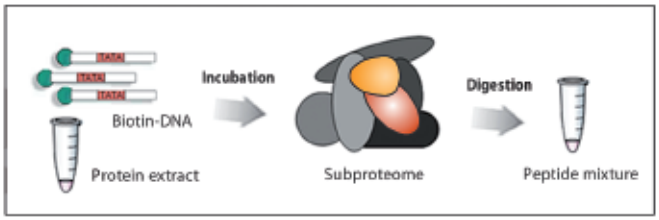
Support Scientists: *Emilio Camafeita*
Enrique Calvo

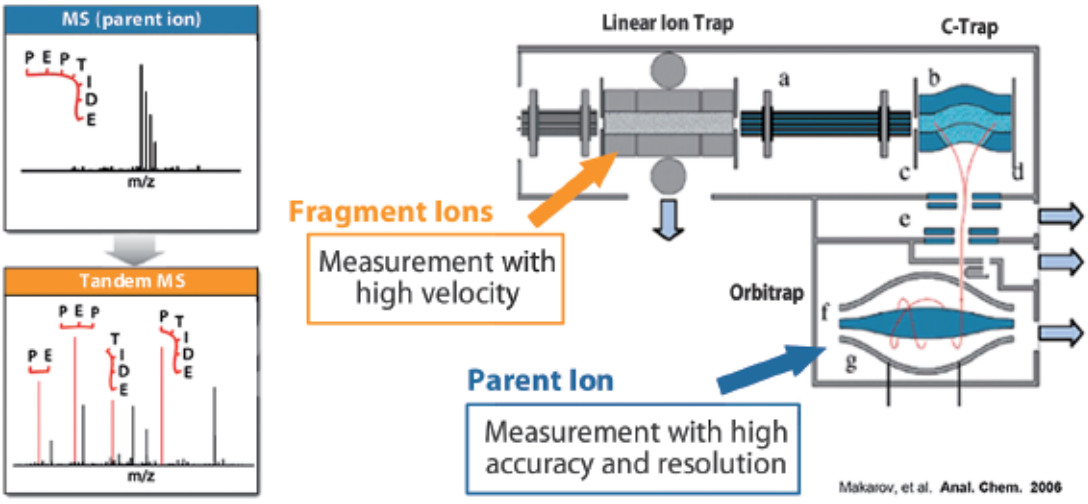
RESEARCH INTEREST

The Proteomics Unit was established in June 2003. Since then, the Unit has grown with the needs of researchers at the CNIC and other public and private institutions, providing services for the separation, quantification, identification and characterization of proteins in biological systems by 2-dimensional gel electrophoresis, multidimensional liquid chromatography, and mass spectrometry (MALDI-TOF and ESI Ion-Trap).

The Unit's biomedical research support is founded on the search for appropriate experimental design, constant protocol optimization, and the continuous update of equipment and expertise. The Unit has a comprehensive system for the separation of proteins by 2-dimensional gel electrophoresis, including the necessary technology for difference gel electrophoresis (DIGE: 2-dimensional gel electrophoresis with pre-labelling of samples with differential fluorochromes). As an alternative "gel-free" approach, a complete nano-HPLC system enables analysis by multidimensional liquid chromatography. Proteins are identified and characterized with MALDI-TOF/TOF and ESI Ion-Trap mass spectrometers (Ion Trap; Triple Quadrupole; and Linear Ion Trap coupled to the Orbitrap high-resolution mass analyser), while a protein picker for protein retrieval from gels and an automatic sample digester contribute to high throughput, automated sample processing.

This robust technology platform and our long experience enable us to tackle research projects demanding both qualitative and quantitative proteomic approaches, such as the assessment of differential protein expression, the study of chemical and post-translational modifications, and the mapping of protein-protein interactions in a wide variety of biological systems.





Protein identification of a complex mixture in the Thermo LTQ Orbitrap XL mass spectrometer

SELECTED PUBLICATIONS

Lizarbe TR, Garcia-Rama C, Tarin C, Saura M, Calvo E, Lopez JA, Lopez-Otin C, Folgueras AR, Lamas S and Zaragoza C. **Nitric oxide elicits functional MMP-13 protein-tyrosine nitration during wound repair**. *FASEB J* (2008) 22: 3207-3215

Coiras M, Camafeita E, Lopez-Huertas MR, Calvo E, Lopez JA and Alcami J. **Application of proteomics technology for analyzing the interactions between host cells and intracellular infectious agents**. *Proteomics* (2008) 8: 852-873

Rollin R, Marco F, Camafeita E, Calvo E, Lopez-Duran L, Jover JA, Lopez JA and Fernandez-Gutierrez B. **Differential proteome of bone marrow mesenchymal stem cells from osteoarthritis patients**. *Osteoarthr Cartilag* (2008) 16: 929-935

Castillo L, Calvo E, Martinez AI, Ruiz-Herrera J, Valentin E, Lopez JA and Sentandreu R. **A study of the Candida albicans cell wall proteome**. *Proteomics* (2008) 8: 3871-3881

Corton M, Botella-Carretero JI, Lopez JA, Camafeita E, San Millan JL, Escobar-Morreale HF and Peral B. **Proteomic analysis of human omental adipose tissue in the polycystic ovary syndrome using two-dimensional difference gel electrophoresis and mass spectrometry**. *Hum Reprod* (2008) 23: 651-661

Genomics

Head of Unit: ***Ana Dopazo***

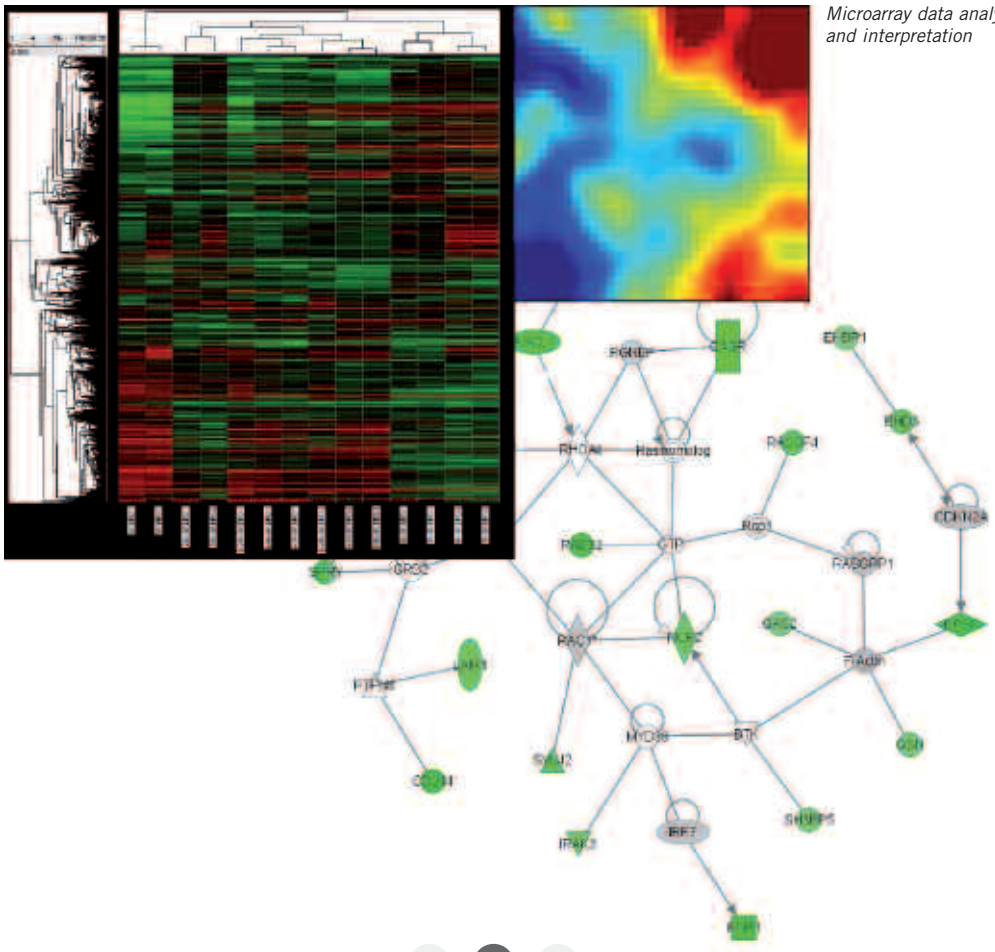
Support Scientists: *Sergio Callejas*
Alberto Benguría
Fátima Sánchez Cabo
Pedro López Romero

Technician: *Rebeca Álvarez*

RESEARCH INTEREST

The Genomics Unit is dedicated to providing high-quality genomic technology, service and support to the scientific community at the CNIC and beyond. The Unit, equipped with Agilent and Affymetrix microarray platforms, has extensive experience in the study of transcriptomes by means of DNA microarrays. Major services and array-based applications include genome-wide gene (mRNA) and microRNA expression analysis, and our service portfolio has recently been expanded to incorporate whole-genome microarray differential gene expression analysis at the exon level. The Unit's expertise in array-based transcriptome analysis encompasses all the necessary steps required by this approach, including experimental design, sample preparation and processing and statistical data analysis

Other services include the maintenance and management of real-time PCR instruments (one AB 7000 and two ABI 7900HT), a TaqMan array processing service and user advice and training on topics related to the Unit's activity (particularly experimental design and statistical data analysis in qPCR and microarray experiments).



Microarray data analysis and interpretation

MAJOR GRANTS

MEC (REDESPROFITMEC07 Splired to AD).

EC (016354 INDIGO to AD).

SELECTED PUBLICATIONS

Gamundi MJ, Hernan I, Muntanyola M, Maseras M, Lopez-Romero P, Alvarez R, Dopazo A, Borrego S and Carballo M. **Transcriptional expression of cis-acting and trans-acting splicing mutations cause autosomal dominant retinitis pigmentosa**. *Hum Mutat* (2008) 29: 869-878

Scheideler M, Elabd C, Zaragosi LE, Chiellini C, Hackl H, Sanchez-Cabo F, Yadav S, Duszka K, Friedl G, Papak C, Prokesch A, Windhager R, Ailhaud G, Dani C, Amri EZ and Trajanoski Z. **Comparative transcriptomics of human multipotent stem cells during adipogenesis and osteoblastogenesis**. *BMC Genomics* (2008) 9: 340

Bermudo R, Abia D, Ferrer B, Nayach I, Benguria A, Zaballos A, Del Rey J, Miro R, Campo E, Martinez-A C, Ortiz AR, Fernandez PL and Thomson TM. **Co-regulation analysis of closely linked genes identifies a highly recurrent gain on chromosome 17q25.3 in prostate cancer**. *BMC Cancer* (2008) 8: 315

Training is one of the CNIC's core activities, and the centre has devised a comprehensive training plan, called CNIC-JOVEN, which includes programmes intended 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 Programme

The ACÉRCATE Programme 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 2008

Name	Secondary School	Comunidad Autónoma
Rocío Alberich	Colegio Hispano Inglés	Canarias
Javier Álvarez	Colegio Santo Tomás de Aquino	Asturias
María Dolores Espinos	IES Cristóbal Lozano	Castilla La Mancha
David García	IES Ramón y Cajal	Castilla La Mancha
Paula Marrero	IES Canarias Cabrera Pinto	Canarias
Sara Miralles	Santísima Trinidad	Madrid
Miguel Molina	IES Pintor Juan Lara	Andalucía
José Manuel Núñez	IES Caura	Andalucía

CICERONE Programme

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

Fellowships in 2008

Name	Degree	University
Laura Gómez	Biology	Complutense, Madrid
María Inmaculada Mejía	Medicine	Complutense, Madrid
Miguel Foronda	Biochemistry	Autónoma, Madrid
Juan Manuel González	Biology	Málaga
Alejandro Prados	Biology	Granada
Ana María Martínez	Biology	Valencia
Marta Cedenilla	Biology	Complutense, Madrid
Diego Balboa	Biotecnology	León
Iñigo Valiente	Biology	Valencia
Verónica Uribe	Biochemistry	Complutense, Madrid
Lucía Morgado	Biochemistry	Autónoma, Madrid
María Martín	Biology	Complutense, Madrid
Isabel Melgare	Medicine	Granada
Damián Lobato	Biology	Complutense, Madrid
María Emilia Tomé	Biotechnology	Francisco de Vitoria, Madrid
Raquel Espín	Biology	Murcia
David Ruano	Biochemistry	Autónoma, Madrid
Alba Vicario	Chemistry	Autónoma, Madrid
Sergio Candel	Biology	Murcia
Ana María Orive	Biology	Autónoma, Madrid

PRÁCTICAS Programme

Through agreements with Spanish universities, this programme offers students the chance to carry out their undergraduate laboratory project at the CNIC.

The aim is to equip university students with in-depth knowledge of biomedical science so that they can make informed choices about a possible career in research.

Fellowships in 2008

Name	Degree	University
Jesús García	Biochemistry	Autónoma, Madrid
Marina Peralta	Biology	Granada
María Pimentel	Biotechnology	Francisco de Vitoria, Madrid

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

This group of lectures provides a general introduction to cardiovascular research in Spain, and also gives participants the chance to ask questions to key researchers and opinion leaders in the field.

Dates: 19 and 20 September 2008

Venue: CNIC Auditorium

Attendees: 141

VASCULAR BIOLOGY Course

Dr Valentín Fuster delivers this lecture series, sponsored by the pharmaceutical company Esteve, on "Vascular biology: basic and clinical research" as part of the summer programme of the Universidad Internacional Menéndez Pelayo (UIMP).

Dates: 21 and 22 July 2008

Venue: CNIC Auditorium

Attendees: 234

Recent Graduates

CARDIOVASCULAR POSGRADUATE Programme

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

In 2008, 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: 12-29 January 2009

Venue: CNIC

Students: 13

MASTERS Programme

This grants programme provides individual funding for study towards a Masters degree at a Spanish university. The programme is directed at students who are going to study for a PhD in one of the CNIC's labs: 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 2008

Name	Masters studies	University
Marta Cedenilla	Cell and Molecular Biology	Autónoma, Madrid
Miguel Foronda	Cell and Molecular Biology	Autónoma, Madrid
Laura Gómez	Cell and Molecular Biology	Autónoma, Madrid
Juan Manuel González	Cell and Molecular Biology	Autónoma, Madrid
David Lara	Cell and Molecular Biology	Autónoma, Madrid
Nerea Méndez	Molecular Biomedicine	Autónoma, Madrid
Verónica Uribe	Cell and Molecular Biology	Autónoma, Madrid
Iñigo Valiente	Molecular Biomedicine	Autónoma, Madrid

PREDOCTORAL (PhD) Programme

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

The aims of the programme are as follows:

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

Graduate students conducting their PhD research at the CNIC during 2008

Name	Funding Agency	CNIC Department
Patricia García	CNIC-Bancaja	Atherothrombosis and Cardiovascular Imaging
Sales Ibiza	CNIC-Bancaja	Regenerative Cardiology
Enara Aguirre	FIS (Spanish Ministry of Health)	Regenerative Cardiology
Oscar A. Marcos	FPU/MEC Grant (Spanish Ministry of Education and Science)	Atherothrombosis and Cardiovascular Imaging
Araceli Grande	CNIC	Vascular Biology and Inflammation
Vanessa Romero	FPU (Spanish Ministry of Education and Science)	Vascular Biology and Inflammation
Raquel López	FIS (Spanish Ministry of Health)	Regenerative Cardiology
Katia Urso	FIS (Spanish Ministry of Health)	Vascular Biology and Inflammation
Aranzazu Cruz	FPU (Spanish Ministry of Education and Science)	Vascular Biology and Inflammation
Carlos A. Tarín	FPI (Spanish Ministry of Education and Science)	Atherothrombosis and Cardiovascular Imaging
Marta C. Guadañillas	FPI (Spanish Ministry of Education and Science)	Vascular Biology and Inflammation
María Victoria Hernández	FPI (Spanish Ministry of Education and Science)	Vascular Biology and Inflammation
Olivia Muriel	FIS (Spanish Ministry of Health)	Vascular Biology and Inflammation
Mónica Sala	FIS (Spanish Ministry of Health)	Vascular Biology and Inflammation
Alberto Izarra	FPI (Spanish Ministry of Education and Science)	Regenerative Cardiology
Beatriz Escudero	FIS (Spanish Ministry of Health)	Regenerative Cardiology
Silvia García	FPU (Spanish Ministry of Education and Science)	Regenerative Cardiology
Alberto Roselló	FPI (Spanish Ministry of Education and Science)	Cardiovascular Developmental Biology
Clara García	FPU (Spanish Ministry of Education and Science)	Cardiovascular Developmental Biology
Emilio Tejera	FIS (Spanish Ministry of Health)	Vascular Biology and Inflammation
Amelia Escolano	FPI (Spanish Ministry of Education and Science)	Vascular Biology and Inflammation
Cristina Sánchez	FPI (Spanish Ministry of Education and Science)	Regenerative Cardiology
Bárbara Pemaute	FPU (Spanish Ministry of Education and Science)	Cardiovascular Developmental Biology
Beatriz Fdez.-Tresquerres	FPI (Spanish Ministry of Education and Science)	Cardiovascular Developmental Biology
Daniel Alameda	FPI (Spanish Ministry of Education and Science)	Regenerative Cardiology
Teresa Rayón	FPU (Spanish Ministry of Education and Science)	Cardiovascular Developmental Biology

CARDIO-IMAGE Programme

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

Fellowships in 2008

Name	Institution
Ana María García	Cardiología-Quirón, Madrid
Gabriela Guzmán	Hospital Universitario La Paz, Madrid
Teresa López	Hospital Universitario La Paz, Madrid

Postgraduate Students & Medical Professionals

INVESMIR Programme

The INVESMIR programme 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 programme is that participants will 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 centres within the Spanish National Health System.

Fellowships in 2008

Name	Hospital	CNIC Department
Aida Esperanza Ballén	Hospital Universitario 12 de Octubre, Madrid	Regenerative Cardiology
Eduardo Barge	Hospital Juan Canalejo, A Coruña	Regenerative Cardiology
Gustavo Andrés Prieto	Hospital Universitario 12 de Octubre, Madrid	Cardiovascular Developmental Biology

CARDIOJOVEN Programme

The CARDIOJOVEN Programme provides theoretical and practical training for medical practitioners in the cardiovascular area who are interested in research. The aim is to promote high-quality translational research at centres within the Spanish National Health System.

The programme offers quality training for up to three years in three strands: clinical research methodology (including statistical analysis), the latest basic research techniques used in cardiovascular medicine, and the possibility of further specialization in any clinical area of cardiology.

The Programme includes a fellowship at the Johns Hopkins University and the possibility of fellowships at Mount Sinai Medical School or other international centres.

Fellowships in 2008

Name	Institution
Ana García Álvarez	Hospital Clínico, Barcelona

CARDIOVASCULAR PATHOPHYSIOLOGY Course: "From symptoms to genes"

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

Dates: 14 and 15 November 2008

Venue: CNIC Auditorium

Attendees: 116

Physicians & Researchers

POSTDOCTORAL Programme

The POSTDOCTORAL programme is designed to attract young researchers (both Spanish and citizens of other countries) to receive top level training in one of the areas of cardiovascular research being carried out in the laboratories at our centre. Research projects can also be carried out in collaboration with international centres with which the CNIC has established training agreements. With this programme the CNIC aims to make a significant contribution to the creation of a strong base of internationally-trained scientists specialized in areas of interest in cardiovascular research.

Fellowships in 2008

Name	CNIC Department	Research line
Kausalia Vijayaragavan	Regenerative Cardiology	Cellular characterization of cardiac development potential from a novel non-canonical Wnt11 responsive mesoderm population derived from human embryonic stem cells

TRANSLATIONAL RESEARCH Forum

This forum on Translational Research provides a shared space where basic and clinical researchers in the cardiovascular area can exchange ideas and scientific interests. The aim is to stimulate the development of translational projects that will permit a rapid transfer of research findings to the clinic, to the benefit of patients.

Dates: 28 and 29 November 2008

Venue: CNIC Auditorium

Attendees: 137

Seminars and Events

JANUARY

14	**María Blasco** (Centro Nacional de Investigaciones Oncológicas, Madrid, Spain)
21	**Robert Roberts** (University of Ottawa Heart Institute, Ottawa, Canada)

FEBRUARY

04	**Juan Carlos Kaski** (St Georges Hospital Medical School, London, UK)
07	**Francesco Blasi** (Firc Institute of Molecular Oncology, Milan, Italy)
11	**Johanna Ivaska** (VTT Medical Biotechnology, Turku, Finland)
18	**Xosé Bustelo** (Centro de Investigación del Cáncer, Instituto de Biología Molecular y Celular del Cáncer, CSIC-University of Salamanca, Salamanca, Spain)
25	**Brant Weinstein** (National Institute of Child Health and Human Development, NIH, Bethesda, USA)

MARCH

03	**Lars Ryden** (Karolinska University Hospital, Stockholm, Sweeden)
04	**José Luis Gómez-Skarmeta** (Centro Andaluz de Biología del Desarrollo, Sevilla, Spain)
06	**Patricia Ruiz** (Max Planck Institute for Molecular Genetics, Berlin, Germany)
10	**Robert Hegele** (Schulich School of Medicine and Dentistry, University of Western Ontario, Canada)
17	**Jonathan Epstein** (Penn Cardiovascular Institute, University of Pennsylvania, Philadelphia, USA)

26	**Fred Brancati** (Johns Hopkins University, Baltimore, USA)
28	**Martin Schawrtz** (Mellon Prostate Cancer Research Institute, University of Virginia, Charlottesville, USA)
31	**Marcos González-Gaitán** (Geneva University, Geneva, Switzerland)

APRIL

03	**Zoltan Ivics** (Max Delbruck Centre for Molecular Medicine, Berlin, Germany)
07	**Christopher K. Glass** (University of California, San Diego, USA)
14	**Klaus Ley** (La Jolla Institute for Allergy & Immunology, La Jolla, USA) **William Sessa** (Yale University School of Medicine, New Haven, USA)
16	**Jesús Salvador** (Centro Nacional de Biotecnología, Madrid, Spain)
17	**Christian Hellriegel** (University of California, Berkeley, USA)
18	**Enrique Lara-Pezzi** (Imperial College London, Londo, UK)
21	**Felipe Prosper** (Universidad de Navarra, Pamplona, Spain)
22	**Alfonso Martínez-Arias** (University of Cambridge, Cambridge, UK)
28	**Carl Figdor** (Nijmejen Centre for Molecular Life Sciences, Nijmegen, The Netherlands)
30	**Steve Albelda** (University of Pennsylvania, Philadelphia, USA)

MAY

05	**Josep Brugada** (Hospital Clínic de Barcelona, Barcelona, Spain)
06	**Carmen Guerra** (Centro Nacional de Investigaciones Oncológicas, Madrid, Spain)
12	**Gordon Keller** (McEwen Centre for Regenerative Medicine, Toronto, Canada)
19	**Thomas Graf** (Centre for Genomic Regulation, Barcelona, Spain)

JUNE

02	**Carlo Patrono** (University of Rome, Rome, Italy)
03	**Ignacio Flores** (Centro Nacional de Investigaciones Oncológicas, Madrid, Spain)
09	**Didier Stainier** (University of California, San Francisco, USA)
23	**Anne Eichmann** (College de France, Paris, France)
27-28	*CNIC First Cardiovascular Symposium* **Mark H. Ginsberg** (University of California, San Diego, La Jolla, USA) **Timothy A. Springer** (Immune Disease Institute, Harvard Medical School, Boston, USA) **Eric Rimm** (Harvard Medical School, Boston, USA) **Chris O´Donnell** (National Heart, Lung and Blood Institute, Framingham Heart Study, Bethesda, USA) **Ihor R. Lemischka** (Mount Sinai School of Medicine, New York, USA) **Kenneth R. Chien** (MGH Cardiovascular Research Center, Massachusetts General Hospital, Richard B. Simches Research Center, Boston, USA)

27-28 *CNIC First Cardiovascular Symposium*

Peter Carmeliet
(Centre for Transgene Technology and Gene Therapy, Flanders Interuniversity Institute for Biotechnology, Leuven, Belgium)

Robert Roberts
(University of Ottawa Heart Institute, Ottawa, Canada)

Zahi Fayad
(Mount Sinai School of Medicine, New York, USA)

James Rudd
(Addenbrooke's Hospital, Cambridge, UK)

Bernard Gersh
(Mayo Clinic, Rochester, USA)

Michael Bristow
(University of Colorado Cardiovascular Institute, Health Sciences Center, Denver, USA)

JULY

21 **Valentín Fuster**
(Centro Nacional de Investigaciones Cardiovasculares and Cardiovascular Institute, Mount Sinai Medical Center, New York, USA)

AUGUST

13-16 *10th International Conference on Limb Development and Regeneration*

Denis Duboule
(University of Geneva, Geneva, Switzerland)

John Fallon
(School of Medicine. University of Wisconsin, Wisconsin, USA)

Juan Hurlé
(Faculty of Medicine. University of Cantabria, Santander, Spain)

Malcolm Logan
(National Institute for Medical Research, London, UK)

Susan Mackem
(National Cancer Institute, Bethsheda, Bethesda, USA)

Gail Martin
(University of California, San Francisco, USA)

13-16 *10th International Conference on Limb Development and Regeneration*

Lee Niswander
(University of Colorado Health Sciences Center, Denver, USA)

Sumihare Noji
(Faculty of Engineering, The University of Tokushima, Tokushima, Japan)

Marian Ros
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(Harvard Medical School, Boston, USA)

Cheryll Tickle
(University of Bath, Bath, UK)

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(Centro Nacional de Investigaciones Cardiovasculares, Madrid, Spain)

Lewis Wolpert
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Rolf Zeller
(Centre for Biomedicine. University of Basel Medical School, Basel, Switzerland)

SEPTEMBER

10 **Wolfgang Link**
(Centro Nacional de Investigaciones Oncológicas, Madrid, Spain)

11 **María Montoya**
(Centro Nacional de Investigaciones Oncológicas, Madrid, Spain)

12 **Juan Manuel Domínguez Correa**
(GlaxoSmithKlin, Madrid, Spain)

23 **Eric Olson**
(University of Texas, Austin, USA)

30 **Doug Hanahan**
(UCSF Helen Dillier Family Comprehensive Cancer Centre, San Francisco, USA)

OCTOBER

06	**Francisco Fernández-Avilés** (Hospital Gregorio Marañón, Madrid, Spain)
24	**Antoon Moorman** (Academy Medical Centre, University of Amsterdam, Amsterdam, The Netherlands)
28	**Diego Franco** (University of Jaen, Jaen, Spain)

NOVEMBER

10	**José Manuel Sánchez Morgado** (Centro Nacional de Investigaciones Cardiovasculares, Madrid, Spain)
17	**Angela Nieto** (Neurosciences Institute, Alicante, Spain)
24	**Benoit Bruneau** (Gladstone Institute of Cardiovascular Disease, San Francisco, USA)

DECEMBER

01	**Ramón Estruch** (Fundació Cliníc, Hospital Cliníc de Barcelona, Barcelona, Spain)
09	**Natalia Jiménez** (Centro Nacional de Biología-Consejo Superior de Investigaciones Científicas, Madrid, Spain)
15	**Tim Wai** (McGill University, Montreal, Canada)
23	**Daniel Lucas- Alcaraz** (Mount Sinai School of Medicine, New York, USA)

CNIC Scientific Publications in 2008, by Department

(IF = Thompson ISI impact factor, 2007)

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CNIC Publications 2008

	Nº articles	Cumulative IF	Mean IF
VASCULAR BIOLOGY AND INFLAMMATION	18	143.877	7.993
REGENERATIVE CARDIOLOGY	13	106.056	8.158
CARDIOVASCULAR DEVELOPMENTAL BIOLOGY	5	33.142	6.628
ATHEROTHROMBOSIS AND CARDIOVASCULAR IMAGING	19	146.045	7.687
CARDIOVASCULAR EPIDEMIOLOGY AND POPULATION GENETICS	15	90.416	6.028
TRANSLATIONAL CARDIOVASCULAR RESEARCH	3	5.210	1.303
TECHNICAL UNITS	18	76.724	4.262
ALL PUBLICATIONS	**91**	**601.470**	**6.610**

The CNIC: An innovative joint venture between the state and the private sector

On December 15 2005, the Spanish Prime Minister, José Luis Rodríguez Zapatero, presided at the creation of the Pro CNIC Foundation, through which some of the most important Spanish companies agreed to provide a significant part of the funding for the research activity at the Centro Nacional de Investigaciones Cardiovasculares (CNIC).

Through the creation of the Pro CNIC Foundation, some of the largest companies in the country have made a long-term commitment to biomedical research. One of the central elements of the agreement was the incorporation of Valentín Fuster as president of the Centre's External Scientific Advisory and Evaluation Committee. The creation of the Pro CNIC Foundation represents the most significant act of business sponsorship in recent years in terms of the amount of funding it provides, its social significance, the group of companies involved, and the anticipated outcomes.

Participating companies take part in important decisions about the CNIC's activities through their representation on the CNIC's Board of Trustees. The CNIC project is thus structured as a joint venture between the state and the private sector, ensuring stable funding.

If you would like to know more about the funding of the CNIC you can read the following article published in Nature Clinical Practice Cardiovascular Medicine, CNIC Edition (November 2008, Vol 5, CNIC-2): "Spanish National Centre for Cardiovascular Research (CNIC): pioneering a new model for funding biomedical research", by Ginés Sanz and Valentín Fuster.

Scheme of CNIC Funding

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Investigaciones
Cardiovasculares
Carlos III

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