RESEARCH AREAS

TRANSLATIONAL COORDINATION

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

2. Vascular Pathophysiology

AREA COORDINATORS:

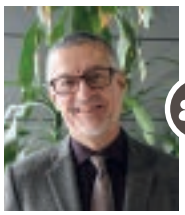
ALMUDENA RAMIRO

ANTONIO FERNÁNDEZ-ORTIZ

RESEARCH INTEREST

Research in the Vascular Pathophysiology Area (VPA) focuses on the biology of the vascular system in health and disease, using a multidisciplinary and transverse approach, embracing molecular and cellular biology as well as translational and clinical research. Our research groups use a wide variety of techniques, including animal, tissue, cell and molecular models, to investigate normal vascular function and the key steps in the vascular alterations that underlie cardiovascular diseases. We are also interested in the cellular and molecular mechanisms regulating striated muscle regeneration and growth in physiology and pathology, as well as in aging. VPA groups also work on translational and clinical research through several research projects, including Secure and PESA. We also have a major interest in cardiovascular proteomics. The VPA hosts three technical units: Genomics, Proteomics/Metabolomics, and Bioinformatics.

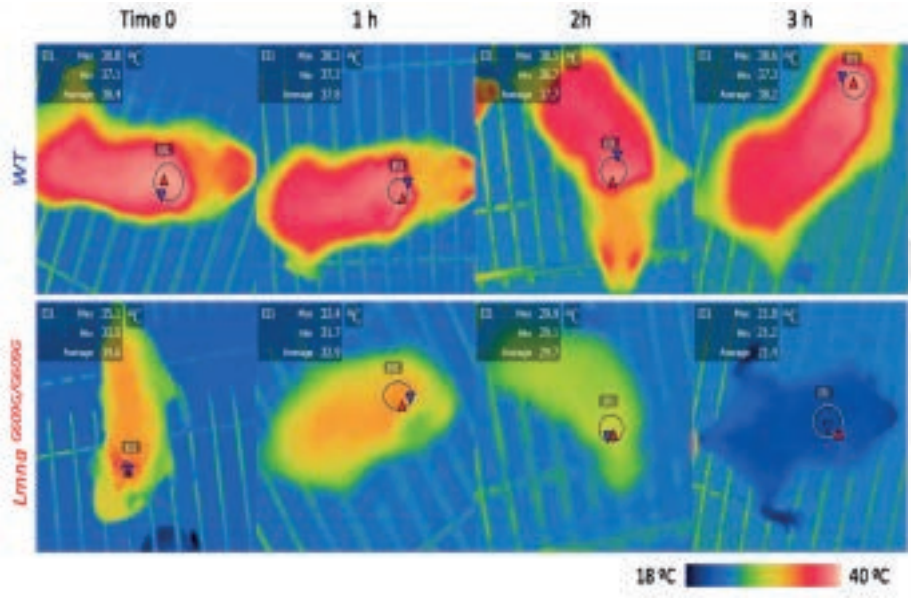
Molecular and genetic cardiovascular pathophysiology



RESEARCH INTEREST

The World Health Organization has estimated that cardiovascular disease (CVD) will by 2020 be the main health and socioeconomic problem worldwide, in part due to the progressive aging of the world population. Atherosclerosis and heart failure contribute significantly to CVD-related morbimortality in the elderly. These anomalies and the aging process are greatly accelerated in Hutchinson-Gilford progeria syndrome (HGPS), a rare genetic disorder caused by the expression of progerin, a mutant form of lamin A. The most serious aspect of HGPS is extensive atherosclerosis and cardiac electrophysiological alterations that are associated with early death (average lifespan, 14.6 years; range, 8-21 years), predominantly from myocardial infarction or stroke. Progerin is also expressed at low level in aged tissues of non-HGPS individuals, suggesting a role in normal aging. Understanding how this mutant form of lamin A causes CVD and premature aging may therefore shed light on normal aging.

Our current research focuses on the following areas: 1) The role of nuclear A-type lamins in atherosclerosis and aging; 2) Cellular and molecular mechanisms underlying progerin-induced cardiovascular damage; 3) Generation of a new HGPS mouse model to assess the reversibility and tissue-specificity of progerin-induced damage; 4) Generation of a porcine model of HGPS using CRISPR/Cas9 technology to accelerate translational research in HGPS; and 5) The molecular mechanisms common to premature and physiological aging and specific to each process.



Representative thermographs showing basal hypothermia and impaired heat production in the dorsal brown adipose tissue of 4-month-old progeroid *Lmna*$^{G609G/G609G}$ mice exposed to 4ºC for 3 h, resulting in accelerated loss of body temperature compared with age-matched WT controls.

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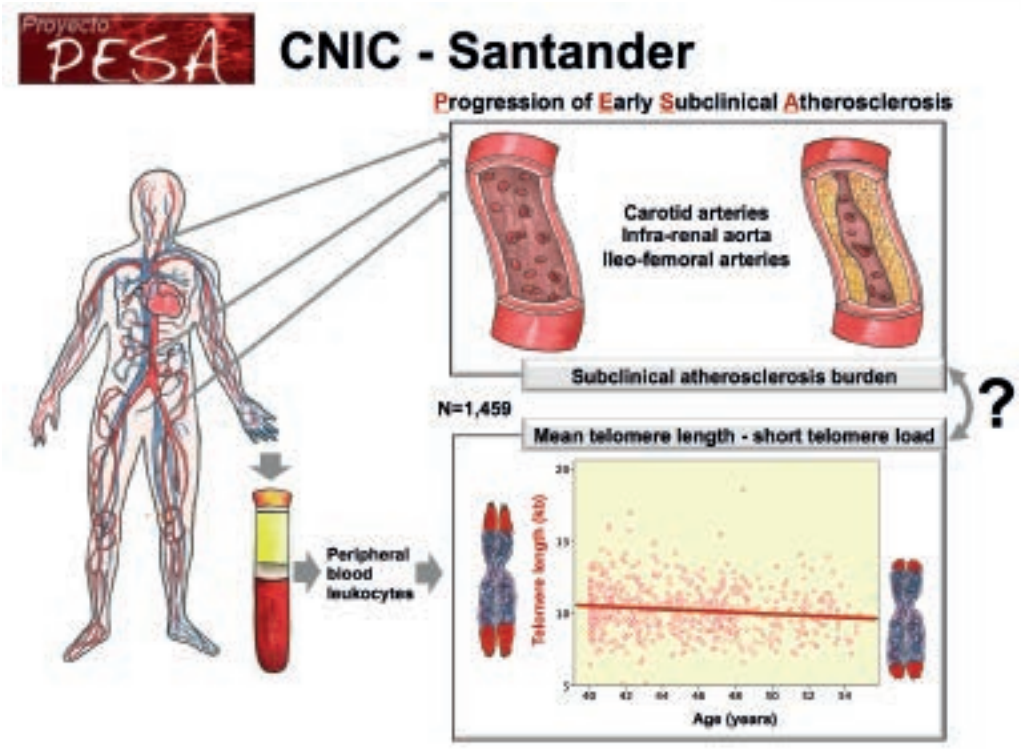

In a cross-sectional study, different vascular territories were analyzed by 2-dimensional and 3-dimensional ultrasound to quantify subclinical atherosclerosis burden and examine possible associations with mean telomere length and short telomere load in peripheral blood leukocytes examined by high-throughput quantitative fluorescence in situ hybridization (Fernández-Alvira et al. *J Am Coll Cardiol* 67: 2467-76, 2016).

MAJOR GRANTS

- Progeria Research Foundation (Established Investigator Award PRF 2014)
- Ministerio de Economía y Competitividad. Modalidad Retos Investigación (SAF2013-46663-R)
- Ministerio de Economía y Competitividad. Modalidad Retos Investigación (SAF2016-79490-R)
- Marató TV3 (20153731).
- Ministerio de Economía y Competitividad. FIS RETICS (RiC, RD12/0042/0028)
- Ministerio de Economía y Competitividad. FIS (CP11/00145) PI: J.M. González Granado
- Fundación Ramón Areces (XVII Concurso Nacional para la Adjudicación de Ayudas a la Investigación en Ciencias de la Vidas y de la Materia). PI: J.M. González Granado

SELECTED PUBLICATIONS

Rivera-Torres J, Calvo CJ, Llach A, Guzmán-Martínez G, Caballero R, González-Gómez C, Jiménez-Borreguero LJ, Guadix JA, Osorio FG, López-Otín C, Herraiz-Martínez A, Cabello N, Vallmitjana A, Benítez R, Gordon LB, Jalife J, Pérez-Pomares JM, Tamargo J, Delpón E, Hove-Madsen L, Filgueiras-Rama D, Andrés V. **Cardiac electrical defects in progeroid mice and Hutchinson-Gilford progeria syndrome patients with nuclear lamina alterations**. *Proc Natl Acad Sci U S A* (2016) 113: E7250-E7259

Fernández-Alvira JM, Fuster V*, Dorado B, Soberón N, Flores I, Gallardo M, Pocock S, Blasco MA, Andrés V*. **Short telomere load, telomere length, and subclinical atherosclerosis: the PESA study**. *J Am Coll Cardiol* (2016) 67: 2467-76 (* corresponding authors)

Villa-Bellosta R, Hamczyk MR, Andrés V. **Alternatively activated macrophages exhibit an anti-calcifying activity dependent on extracellular ATP/pyrophosphate metabolism.** *Am J Physiol Cell Physiol* (2016) 310: C788-99

Fuster V, Ibáñez B, Andrés V. **The CNIC: a successful vision in cardiovascular research.** *Circ Res* (2016) 119: 785-9

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

Experimental pathology of atherosclerosis

RESEARCH INTEREST

Living a long life is the main risk factor for suffering atherosclerotic heart attack or stroke; the longer you survive other threats, the more likely you are to face the consequences of atherosclerosis developing insidiously within your arteries. Consequently, as lifespan increases around the world due to improvements in socioeconomic conditions and health care, so does the need to find efficient ways of retarding atherosclerosis.

The goals of our laboratory are to improve understanding of the mechanisms underlying initiation and progression of atherosclerosis and to develop tools that can eventually monitor atherosclerosis in humans. Our work relies heavily on genetic tools to induce atherosclerosis in mice and minipigs by increasing the principal causal factor for atherosclerosis, apoB-containing lipoproteins (apoB-LP).

Current work in the lab focuses on the interaction of apoB-LP with the vascular wall and how local arterial wall cells transform their phenotype and engage in atherosclerotic lesion development. Recently we have described how blood flow forces modulate the artery wall to sequester more apoB-LP from the bloodstream. Furthermore, we have characterized the clonal architecture of smooth muscle cells in atherosclerotic lesions, showing that these cells are derived from a surprisingly low number of pre-existing cells undergoing substantial clonal expansion during disease development.

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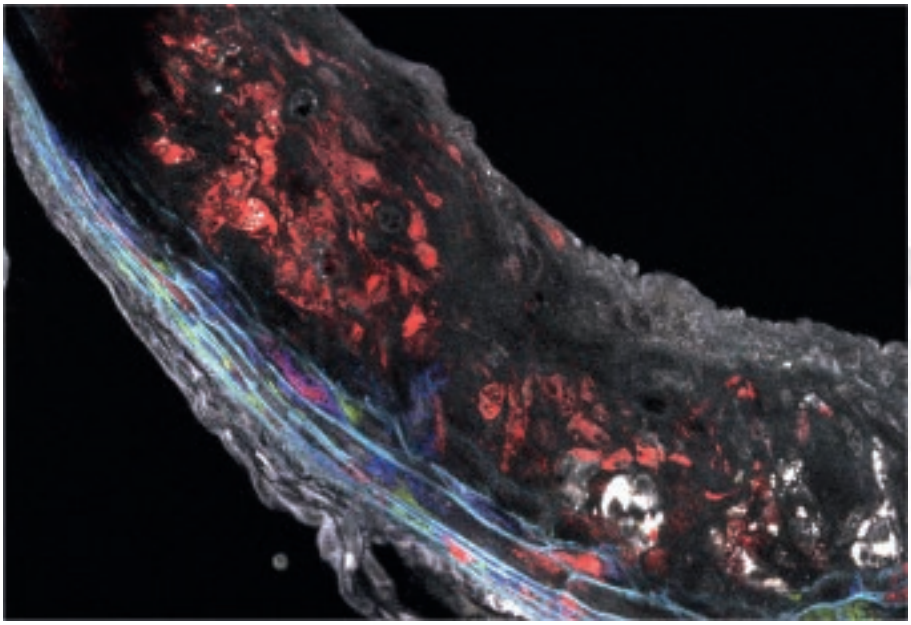


Clone in atherosclerosis. Atherosclerosis induced in mice with mosaic expression of fluorescent proteins in smooth muscle cells (SMC). The large population of red fluorescent SMCs is descended with high probability from a single cell that underwent massive clonal expansion during atherosclerotic plaque development.

ApoB-LP retention. ApoB-LP retention (black) across the vascular tree of a normal mouse. Locations of ApoB-LP binding in the vascular tree under physiological conditions are the same as those that develop atherosclerosis when ApoB-LP levels are high.

MAJOR GRANTS

- Ministerio de Economía y Competitividad (SAF2016-75580-R)
- Det Frie Forskningsråd, Sapere Aude Level II grant (DFF – 4004-00459). Funds held at Aarhus University.
- Novo Nordisk Fonden, Interdisciplinary Synergy grant (PI: Søren Moestrup). Funds held at University of Southern Denmark

SELECTED PUBLICATIONS

Bentzon JF. **Targeting inflammation in atherosclerosis**. *J Am Coll Cardiol* (2016) 68: 2794-6

Mortensen MB, Nilsson L, Larsen TG, Espeseth E, Bek M, Bjørklund MM, Hagensen MK, Wolff A, Gunnersen S, Füchtbauer EM, Boedtkjer E, Bentzon JF. **Prior renovascular hypertension does not predispose to atherosclerosis in mice**. *Atherosclerosis* (2016) 249: 157-63

Poulsen CB, Mortensen MB, Koechling W, Sørensen CB, Bentzon JF. **Differences in hypercholesterolemia and atherogenesis induced by common androgen deprivation therapies in male mice**. *J Am Heart Assoc* (2016) 5: e002800

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

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

Intercellular signaling in cardiovascular development & disease

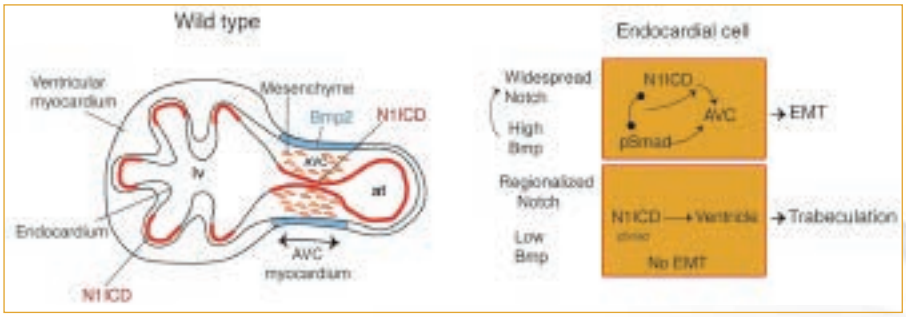
RESEARCH INTEREST

We investigate the signaling pathways regulating cardiovascular development and how their alteration can cause congenital heart disease. We use the mouse as our main experimental model, and also study heart regeneration in the zebrafish. Our experimental approach couples genetics with live imaging, global gene expression analysis, cardiac explant assays, cell biology/biochemistry experiments, and ultimately validation studies in human samples.

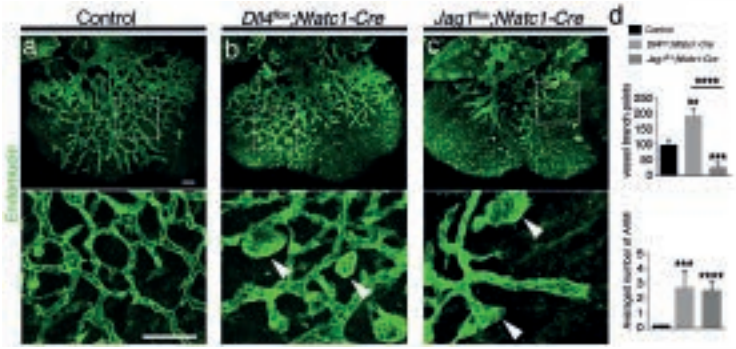
During heart valve development, the myocardial signal Bmp2 activates and functions together with the endocardial signal Notch to pattern the embryonic endocardium as cardiac valve tissue (Figure 1). NOTCH signaling alterations in humans lead to aortic valve dysmorphogenesis; we are currently investigating the potential interplay between NOTCH and WNT signaling in a cohort of aortic valve disease patients.

The coronary vasculature develops to satisfy the increasing oxygen demand of the expanding ventricular walls. We have found that dynamic Notch ligand-receptor signaling regulates capillary sprouting, coronary artery specification, and vascular tree remodeling. The absence of a functional primary coronary plexus in mutant mice leads to an adaptive hypoxia response and reduced cardiomyocyte proliferation, resulting in a thinner myocardial wall and ultimately heart failure and embryonic death (Figure 2).

In adult life, the combination of genetic predisposition and poor dietary habits leads to atherosclerosis, which can ultimately result in obstruction of the main coronary arteries and myocardial infarction. Notch regulates the inflammatory response associated with atherosclerosis and, in the coronaries of atherosclerotic patients, expression of the NOTCH ligand JAG1 is increased, suggesting that this (together with NOTCH-dependent metabolites) may be diagnostic markers of disease progression (Figure 3; Nus et al., 2016).



Model of Bmp2 and Notch1 interplay. E9.5 wild-type heart. Bmp2 expression (blue) restricted to the AVC myocardium induces Notch1 activity and EMT in the AVC endocardium. Uniform N1ICD expression in AVC endocardium and in the atrium (red); in ventricular endocardium N1ICD is restricted to the base of the forming trabeculae. In the ventricles, low Bmp and N1ICD signaling prevent physical interaction of the effectors.



Dorsal view of E12.5 whole-mount hearts stained for the endothelial marker endomucin. (**a**) Control, (**b**) *Dll4^{flox};Nfatc1-Cre*, and (**c**) Jag1^{flox}; Nfatc1-Cre mutant embryos. Mutant embryos show defective vessel branching (**d**) and arterio-venous malformations (AVM, **d**).

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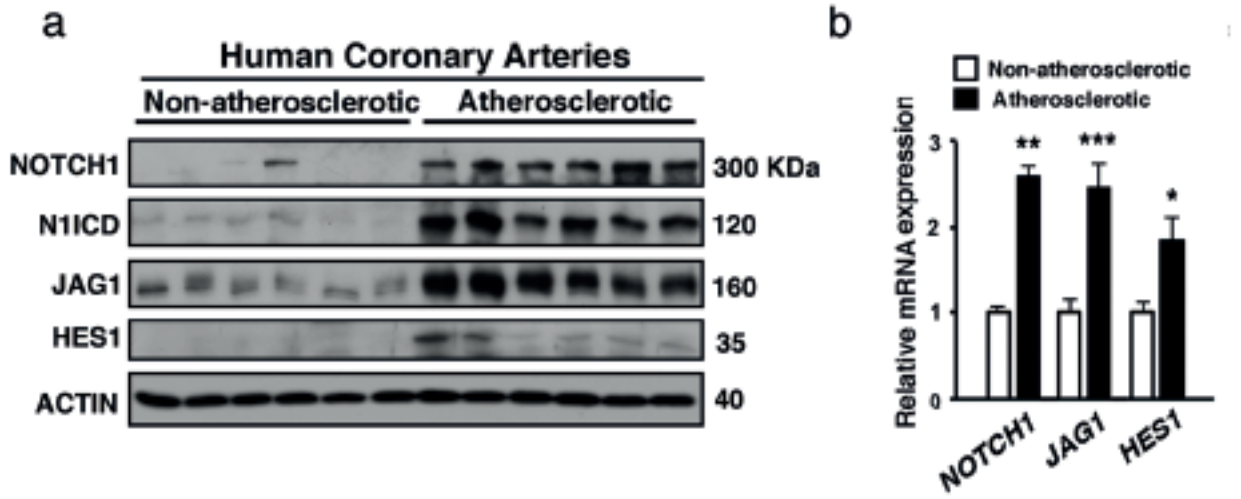

NOTCH signaling upregulation in human atherosclerosis. (**a**) Western blot showing NOTCH1 and N1ICD, JAG1, and HES1 in atherosclerotic and nonatherosclerotic human coronary arteries. (**b**) qPCR analysis of NOTCH signaling genes in atherosclerotic and nonatherosclerotic human coronary arteries.

MAJOR GRANTS

- Ministerio de Economía, Industria y Competitividad (SAF2016-78370-R)
- Ministerio de Economía y Competitividad. Red de excelencia Temática (SAF2015-71863-REDT)
- Ministerio de Economía y Competitividad. FIS RETICS (TERCEL: RD12/0019/0003 and RIC: RD12/0042/0005)
- Ministerio de Economía y Competitividad (SAF2013-45543-R)
- Fundación BBVA (Ref. : BIO14_298)
- Fundació La Marató (Ref.: 20153431)
- European Commission. International IPP (Ref.: UE0COF1214) . PI: L. Luna Zurita
- Ministerio de Economía y Competitividad. (BES-2014-068818) PI: P. Gómez Apiñaniz
- Ministerio de Economía y Competitividad. (SVP-2014-068723) PI: M. Siguero Álvarez
- Fundación La Caixa. (CX_E-2015-04). PI: R. Torregrosa Carrión
- Ministerio de Educación, Cultura y Deporte. (FPU15/01011). PI: A. Salguero Jiménez
- Comunidad de Madrid. (PEJ15/BIO/TL-0428). PI: B Ríos Lara

SELECTED PUBLICATIONS

MacGrogan D, D'Amato G, Travisano S, Martinez-Poveda B, Luxán G, Del Monte-Nieto G, Papoutsi T, Sbroggio M, Bou V, Gomez-Del Arco P, Gómez MJ, Zhou B, Redondo JM, Jiménez-Borreguero LJ, de la Pompa JL. **Sequential ligand-dependent Notch signaling activation regulates valve primordium formation and morphogenesis**. *Circ Res* (2016) 118:1480-97

D'Amato G, Luxán G, Del Monte-Nieto G, Martínez-Poveda B, Torroja C, Walter W, Bochter MS, Benedito R, Cole S, Martinez F, Hadjantonakis AK, Uemura A, Jiménez-Borreguero LJ, de la Pompa JL **Sequential Notch activation regulates ventricular chamber development**. *Nat Cell Biol* (2016) 18: 7-20

de Luxán G, D'Amato G, MacGrogan D, de la Pompa JL. **Endocardial Notch signaling in cardiac development and disease**. *Circ Res* 118: e1-e1

D'Amato G, Luxán G, de la Pompa JL. **Notch signalling in ventricular chamber development and cardiomyopathy**. *FEBS J* (2016) 283: 4223-4237

Gómez-Del Arco P, Perdiguero E, Yunes-Leites PS, Acín-Pérez R, Zeini M, Garcia-Gomez A, Sreenivasan K, Jiménez-Alcázar M, Segalés J, López-Maderuelo D, Ornés B, Jiménez-Borreguero LJ, D'Amato G, Enshell-Seijffers D, Morgan B, Georgopoulos K, Islam AB, Braun T, de la Pompa JL, Kim J, Enriquez JA, Ballestar E, Muñoz-Cánoves P, Redondo JM. **The chromatin remodeling complex Chd4/NuRD controls striated muscle identity and metabolic homeostasis**. *Cell Metab*. (2016) 23: 881-92

Matrix metalloproteinases in angiogenesis and inflammation

RESEARCH INTEREST

The vasculature ensures optimal delivery of nutrients and oxygen throughout the body, and to achieve this function must continually adapt to varying tissue demands, particularly after tissue damage. Our group studies the cellular and molecular mechanisms that govern vascular responses during inflammation and how these mechanisms contribute to tissue repair. We focus mainly on the actions in these responses of membrane-type matrix metalloproteinases in endothelial and vascular smooth muscle cells and macrophages.

We previously showed that the protease MT1-MMP is required for endothelial cell sprouting by processing extracellular matrix components and for macrophage migration by regulating intracellular signals. Our recent studies have expanded our knowledge of MT1-MMP actions in these cell types, showing its role in capillary remodeling and also in macrophage-vessel crosstalk in the mouse heart.

Our understanding of the pathophysiology of the vascular system is also benefiting from our current research into MT4-MMP, a GPI-anchored protease whose substrates and functions have previously received scant attention. Our recent analysis of MT4-MMP-deficient mice complemented with proteomics approaches has identified an essential requirement for MT4-MMP in aorta vessel wall development and function and in aneurysm formation. We are following up these findings by extending the analysis to atherosclerosis, an inflammatory arterial disease, and to arteriogenesis, the de novo formation of collateral arteries, after cardiac ischemia.

For these projects, we are using 2D and 3D angiogenic models, high-resolution microscopy, 3D image analysis, proteomics, bioinformatics, protein modeling, lentiviral strategies, and genetically modified mouse lines and disease models. We ultimately intend to apply this knowledge to develop novel angiotherapies aimed at improving tissue perfusion and/or modulating inflammatory responses in various pathophysiological contexts.

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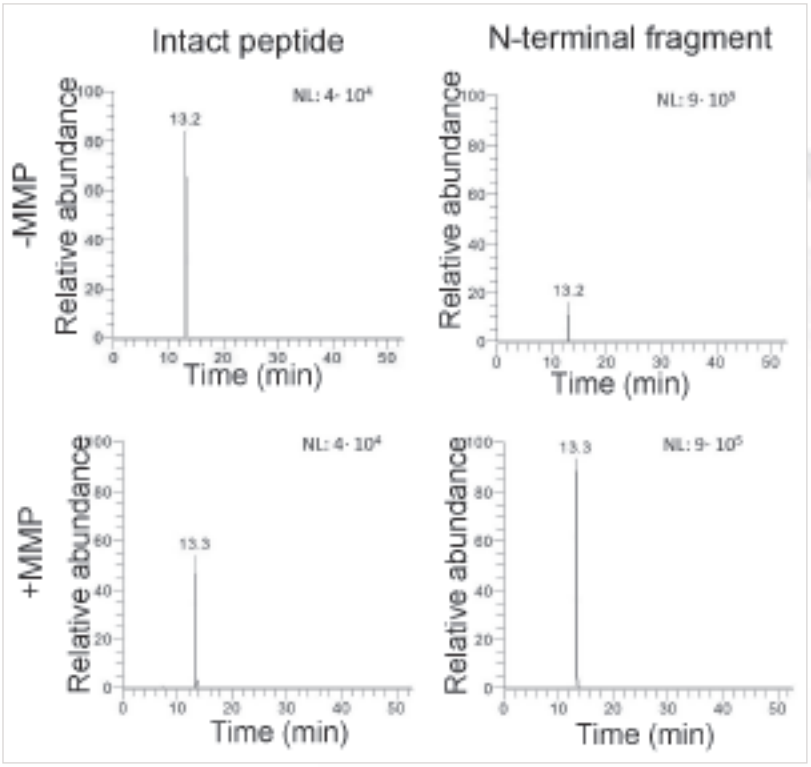
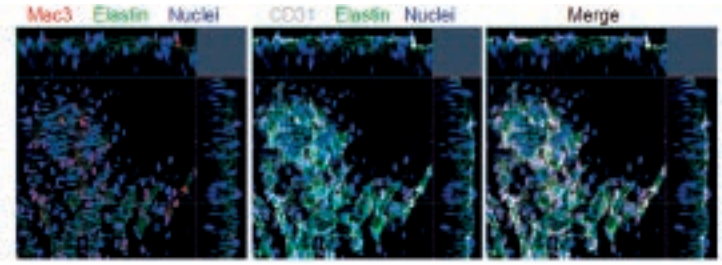
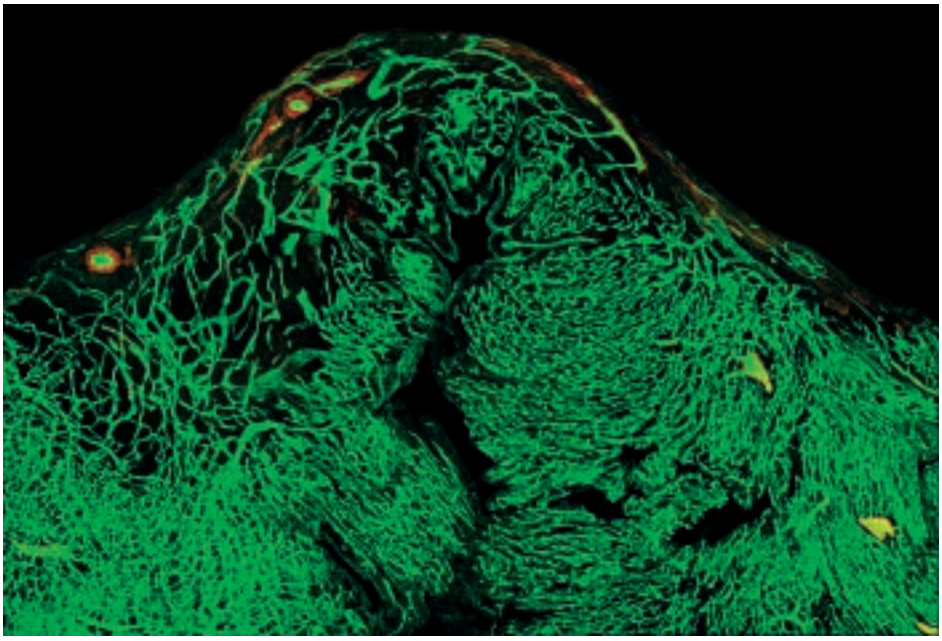


Analysis of protease-mediated substrate cleavage by mass spectrometry. In collaboration with the Proteomics Unit, we are analysing a synthetic peptide containing a putative MMP cleavage site by mass spectrometry in the absence or presence of the recombinant catalytic domain of a given protease. The charts show the profiles obtained for the intact peptide and the N-terminal fragment generated after proteolytic cleavage. This approach is particularly useful for transmembrane protein substrates.



Exploring macrophage-vascular communication in vivo. Whole-mount staining of the aortic arch from Ldlr$^{-/-}$ mice fed a high-fat diet for 3 days allows visualization of macrophage entrapment in the inflamed aortic vessel wall, particularly in the athero-prone lesser curvature of the aorta. The panel shows a representative orthogonal view compiled from confocal images of macrophages (Mac3, red), endothelial cells (CD31, white), vascular elastin (autofluorescence, green), and nuclei (blue).

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



MAJOR GRANTS

- Ministerio de Economía y Competitividad (SAF2014-52050R)
- Ministerio de Economía y Competitividad FIS RETICS (Red de Investigación Cardiovascular: RD12/0042/0023)
- Fundació La Marató TV3 (165/C/2012)
- European Union (PITN-GA-2013-608027) (CardioNext) (Coordinator)

SELECTED PUBLICATIONS

Barreiro O, Cibrian D, Clemente C, Alvarez D, Moreno V, Valiente Í, Bernad A, Vestweber D, Arroyo AG, Martín P, von Andrian UH, Sánchez Madrid F. **Pivotal role for skin transendothelial radio-resistant anti-inflammatory macrophages in tissue repair**. *Elife* (2016) 5: e15251

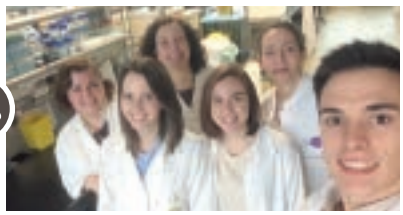
Gkontra P, Żak MM, Norton K-A, Santos A, Popel AS, Arroyo AG. **A 3D fractal-based approach towards understanding changes in the infarcted heart microvasculature**. Medical Image Computing and Computer-Assisted Intervention-MICCAI 2015. *Lecture Notes in Computer Science* (2015) 9351: 173-80

Oller J, Alfranca A, Méndez-Barbero N, Villahoz S, Lozano-Vidal N, Martín-Alonso M, Arroyo AG, Escolano A, Armesilla AL, Campanero MR, Redondo JM. **C/EBPβ and nuclear factor of activated T cells differentially regulate Adamts-1 induction by stimuli associated with vascular remodeling**. *Mol Cell Biol* (2015) 35: 3409-22

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

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

Regulatory molecules of inflammatory processes



RESEARCH INTEREST

Cardiovascular diseases (CVD) are a leading cause of death worldwide and are increasing due to unhealthy modern lifestyles. While a number of treatments are available to address the many risk factors associated with CVD, surgical intervention remains the primary treatment option to prevent or treat an episode of acute myocardial injury. Heart failure can progress to end-stage dilated cardiomyopathy requiring heart transplantation. This process is characterized by inflammation and loss of cardiomyocytes combined with impaired function of the remaining cells, leading to decreased blood flow and increased risk of morbidity and mortality. Inflammation and autoimmune abnormalities play an important role in the progression of heart and vascular failure.

Our group is interested in the peripheral mechanisms operating in autoimmune and chronic inflammation and their exploitation for the design and development of novel therapies. Our work has shown that exacerbated Th17 responses or suboptimal behaviour of regulatory T (Treg) cells increase inflammation and fibrosis of the heart, arteries, peritoneum, and kidneys, resulting in exacerbated myocarditis, atherosclerosis, or hypertension-driven renal dysfunction and associated comorbidities. Our recent work shows that Tregs are a first line defense against CVD. Tregs can directly mediate neutrophil apoptosis, thereby protecting the tissue from damage, or can inhibit Th17 responses, controlling the recruitment of inflammatory cells to the target tissues. Detailed knowledge of Th17 and Treg biology will pave the way to the development of new therapeutic and prevention strategies to control inflammation and fibrosis related to cardiovascular diseases.

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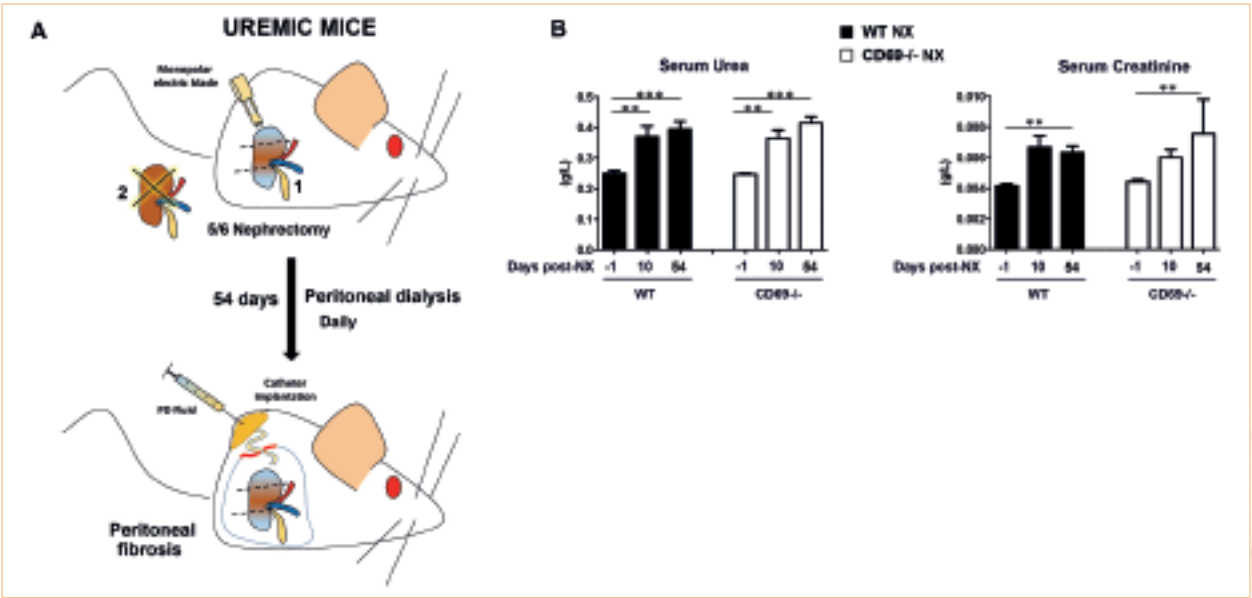
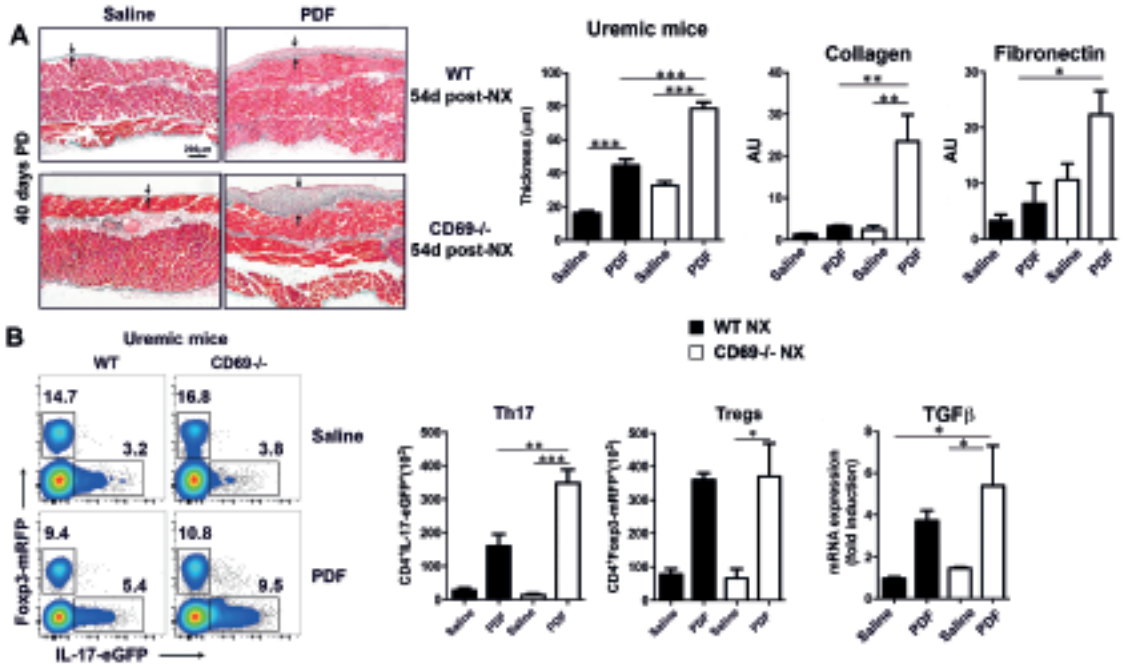
Tania Sonia Luque Díaz



Generation of uremic mice. (A) 5/6 nephrectomy (NX) was performed by laparotomy of the right kidney, and the anterior and posterior 1/3 parts of the left kidney were injured using a monopolar electric blade. The remaining functional 1/3 of the left kidney was replaced in its original position in the abdominal cavity before treatment with saline or standard peritoneal dialysis fluid (PDF) for an additional period of 40 days, starting 14 days after NX. (B) Serum levels of urea and creatinine were measured before and 10 and 54 days after 5/6 nephrectomy throughout the treatment period.



CD69 regulates fibrosis in mice with abnormal renal function. (A) Peritoneal membrane fibrosis assessed by Masson's Trichrome staining 54 days post-nephrectomy. Arrows indicate peritoneal membrane thickness. Right, quantification of peritoneal fibrosis in uremic mice (n≥10). Fibrosis in peritoneal tissue from uremic WT and CD69$^{-/-}$ mice was assessed by qPCR analysis of collagen I and fibronectin. Bars represent means ± SD (n≥6). (B) Density plots of flow-cytometry analysis in peritoneal effluents from uremic CD69$^{-/-}$ double reporter mice (Foxp3-mRFP in the foxp3 locus and IL-17A-eGFP in the Il17a locus) or wt littermates. Panels show analysis of CD4+FoxP3-RFP and IL-17eGFP in the indicated groups. TGF levels were assessed by qPCR.

MAJOR GRANTS

- Comunidad de Madrid. Redes de Excelencia (S2010/BMD-2332)
- Ministerio de Economía y Competitividad. FIS RETICS (RIC: RD12/0042/0056)
- Fundación BBVA (IN[16]_BBM_TRA_0365)
- Ministerio de Economía y Competitividad. Proyectos de investigacion en salud (AES 2016). Modalidad proyectos en salud (PI16/01956)
- CIBER de Enferemedades Cardiovasculares, ISCIII.

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Borroto A, Reyes-Garau D, Jimenez MA, Carrasco E, Moreno B, Martinez-Pasamar S, Cortes JR, Perona A, Abia D, Blanco S, Fuentes M, Arellano I, Lobo J, Heidarieh H, Rueda J, Esteve P, Cibrian D, Martinez-Riano A, Mendoza P, Prieto C, Calleja E, Oeste CL, Orfao A, Fresno M, Sanchez-Madrid F, Alcami A, Bovolenta P, Martin P, Villoslada P, Morreale A, Messeguer A, Alarcon B. **First-in-class inhibitor of the T cell receptor for the treatment of autoimmune diseases**. *Sci Transl Med* (2016) 8: ra184

Barreiro O, Cibrian D, Clemente C, Alvarez D, Moreno V, Valiente Í, Bernad A, Vestweber D, Arroyo AG, Martín P, von Andrian UH, Sánchez Madrid F. **Pivotal role for skin transendothelial radio-resistant anti-inflammatory macrophages in tissue repair**. *Elife* (2016) 5: e15251

Liappas G^, González-Mateo GT^, Sánchez-Díaz R^, Lazcano JJ, Lasarte S, Matesanz-Marín A, Zur R, Ferrantelli E, Ramírez LG, Aguilera A, Fernández-Ruiz E, Beelen RH, Selgas R, Sánchez-Madrid F, Martín P*, López-Cabrera M*. **Immune-regulatory molecule CD69 controls peritoneal fibrosis**. *J Am Soc Nephrol.* (2016) 27(12):3561-3576. ^Co-first authors. *Co-corresponding authors.

Fabbiano S, Menacho-Márquez M, Robles-Valero J, Pericacho M, Matesanz-Marín A, García-Macías C, Sevilla MA, Montero MJ, Alarcón B, López-Novoa JM, Martín P, Bustelo XR. **Immunosuppression-independent role of regulatory T cells against hypertension-driven renal dysfunctions**. *Mol Cell Biol* (2015) 35: 3528-46

Tissue regeneration

RESEARCH INTEREST

Autophagy is required for the maintenance of muscle stem cell function

During aging, there is a decline in the regenerative function of muscle stem cells (satellite cells). This decline intensifies with advanced old age as cellstransition from quiescence to irreversible senescence. How satellite cells maintain quiescence and avoid senescence during their long life remains largely unknown. We have shown that basal autophagy is indispensable for maintaining the quiescent stem-cell state. Autophagy failure in physiologically aged satellite cells causes senescence entry due to loss of proteostasis and increased mitochondrial dysfunction, resulting in a decline in the number of functional satellite cells. Reestablishment of autophagy reverses senescence and restores regenerative functions in geriatric satellite cells. Since autophagy also declines in human geriatric satellite cells, these findings uncover autophagy as a decisive stem-cell-fate regulator and have implications in sarcopenia.

Myeloid cells are essential in the advanced stage of muscle regeneration

In response to tissue damage, innate immune cells phagocytose cellular debris and secrete factors that promote repair. In the initial response to muscle injury, M1 macrophages infiltrate the damaged tissue concomitantly with the expansion of the resident muscle stem cells and mesenchymal progenitors. Subsequently, M1 cells are substituted by M2-like macrophages, coinciding with growth of the newly formed myofibers and new vascularization. How this late repair process is coordinated is poorly understood. We have found an essential role for myeloid-produced p38alpha in the late resolving phase of muscle injury. Deletion of p38alpha MAPK in M2-like macrophages seriously compromised muscle tissue regeneration by causing defective angiogenesis and aberrant fat accumulation.

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Masters Student:
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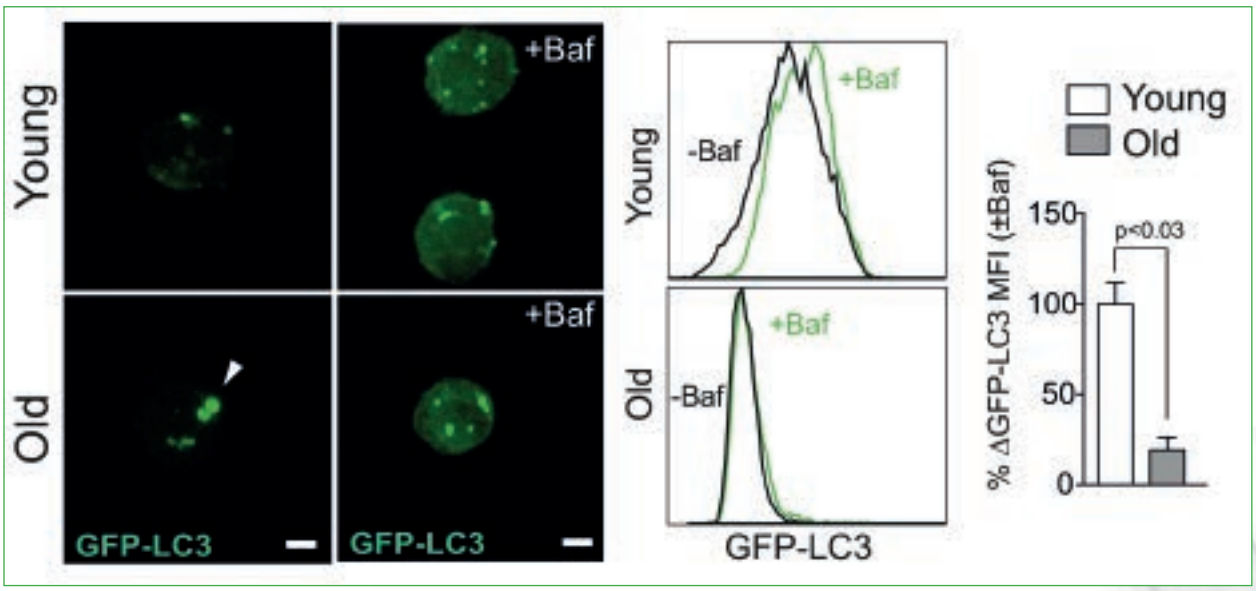
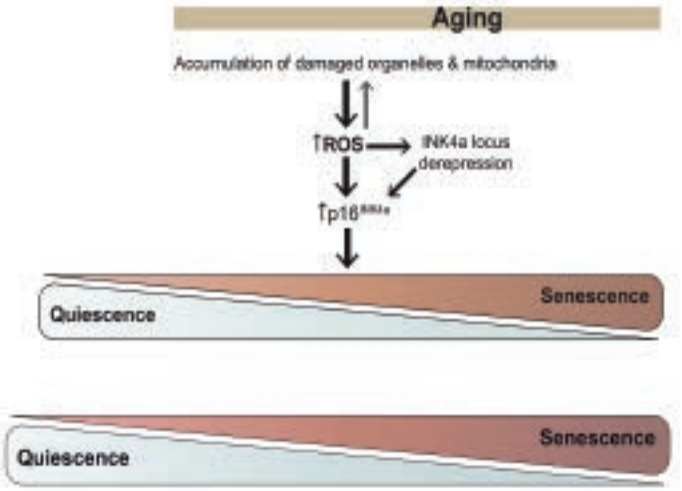
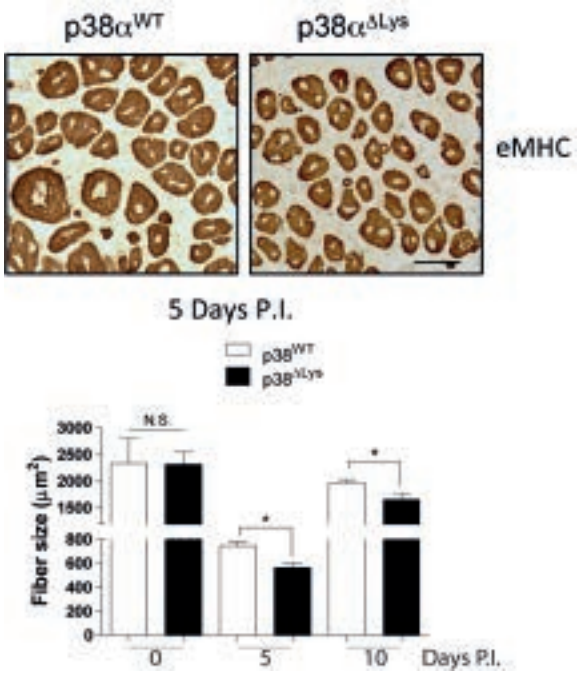




Model of how autophagy decline leads to muscle stem cell senescence and tissue regenerative decline with aging



Delayed muscle regeneration in mice with deletion of p38alpha MAPK in the myeloid lineage. Analysis of muscle regeneration (indicated by the size of new myofibers expressing embryonic myosin heavy chain (eMHC)) after an acute injury in wild type (WT) and p38alpha deficient mice. Absence of p38alpha in the myeloid compartment causes a delay in muscle regeneration that persists until at least 10 days post-injury (P.I.). eMHC staining is shown at 5 days P.I.

MAJOR GRANTS

- Association française pour les myopaties (AFM)-France (MDA418174). Funds held at UPF and CNIC.
- Ministerio de Economía y Competitividad e Instituto de Salud Carlos III (PIE14/00061). Funds held at UPF.
- European Commission. European Research Projects on Rare Diseases. Funds held at UPF
- Ministerio de Economía y Competitividad e Instituto de Salud Carlos III (CIBERNED 2015-2). Funds held at UPF
- Ministerio de Economía y Competitividad (SAF2015-67369-R). Funds held at UPF.
- Muscular Dystrophy Association (MDA)-USA. Funds held at UPF.

SELECTED PUBLICATIONS

García-Prat L, Martínez-Vicente M, Perdiguero E , Ortet L, Garcia-Ubreva J, Rebollo E, Ruiz-Bonilla V, Gutarra S, Ballestar E, Serrano AL, Sandri M, Muñoz-Cánoves P. **Autophagy maintains stemness by preventing senescence.** *Nature* (2016) 529: 37-42

Segalés J, Islam AB, Kumar R, Liu QC, Sousa-Victor P, Dilworth FJ, Ballestar E, Perdiguero E, Muñoz-Cánoves P. **Chromatin-wide and transcriptome profiling integration uncovers p38α MAPK as a global regulator of skeletal muscle differentiation.** *Skelet Muscle* (2016) 6:9 doi: 10.1186/s13395-016-0074-x.

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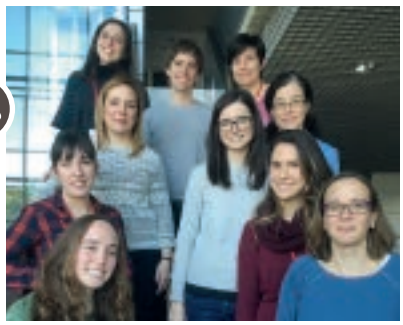
Sousa-Victor P, García-Prat L, Serrano AL, Perdiguero E, Muñoz-Cánoves P. **Muscle stem cell aging: regulation and rejuvenation.** *Trends Endocrinol Metab.* (2015) 26287-96



B lymphocyte biology

RESEARCH INTEREST

B cells execute the humoral immune response, an essential defence mechanism that relies on the generation of a huge repertoire of antibodies that will selectively and specifically bind and mark pathogens for destruction. A critical step in antibody diversification occurs during the germinal center reaction, whereby B cells that have been activated by an infectious agent generate high affinity memory B cells and antibody-secreting plasma cells. Antibody diversification, while enabling the humoral immune response, is also linked to various health problems, including autoimmunity and cancer.

In our lab we are particularly interested in the molecular characterization of the humoral immune response and the germinal center reaction. In recent years our work has covered the molecular biology of secondary antibody diversification by activation induced deaminase (AID) and the regulation of B cell function by microRNAs, and the links between these events and human disease through the generation and characterization of genetically modified animal models.

Current research projects of the lab include 1) analysis of the specificity of AID activity during antibody remodeling in germinal centers and its impact on B cell lymphomagenesis; 2) the role of the chromatin organizer CTCF during the germinal center reaction and terminal B cell differentiation; and 3) characterization of the antibody repertoire associated with atherogenesis.

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María Inmaculada Martos Folgado *(from March 2016)*
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Arantxa Pérez *(until July 2016)*
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Dobromira Veselinova Stoycheva

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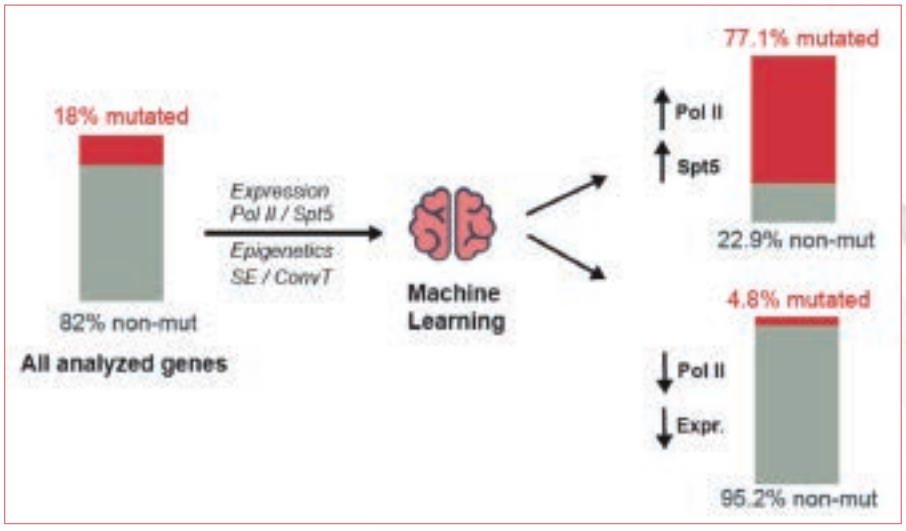
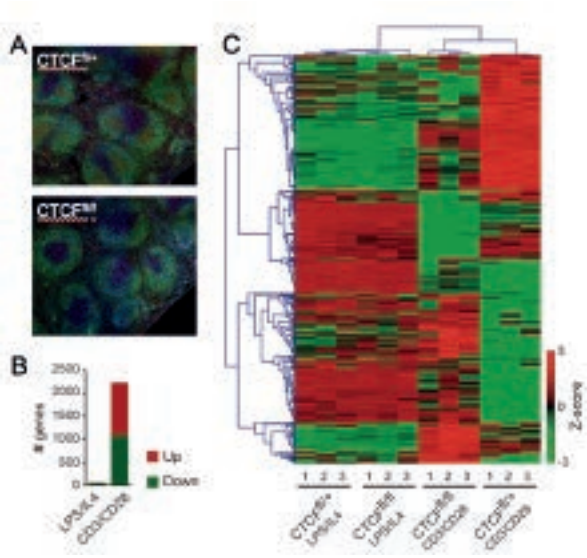
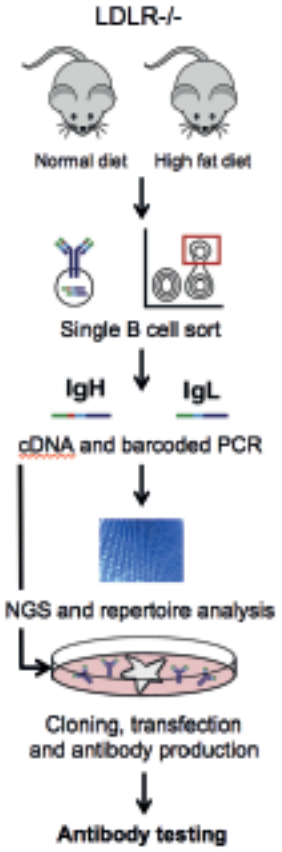


A gene selection representing ≈10% of the mouse genome was enriched with an RNA probe library and sequenced by next generation sequencing. Mutation frequency was determined to identify genes undergoing AID-mediated mutagenesis and a machine learning algorithm was used to integrate mutation data with expression and PolII and Spt5 binding, among other features, to predict gene mutability.



B cell sensitivity to CTCF loss is determined by the B cell activation pathway. A. CTCF is absolutely required for germinal center formation in vivo. CTCF control ($CTCF^{fl/+}$) and deficient ($CTCF^{fl/fl}$) mice were immunized and spleens were analyzed by immunofluorescence (Blue, DAPI; Green, B220; Red, PNA). B-C. LPS/IL4 stimulated B cells are refractory to CTCF loss. B. Number of transcriptionally altered genes in CTCF-deficient B cells activated in the presence of LPS and IL4 or of CD3/CD28 activated T cells. C. Representative heatmap of genes in B.



Experimental pipeline for analysis of atherosclerosis-associated antibody repertoires. Individual spleen B cells isolated from $LDLR^{-/-}$ mice fed a normal or high-fat diet are isolated by single-cell FACS. Heavy and light antibody cDNAs are amplified, barcoded and sequenced by next generation sequencing. For functional analysis, amplified heavy and light cDNAs are cloned in expression vectors and transfected into cells, and secreted antibodies are collected and assayed.

MAJOR GRANTS

- Ministerio de Economía y Competitividad (SAF2016-75511-R)
- European Commission. ERC PROOF OF CONCEPT GRANT 2015 (ERC-2015-PoC- 713728)
- Ministerio de Economía y Competitividad (SAF2013-42767-R)

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Thornton TM, Delgado P, Chen L, Salas B, Krementsov D, Fernandez M, Vernia S, Davis RJ, Heimann R, Teuscher C, Krangel MS, Ramiro AR, Rincón M. **Inactivation of nuclear GSK3β by Ser(389) phosphorylation promotes lymphocyte fitness during DNA double-strand break response**. *Nat Commun* (2016) 7:10553

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

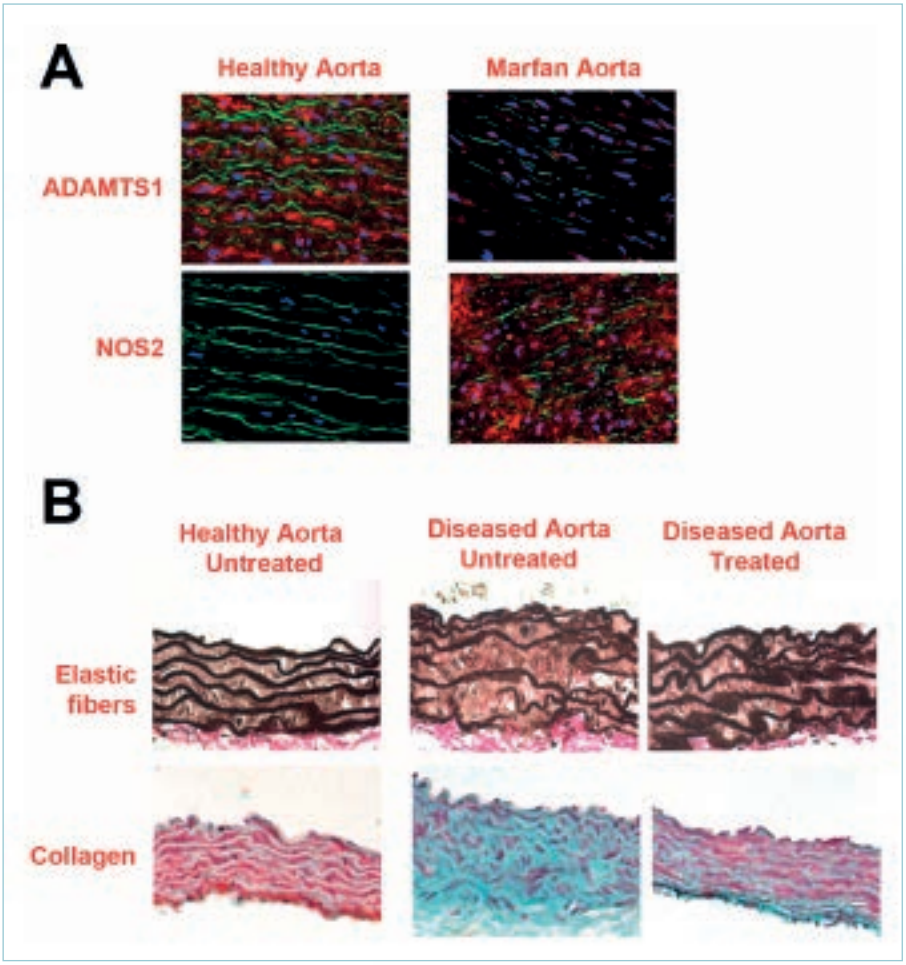
Ramiro AR, Barreto VM. **Activation-induced cytidine deaminase and active cytidine demethylation**. *Trends Biochem Sci* (2015) 40:172-81

Gene regulation in cardiovascular remodeling and inflammation

RESEARCH INTEREST

Much of our recent effort has centered on the mechanisms mediating aortic diseases such as familial forms of thoracic aortic aneurysm and dissection (TAAD), including Marfan syndrome.We have identified new pathophysiological mechanisms and targets in aortic diseases, showing that Adamts1 is a major mediator of vascular homeostasis and that inhibition of inducible nitric oxide synthase (Nos2) is able to prevent and reverse aortic dilation and medial degeneration in a mouse model of Marfan syndrome and other types of aneurysm. These findings suggest a major potential for NOS2 inhibitors in the treatment of thoracic aortic aneurysm.

Our group has an established history in the study of the regulation of calcineurin (CN) signaling in angiogenesis and inflammation. We have characterized the mechanisms and sequences involved in the interactions of CN with a range of substrates, including immunosuppressive drugs, and have shown how specific CN targeting modulates inflammatory responses. In addition, we have studied mediators of vascular and cardiac remodeling related to the angiotensin II and CN pathways. We are currently using conditional mice deficient for CN and Rcan1 isoforms in the endothelial, vascular smooth muscle, and cardiomyocyte compartments to elucidate the mechanisms that mediate this remodeling. We have already identified CN-regulated genes in different mouse models of cardiac hypertrophy (CH) and are characterizing their roles in CH using mice conditionally lacking CN and Rcan1 in cardiac tissue. We are also elucidating the role of Chd4/NuRD in cardiac homeostasis and have found that the NuRD complex determines skeletal muscle identity by silencing the skeletal muscle program in cardiomyocytes and the cardiac program in skeletal muscle.



Inhibition of NOS2 protein has therapeutic potential in Marfan syndrome. (**A**) Comparison of the expression of ADAMTS1 and NOS2 proteins (both in red) and elastic fibers (green) in the aortic wall of a healthy donor and a Marfan syndrome patient. (**B**) Staining showing elastic fiber organization (dark brown) and collagen deposits (blue) in the aortic wall of a healthy mouse (Healthy Aorta Untreated), a mouse with untreated syndromic aortic disease (Diseased Aorta Untreated), and a diseased mouse treated with a NOS2 inhibitor (Diseased Aorta Treated). The images show how this treatment restores the blood vessel wall structure to the pre-disease state.

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MAJOR GRANTS

- Ministerio de Economía y Competitividad (SAF2015-63633-R)
- Ministerio de Economía y Competitividad. FIS RETICS (Red de Investigación Cardiovascular: RD12/0042/0022)
- Fundació La Marató TV3 (20151331)
- Fundació La Marató TV3 (264/C/2012) (PI: Sara Martínez)

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González-Terán B, López JA, Rodríguez E, Leiva L, Martínez-Martínez S, Bernal JA, Jiménez-Borreguero LJ, Redondo JM, Vazquez J, Sabio G. **p38γ and δ promote heart hypertrophy by targeting the mTOR-inhibitory protein DEPTOR for degradation**. *Nat Commun* (2016) 7:10477

Oller J, Alfranca A, Méndez-Barbero N, Villahoz S, Lozano-Vidal N, Martín-Alonso M, Arroyo AG, Escolano A, Armesilla AL, Campanero MR, Redondo JM. **C/EBPβ and nuclear factor of activated T cells differentially regulate Adamts-1 induction by stimuli associated with vascular remodeling**. *Mol Cell Biol* (2015) 35:3409-22

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

CNIC-UAM COLLABORATIVE PROGRAM

Intercellular communication in the inflammatory response

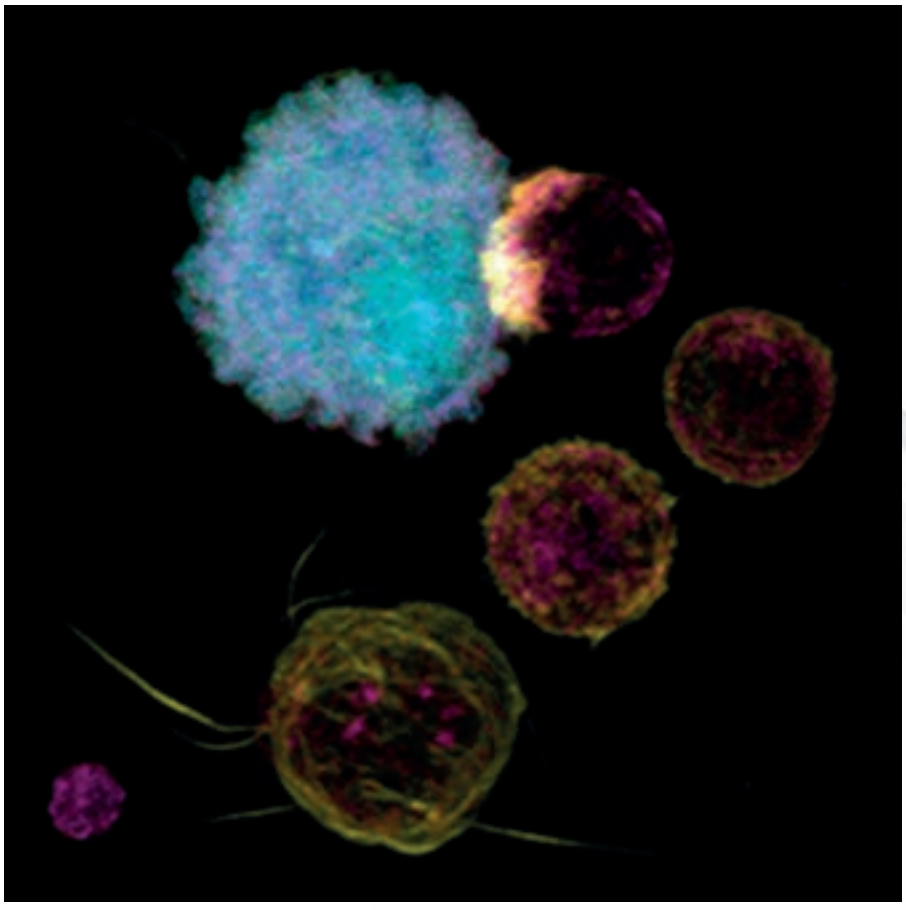
RESEARCH INTEREST

The group pursues three main lines of research.

1) **Regulation of immune synapse formation and function**. We are exploring precise roles of centrosomal proteins in IS formation, especifically the role of posttranslational modifiers such as Ser/Thr kinases. In addition, we are analyzing the role of mitochondrial components in the biogenesis and secretion of exosomes and their impact on macrophage and dendritic cell function.

2) **Fine tuning of T cell biology by controlling exosome biogenesis**. Exosome production and their specific constituents are being examined with the aim of identifying and characterizing specific proteins that are sorted into exosomes through ISG-ylation, a posttranslational modification.

3) **Immunoregulatory molecules and cells in steady state and inflammatory diseases**. We are analyzing the role of the immunoregulatory molecule CD69 and newly described partners such as aminoacid transporters in animal models of atherosclerosis and psoriasis and in patients. These studies are aimed at identifying the molecular basis of these inflammatory diseases. This includes the study of the role of specific subsets of macrophages in the immunesurveillance of blood vessels at steady-state.



Aurora A regulates T cell activation. Aurora A accumulates at the immune synapse and regulates the activity of Lck and the centrosome as a microtubule-organizing center in T cells

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Olga Moreno
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Javier Silván

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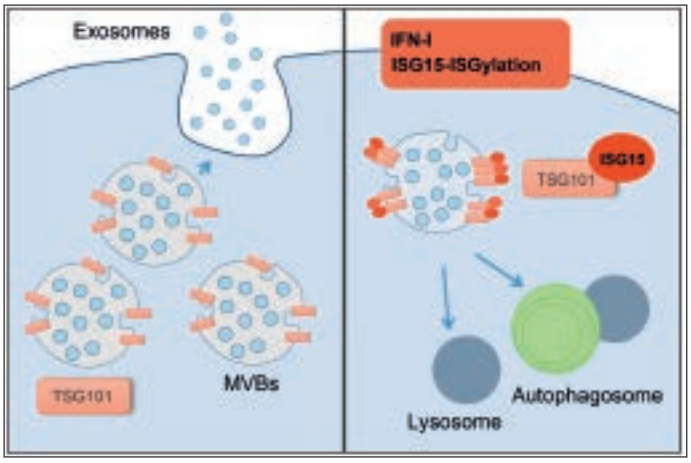
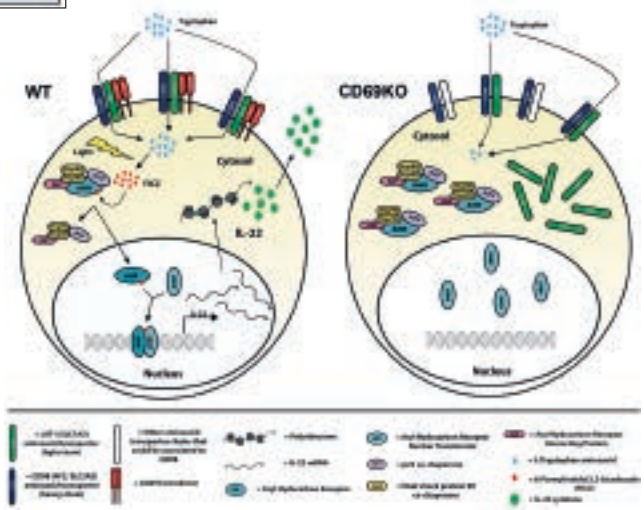
Diego Calzada



ISG-ylation: a posttranslational modification regulating proteostasis. ISG-ylation controls the activity of multivesicular bodies for protein degradation. From *Nat Commun* (2016) 7: 13588.



CD69 and LAT1 act as immuno-shuttles in psoriasis. CD69 interaction with the aminoacid transporter LAT1 regulates the production of IL-22 and psoriasis severity. From Nat Immunol. 2016. 17:985-96

MAJOR GRANTS

- European Commission. ERC Advanced Investigators Grant (ERC-2011-AdG 20110310) (GENTRIS)
- Ministerio de Economía y Competitividad (SAF2014-55579-R)
- Ministerio de Economía y Competitividad. FIS RETICS (RIC: RD12/0042/0056)
- Redes de Excelencia de la Comunidad de Madrid (P2010/BMD-2332)
- Fundación La Marató-2015 (281/C/2015).
- European Union. COST-Action BM1202.

SELECTED PUBLICATIONS

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Torralba D, Baixauli F, Sánchez-Madrid F. **Mitochondria Know No Boundaries: Mechanisms and Functions of Intercellular Mitochondrial Transfer**. *Front Cell Dev Biol* (2016) 4: 107.

Cardiovascular proteomics

RESEARCH INTEREST

We are working on novel high-throughput quantitative approaches for the dynamic analysis of the deep proteome, including novel bioinformatics algorithms for protein identification, quantification, and systems biology interpretation in very large numbers of samples and for the study of posttranslational modifications using novel hypothesis-free approaches.

Using a pig model of ischemia reperfusion, we are applying these proteomics technologies to study the molecular events taking place in the heart after infarction and the molecular effects of protective treatments, including the impact on postranslational modifications.

We have also developed a translational proteomics platform, with which we are studying the molecular mechanisms implicated in early atherosclerosis. This platform is being applied to the search for protein, metabolic and lipid factors correlating with subclinical atherosclerosis markers such as calcium deposition and plaque formation and activity in the PESA project. We are also studying atherosclerosis models in other clinical stages, including molecular changes taking place in the aorta at early stages of plaque formation, including the plaque itself and its secretome.

Finally, we are studying the molecular mechanisms that regulate assembly and superassembly of the electron transport chain complexes in mitochondria, using Blue-DiS, an advanced technology that allows analysis of the interactome and the implication of protein factors, isoforms, and postranslational modifications with unprecedented molecular detail.

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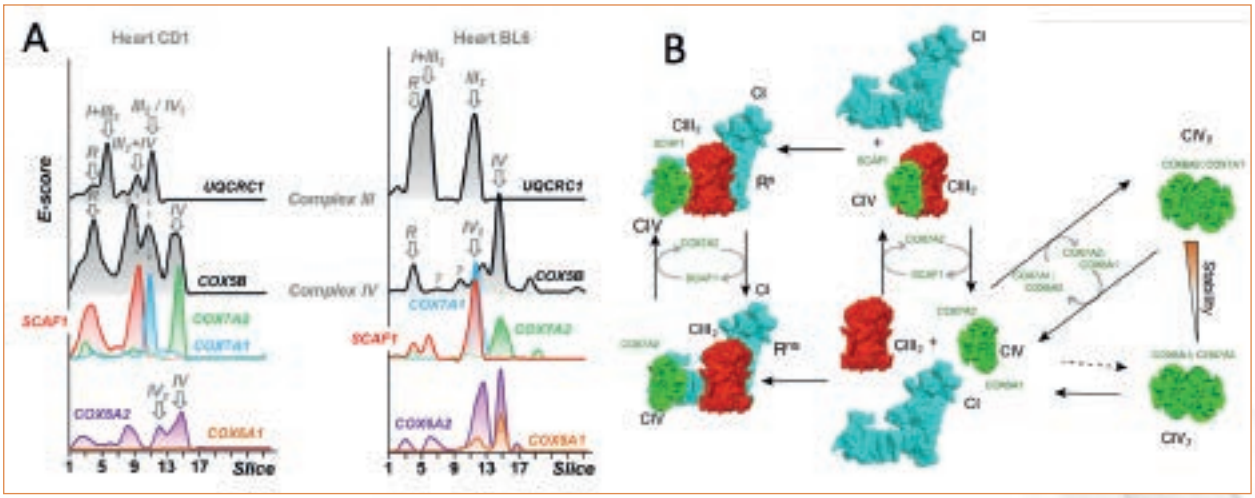
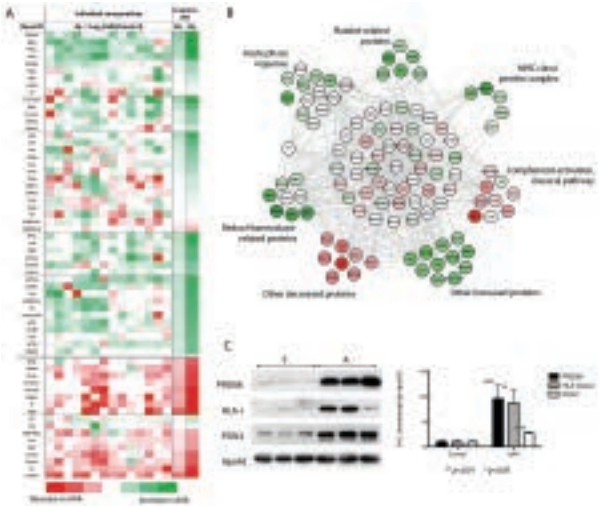
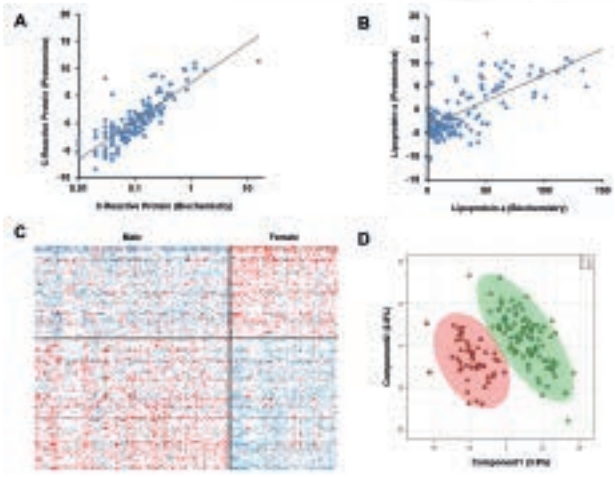


Super-assembly of CIII and CIV into different structures depends on the subunit composition of CIV. (A) Quantitative protein profiles of mitochondrial supercomplexes separated by blue native gel electrophoresis and analyzed by DiS, a novel data-independent mass spectrometry technology developed in our laboratory. (B) Model of CIV dimerization and superassembly driven by the exchange of CIV subunit isoforms.



Quantitative proteomics reveals high-density-lipoprotein (HDL) alterations in human abdominal aortic aneurysm (AAA) highlighted by increased peroxiredoxin-6 (PRDX6) levels and consistent with systemic redox imbalance. A. Altered HDL-associated proteins in AAA patients. B. Network interaction analysis of dynamic changes. C. Western-blot validation of the proteins most altered in AAA.



Performance of the Translational Proteomics Platform. Agreement between biochemistry and proteomics for levels of C-reactive protein (A) and lipoprotein a (B). (C) The platform allows gender determination from quantitative data (red: increase; blue: decrease). (D) PLS-DA analysis showing good discrimination between male (green) and female participants (red).

MAJOR GRANTS

- Ministerio de Economía y Competitividad (BIO2012-37926)
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- Ministerio de Economía y Competitividad. FIS RETICS (RIC: RD12/0042/0056)
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- Progeria Research Fund Specialty Award (USA)
- Fundació La Marató de TV3

SELECTED PUBLICATIONS

Burillo E, Jorge I, Martínez-López D, Camafeita E, Blanco-Colio LM, Trevisan-Herraz M, Ezkurdia I, Egido J, Michel JB, Meilhac O, Vázquez J *, Martin-Ventura JL* (*co-corresponding authors). **Quantitative HDL proteomics identifies peroxiredoxin-6 as a biomarker of human abdominal aortic aneurysm**. *Sci Rep* (2016) 6: 38477

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