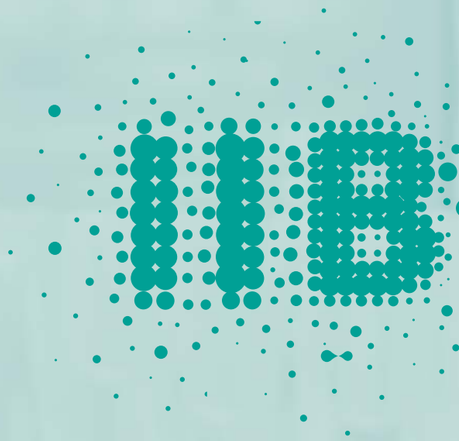
SCIENTIFIC REPORT 2025



MaX
MARCO DE AUTOEVALUACIÓN DE LA EXCELENCIA

SOLS MORREALE

CSIC
CONSEJO SUPERIOR DE INVESTIGACIONES CIENTÍFICAS

UAM Universidad Autónoma de Madrid

INSTITUTO DE
INVESTIGACIONES
BIOMÉDICAS
SOLS-MORREALE

Index *interactive*

Welcome from the director

It is my pleasure to present this report summarizing the activities of the Institute for Biomedical Research Sols-Morreale (IIBM) during the year 2025. Now, after more than two years in the role of Director, I am grateful to witness the continued progress and consolidation of our Institute. The strong foundations built in previous years, together with the collective commitment of our community, have allowed us to maintain a positive trajectory, reflected in both the quality of our scientific output and our success in securing competitive funding.

A defining milestone of 2025 has been the formal launch and implementation of the CSIC's MaX Program at our Institute. Following the successful evaluation completed last year, we have focused on executing the objectives and actions outlined in our Excellence Plan. Throughout the year, we have advanced according to the established roadmap, taking concrete steps to strengthen our research environment, organizational structure, and long-term strategic vision.

In this context, one of the most important activities has been the renewal of our Scientific Advisory Board (SAB), ensuring a panel of internationally recognized experts aligned with our scientific priorities. We were also pleased to host the SAB during their visit to the IIBM, where they conducted a comprehensive evaluation of both the center as a whole and each individual research group. The feedback provided has been thoughtful, constructive, and highly valuable, offering clear guidance to further enhance our scientific performance and institutional development in the coming years.

Beyond these strategic actions, 2025 has been another year of solid scientific achievement. Our researchers have continued to make significant contributions across a wide range of biomedical fields, maintaining a high level of productivity and reinforcing our commitment to addressing major challenges in human health. At the same time, our success in attracting competitive funding at national and international levels reflects both the relevance and the excellence of the work carried out at the IIBM. We are also pleased to have strengthened our scientific community through the incorporation of new talent, including two Ramón y Cajal researchers, as well as by hosting students at different stages of their academic training, from vocational education and undergraduate to Master's level. In parallel, our commitment to advanced training is reflected in the successful defense of 11 doctoral theses during the year, contributing to the development of the next generation of scientists.

We have also continued to strengthen our collaborative networks and to promote knowledge transfer, fostering interactions with both academic and industrial partners. These efforts are essential to ensure that our research not only advances fundamental understanding but also contributes to tangible societal impact.

As we move forward, our priorities remain clear: to consolidate the progress achieved, to fully implement the MaX Program, and to continue fostering a stimulating and supportive environment for scientific innovation. The recommendations of the SAB, together with the dedication of our researchers, technical staff, and administrative teams, will be instrumental in guiding this next phase.

I would like to express my sincere appreciation to all members of the IIBM for their professionalism, commitment, and enthusiasm. Their work is the driving force behind our achievements and the foundation of our future success.

With confidence in the path ahead, I look forward to another year of progress and discovery at the IIBM.

Pilar López Larrubia. Director, IIBM

Key Metrics

Publications

- **1** *Books*
- **10** *Medium impact*
- **7** *Low impact*
- **76** *High impact*

Fundraising

- **4.987.000€** *Funding*
- **214.000€** R&D *Contracts*

Supervision activities

- **11** *Doctoral theses*
- **34** *Master´s theses*
- **40** *Undergraduate theses*

Outreach activities

- **59** *Events*

department of **Cancer**

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Colon Cancer: Organoids, Microenvironment and Vitamin D

TENURED SCIENTISTS

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(Profesor de Investigación, CSIC).
Group Coordinator

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(Científica Titular, CSIC)

González Sancho, José Manuel
(Catedrático, UAM)

CONTRACT RESEARCHERS

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Fernández Barral, Asunción
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Herreros Cabello, Alfonso

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Rejas González, Raquel

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Albandea Rodríguez, David
Rodríguez Marrero, Silvia
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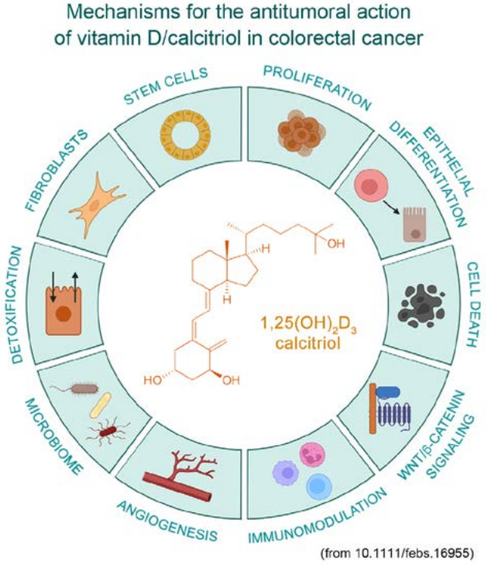
Troya Balseca, Johanna



KEYWORDS

Cancer-associated fibroblasts, Colon cancer, Organoids, Personalized medicine, Tumor microenvironment, Vitamin D

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RESEARCH LINES:

Overview

Colon/colorectal cancer is the most frequent malignancy in Spain and a major neoplasia in terms of incidence and mortality worldwide. Our group uses primary cultures of fibroblasts and stem cell-derived organoids established from healthy and tumor tissue of colorectal cancer patients to investigate the genetic basis and the role of the tumor microenvironment in this neoplasia, and to characterize the antitumor action of vitamin D.

The concept of precision/personalized medicine is a hot topic today. At this respect, our studies using patient-derived organoids and fibroblasts are cutting-edge. We expect that they will contribute to the implementation of these primary culture systems in the clinic to improve the handling of the patients on the basis of their response to available treatments.

The incidence of colorectal cancer in individuals under 50 years of age (early-onset colorectal cancer) is increasing worldwide. The reasons for this rise remain unknown and studies conducted so far are limited, failing to identify differences between these tumors and those developed in older individuals (late-onset colorectal cancer). We are establishing organoids and fibroblast cultures from these two groups of patients to perform comparative analyses (gene expression patterns, pro-tumor properties, drug and vitamin D response) aiming to improve the comprehension of this clinical situation.

Our group is affiliated to the Instituto de Investigación Sanitaria del Hospital Universitario La Paz (IdiPAZ), CIBER de Cáncer (CIBERONC, ISCIII), Conexión Cáncer CSIC and RST Biomed CSIC.

Patient-derived colon organoids: Gene expression and response to drugs and vitamin D

We study the effects of vitamin D on the gene expression, proliferation, and phenotype of patient-derived colon normal and tumor organoids. Organoids are 3D-structures generated by normal or tumor stem cells that are more similar to the tissue-of-origin and reproduce the in vivo situation better than 2D-cultures of established cell lines.

We are focused on the analysis of the effect of the active vitamin D metabolite 1,25-dihydroxyvitamin D3 (calcitriol) on gene expression in organoids, aiming at the identification and study of calcitriol target genes. In addition, we wish to elucidate the effects of calcitriol on cell phenotype and differentiation in colon organoids. Thus, we have established the protocols to differentiate the epithelial stem cells present in human colon healthy tissue-derived organoids towards the main differentiated colon epithelial cell

lineages (absorptive and mucosecretory). In these conditions, our data indicate that calcitriol favors the maintenance of the stem phenotype by attenuating the induction of cell differentiation.

With the aim of contributing to the progress of precision/personalized medicine and to highlight the potential of organoids for anticancer drug testing, we study the response of organoids to several antitumor drugs currently used for the treatment of colon cancer patients and to other drugs in development.

Tumor microenvironment in colorectal cancer: Role of cancer-associated fibroblasts and macrophages and modulation by vitamin D

The tumor microenvironment is crucial for cancer initiation and progression and is involved in tumor relapse and therapeutic resistance. Accordingly, the worst prognosis colorectal cancer consensus molecular subtype is characterized by high stromal infiltration. Cancer-associated fibroblasts are the main cellular component of the tumor microenvironment and play a crucial role in tumorigenesis.

Our work in this area is focused on (i) the role of colon fibroblasts on the mechanisms of resistance to antitumor drugs that carcinoma cells frequently develop; (ii) the paracrine communication among fibroblasts, carcinoma cells and macrophages in the tumor microenvironment, (iii) the metabolism of normal and cancer-associated fibroblasts, and (iv) the regulation of these processes by vitamin D.

We are also carrying out a comparative study of cancer-associated fibroblasts of early- and late-onset colorectal cancer patients in terms of gene expression and vitamin D responsiveness.

Collaborations

We actively collaborate with numerous colleagues from different national and international scientific institutions. As experts in colorectal cancer, we have contributed to the characterization of the protumoral role of cadherin-17 in metastatic colorectal cancer (collaboration with Dr. Jl. Casal, CIB-CSIC, Madrid). Likely, as experts in vitamin D, we have participated in a study on the effect of vitamin D deficiency on erectile dysfunction (collaboration with Dr. F. Pérez-Vizcaíno, UCM, Madrid).

PUBLICATIONS:

Bartolomé, RA.; Pintado-Berninches, L.; Robles, J.; Calvo-López, T.; Boukich, I.; Otero-Núñez, P.; González-Sancho, JM.; Casal, JI. Loss of cadherin 17 downregulates LGR5 expression, stem cell properties and drug resistance in metastatic colorectal cancer cells. *Cell Death Dis.* **2025**, 16, 475. DOI: 10.1038/s41419-025-07811-w.

Olivencia, MA.; Climent, B.; Barreira, B.; Morales-Cano, D.; Sánchez, A.; Fernández, A.; García-Gómez, B.; Romero-Otero, J.; Rodríguez, C.; Moreno, L.; Prieto, D.; Larriba, MJ.; Cogolludo, A.; Angulo, J.; Pérez-Vizcaíno, F. Vitamin D deficiency induces erectile dysfunction: Role of superoxide and Slpi. *Br J Pharmacol*. **2025**, *182*, 3669-3687. DOI: 10.1111/bph.70034.

FUNDING:

Consorcio CIBER Área Temática de Cáncer (CIBERONC). CB16/12/00273. ISCIII. 2017-2026. PI: Alberto Muñoz Terol.

Cáncer colorrectal en población joven. Estudio farmacogenómico en organoides y efectos del microambiente tumoral. ICI20/00057. ISCIII. 2021-2026. PI: Nuria Rodríguez Salas and Alberto Muñoz Terol.

Vitamin D effects on colon cancer stem cells and microenvironment: differentiation, metabolism and intercellular communication. PID2022-136729OB-I00. AEI-MICINN. 2023-2026. PI: Alberto Muñoz Terol and María Jesús Larriba Muñoz.

Hacia la medicina de precisión en cáncer de colon: biomarcadores, microambiente tumoral y microbiota. S2022/BMD-7212. Comunidad de Madrid. 2023-2026. PI: José Manuel González Sancho.

Unidad CIBER de la Plataforma ISCIII de Biomodelos y Biobancos. PT23/00102. ISCIII. 2024-2026. PI: Cristina Villena Portella.

Setting up and validating new strategies for the identification and eradication of the poor prognosis fetal-type colorectal tumors. EP PerMed 2024-149. EU. 2025-2027. PI: Lluís Espinosa Blay.

High-throughput screening platform for precision nutrition in non-responsive celiac disease through 3D organ models and systems biology. TEC-2024/BIO-167. Comunidad de Madrid. 2025-2028. PI: Antonio Barbáchano Becerril.

OUTREACH ACTIVITIES

Interview in the radio program Entre Probetas from Radio 5 (Radio Nacional de España). Organoides: pequeños cuerpecitos de investigación. 07/01/2025.

Public lecture organized by Fundación PharmaMar. ¿Qué es el cáncer?. 03/03/2025.

Public lecture organized by Fundación PharmaMar. Vitamina D y Cáncer. 12/05/2025.

Science Outreach Workshop “Juegos de Ciencia” in the Science Outreach Fair Ventu-Ciencia. 30/05/2025.

Scientific Board Games Event “Juegos de Ciencia” in the game club Mecatol Rex. 22/11/2025.

Prognostic Factors for Hepatocellular Carcinoma Progression.

TENURED SCIENTISTS

Sánchez Pacheco, Aurora
(Profesor Titular). Group Coordinator

PREDOCTORAL SCIENTISTS

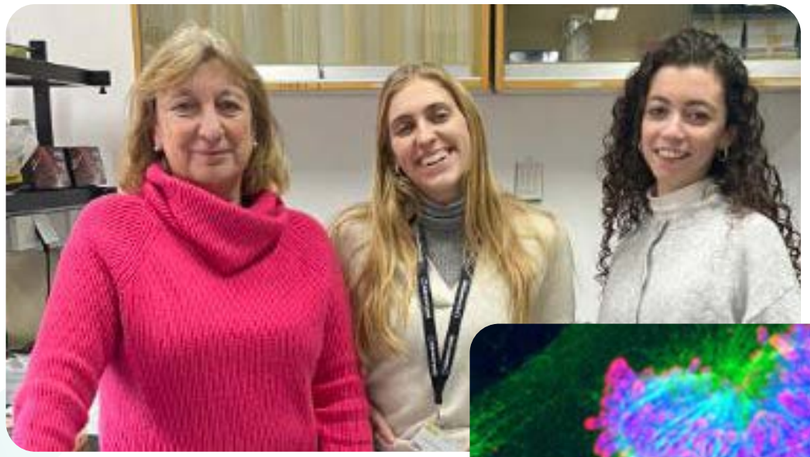
Ricote Cardenal, Carolina
(Predoctoral CAM)

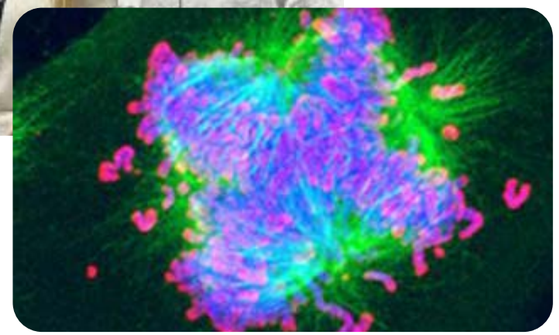
CONTRACT RESEARCHERS

Camblor Murube, Marina
(Postdoctoral Contract)

KEYWORDS

Hepatocellular Carcinoma, Fibrosis, Chirrosis, MASLD, MASH, Aurora Kinase B, Metagenome





RESEARCH LINES:

Overview

Our laboratory is interested in studying the factors associated with the development of hepatocellular carcinoma..

Molecular mechanism of SNPs os AURKB in cirrhosis and HCC development.

Researchers involved: Camblor Murube, Marina.

Cirrhosis constitutes the common final pathway of chronic liver diseases, being an important cause of morbidity and mortality due to the risk of developing end-stage liver disease and hepatocellular carcinoma (HCC). In this regard, the relationship between the overexpression of Aurora Kinase B (AURKB), an activity involved in the regulation of mitosis and cytokinesis, and the development of HCC in patients with chronic liver disease secondary to hepatitis C virus (HCV) infection and steatohepatitis has been described. We are studying the role of SNPs of the AurkB gene.

To this end, we are using CRISPR-Cas9 knock-in techniques to develop stable hepatic cell lines that express the allelic variant of the selected AURKB SNP. The aim is to evaluate by RNAseq techniques the different genetic pathways associated with the molecular mechanism by which genetic variants of AURKB regulate several cellular processes involved in tumor development, such as abscission during cytokinesis, proliferation, cell viability, and gene expression.

These differences could be reflected in a distinct prevalence of these AURKB allelic variants in patients who develop HCC, as we had observed through a clinical pilot study in patients with HCC by HCV infection after DAA treatment.

Genetic factors associated with HCC development induced by steatosis.

Researchers involved: Ricote Cardenal, Carolina

A second research line, recently initiated in our laboratory, is a collaborative project with a food supplements company together with the Oncology and Gastroenterology Departments of La Paz University Hospital–Biomedical Research Foundation of La Paz University Hospital (IdiPAZ), and the Gastroenterology Department of Infanta Sofía University Hospital. The project focuses on evaluating the efficacy and feasibility of a new formulation as a therapeutic option for the prevention and treatment of metabolic dysfunction–associated fatty liver disease (MASLD) and/or metabolic dysfunction–associated steatohepatitis (MASH).

Metabolically–dysfunction–associated steatotic liver disease (MASLD) is the most common chronic liver condition and the leading cause of liver-related mortality in developed countries. It is estimated that 30% of the adult population is affected by MASLD,

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and this percentage is increasing, partly due to rising obesity rates, excess visceral adiposity, and associated conditions such as type 2 diabetes, insulin resistance, hypercholesterolemia, and elevated triglyceride levels. The condition has also been observed in up to 15% of the pediatric population.

The accumulation of lipid deposits within hepatocytes produces significant histological changes that lead to the more severe form of MASLD, metabolic dysfunction–associated steatohepatitis (MASH). MASH is characterized by lobular inflammation and hepatocyte ballooning and is associated with a high risk of liver tissue degeneration and progression to fibrosis. A high percentage of patients with MASLD (20–35%) develop fibrosis and cirrhosis within 10–20 years after diagnosis. Approximately 2% of MASH cases may even progress to hepatocellular carcinoma (MASH-HCC).

To date, clinical interventions for fatty liver disease focus on preventing the development of steatosis and minimizing the risk of hepatic fibrosis by recommending lifestyle modifications, including regular physical exercise (2–3 times per week) and a calorie-restricted Mediterranean diet with low–glycemic index carbohydrates (free of processed foods, sugars, and refined flours), reducing alcohol and saturated fat intake, in accordance with the "Harvard Plate" recommendations. We are collaborating with an enterprise that has developed a new formulation aimed at treating the metabolic factors that trigger MASLD, as well as controlling the disease once established and preventing its progression to MASH.

First, we are analyzing **the molecular mechanism** associated with the administration of the new formulation in human hepatocyte culture systems (Huh7), macrophages from healthy donors, and human hepatic stellate cells (LX-2), to reproduce scenarios similar to MASLD and MASH. Markers of inflammation, apoptosis, autophagy, oxidative stress, endoplasmic reticulum stress, proliferation, and cell viability will be evaluated in the presence and absence of fatty acid treatment, before and after the addition of the formula.

The second aim is to analyze the efficacy of the formulation in patients with MASLD, MASH, and HCC. A pilot study will be conducted, including five patient groups with liver fat accumulation–related pathology. Blood samples will be collected from each patient before and after administration of the formulation or placebo over 12months, with monitoring and sample collection every three months. Usual analytical data, genetic markers (Aurora kinase B and alpha-fetoprotein), and dietary and exercise habits will also be monitored throughout the study. The third aim is **to analyze the liver–gut axis** in human samples from patients with MASLD and MASH. Stool samples will be collected before and after three months of formula administration to evaluate the liver–gut axis. Using third-generation nanopore massive sequencing technology, previously implemented in our laboratory, we aim to establish differences between baseline composition and the evolution of intestinal microbial species associated with the intervention. A statistical correlation study will be conducted to analyze genetic parameters and intestinal microbiota composition in conjunction with biochemical and inflammatory patterns, to determine whether improved disease progression is observed.

DOCTORAL THESES AND OTHER ACADEMIC WORK:

Claudia Fernandéz Folgueira.
Final degree's project. *New drugs for obesity treatment.* Universidad Autónoma de Madrid. 2025. Supervisor: Aurora Sánchez Pacheco. Grade: 8,5.

FUNDING:

Evaluación de la eficacia y viabilidad de un nuevo nutracéutico como opción terapéutica en la prevención y tratamiento de hígado graso (MASLD) y esteatohepatitis (MASH) asociadas a disfunción metabólica. References. Convocatoria 2024 desarrollo de procesos industriales.IND2024/BMD-33899. CAM. 17/09/2025-16/08/2028.. IP: Aurora Sánchez Pacheco

Diseño de algoritmo predictivo de riesgo de desarrollo de CHC en pacientes cirróticos considerando patrones genéticos de polimorfismos de Aurora Quinasa B. Sociedad Española de Oncología Médica SEOM. 11/2025-10/2027 IP.Nuria Rodríguez Sala (Hospital Universitario La Paz).

Desarrollo de sistemas de cribado para detección precoz de cáncer y prevención de efectos secundarios asociados a los tratamientos con inmunoterapia. Genesis Biomed-Universidad Autónoma de Madrid. 03/2024-03/2026. IP: Aurora Sánchez Pacheco.

PATENTS:

A stem-loop primer and a method for short length RNA detection. A. Sánchez-Pacheco; A López-Lopez; M. Camblor Murube. International Patent. EP22383065.03/09/2025.

Cancer Stem Cells and Fibroinflammatory Microenvironment

TENURED SCIENTISTS

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CONTRACT RESEARCHERS

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Contreras Puente, Carmen
Hormigos Gabaldón, Miguel Ángel

KEYWORDS

Cancer stem cells, Pancreatic cancer, Tumor microenvironment, Tumor-associated macrophages, Tumor plasticity, Patient-derived xenografts

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RESEARCH LINES:

Overview

Cancer stem cells (CSCs), also known as tumor-initiating cells or tumor-propagating cells, constitute a biologically unique subset of stem-like cells within the bulk tumor cell population. These cells are believed to be important in metastasis and chemoresistance, and they are hypothesized to be key drivers of the multistep process of oncogenesis, giving rise to the clonogenic core of tumor tissues. In the Cancer Stem Cells and Fibroinflammatory Microenvironment Group, we study CSCs in the context of pancreatic ductal adenocarcinoma (PDAC), the 4th leading cause of cancer related deaths in developed countries. We are running a combined basic and translation research program, which synergistically combines studies on the biology of mouse and human CSCs, including their in vivo microenvironment, in order to enhance our understanding of the regulatory machinery of CSCs.

Cancer Stem Cell Biomarkers

Researchers involved: Alcalá, S; Navarro, D; Maseda, P

Our first main research line involves the identification and characterization of new biomarkers for the detection of CSCs from different solid tumors. In 2014 we discovered a new inherent biomarker present in CSCs across several solid tumors. This biomarker, known as autofluorescence, is the result of riboflavin accumulation in ABCG2-coated intracellular vesicles exclusively found in CSCs. Since then, we have used autofluorescence (and other newly discovered CSC markers) as a means of isolating CSCs for in depth biological and molecular characterization studies. Along these lines, we have used autofluorescence to determine the percentage of CSCs in resected colorectal tumors and correlate these findings with disease relapse at 5 years post-surgery. We have also discovered new CSC biomarkers that identify CSCs with immune-evasive properties, such as the Peptidoglycan recognition protein 1 (PGLYRP1).

Dissecting Cancer Stem Cell Biology

Researchers involved: Alcala, S; Navarro, D; Ruiz, L; Batres, S; Vigiano, V; Maseda, P

Our second main research line focuses on the identification of proteins that govern key CSC phenotypes, such as “stemness”, epithelial to mesenchymal transition (EMT), oxidative phosphorylation (i.e.; mitochondrial respiration) and chemoresistance. By identifying the proteins that mediate these pathways, we can therapeutically target them and test their potential clinical efficacy in advanced murine models of pancreatic cancer (e.g.; patient-derived xenografts). We have discovered that the Interferon Stimulated Gene 15

(ISG15) is not only up-regulated in CSCs, but its function as a Ubiquitin-like modifier is necessary for many CSCs biological processes, such as metabolic plasticity. In addition, we can enrich for CSCs by changing their carbon source (galactose versus glucose), allowing us to study key features such as immune evasion. Using a ruthenium-based compound, we can target CSC mitochondrial respiration, reducing tumor growth in vivo. Lastly, we are studying how polyploidy giant cancer cells (PGCCs), CSCs and senescent cells overlap, share similar properties, and can be targeted in a sequential and orchestrated manner to reduce tumor growth.

The Tumor Microenvironment

Researchers involved: Alcalá, S; Navarro, D; Ruiz, L; Batres, S

Within our third main research line, we want to comprehensively understand the cellular make-up of the CSC niche and the larger more complex tumor microenvironment, specifically the role of tumor-associated macrophages (TAMs) in "activating" CSCs, with respect to the different environmental proteins they can secrete (e.g.; OSM, ISG15) in response to cues from the tumor and how these proteins alter the function of the CSCs at the level of EMT and chemoresistance and the TME (e.g.; LOXL2). Likewise, we interested in how CSCs evade the immune system, by either favoring a pro-tumor environment enriched in TAMs, or by avoiding immune detection via the expression of immune evasion proteins such as PGLYRP1.

Patient-derived Xenografts

Researchers involved: Ruiz, L; Batres, S, Alcalá, S; Fra, S; Contreras, C; Hormigos, MA

As our fourth main research line, we want to establish of one of the largest Biobanks in Spain of Patient-derived PDAC xenografts for in vivo pre-clinical studes and CSC-specific analyses. This tremendous effort is being achieved with collaborations with across Spanish hospitals and their respective biobanks.

PUBLICATIONS:

Liaki V.; Barrambana S.; Kostopoulou M.; Lechuga C.G.; Zamorano-Dominguez E.; Acosta D.; Morales-Cacho L.; Álvarez R.; Sun P.; Rosas-Perez B.; Barrero R.; Jiménez-Parrado S.; López-García A.; San Roman M.; López-Gil J.C.; Drosten M.; Sainz B. Jr.; Musteanu M.; Caleiras E.; Dusetti N.; Poli V.; Sánchez-Bueno F.; Guerra C.; Barbacid M. A targeted combination therapy achieves effective pancreatic cancer regression and prevents tumor resistance. *PNAS*. **2025**, e2523039122. DOI:10.1073/pnas.2523039122

Bakırdöğen D.; Görgülü K.; Xin J.; Alcalá S.; Ruiz-Cañas L.; Frank K.; Wu N; Diakopoulos K.N.; Dai C.; Öztürk H.; Demircioğlu D.; Peschke K.; Ranjan R.; Fusco F.; Martinez-Useros J.; Fernandez-Aceñero M.J.; Chhabra N.F.; López-Gil J.C. ; Ai J. ; Ruess D.A. ; Kaya-Aksoy E.; Steiger K.; Schmidt F.; Kohlmann L.; Berninger A.; Schmid R.M.; Reichert M.; Adli M.; Lesina M.; Sainz B. Jr.; Algül H. c-Rel drives pancreatic cancer metastasis through fibronectin-integrin signaling-induced isolation stress resistance and EMT activation. *Mol Can*. **2025**, 25, 16. DOI:10.1186/s12943-025-02486-5.

Urbanova M.; Viol F.†; Ruiz-Cañas L.; Koniaris E.; Saksis R.; Batres-Ramos S.; Earl J.; Kataki A.; Buocikova V.; Burikova M.; Cihova M.; Rojikova L.; Makovicky P.; Matuskova M.; Kohl Y.; Riedmayer A.; Makova M.; Baciak L.; Gogola D.; Rogoza O.; Rovite V.; Sainz B. Jr.; Smolkova B.; Schrader J. Aurora kinase A inhibition as a synthetic lethality strategy in ARID1A-mutated gastroenteropancreatic neuroendocrine carcinoma. *Can Lett.* **2025**, 634, 218033. DOI:10.1016/j.canlet.2025.218033.

Abal-Sanisidro M.; Palencia-Campos A.; Ruiz-Cañas L.; Cañada-Garcia L.; Batres-Ramos S.; López-López R.; Moreno R.; Martín-Pastor M.; Sainz B. Jr.; de la Fuente M. Biocompatible and Versatile Self-Illuminating Sphingomyelin Nanosystems as Potent Versatile Tools for Photodynamic-based Cancer Therapy. J *Cont Rel.* **2025**, 385, 113982. DOI:10.1016/j.jconrel.2025.113982.

Parejo-Alonso B.; Barneda D.; Trabulo S.; Courtois S.; Compte-Sancerni S.; Zurkovic J.; Ruiz-Cañas L.; Zheng Q.; Tang J.; Schmitz U.; Irun P.; Penin-Peyta L.; Crusz S.; Jagušt P.; Espiau-Romera P.; Gordo-Ortiz A.; Yuneva M.; Lin M.; Yang M.; Lanas A.; Sainz B. Jr.; Thiele C.; Heeschen C.; and Sancho P. PPAR-δ orchestrates a pro-metastatic metabolic response to microenvironmental cues in pancreatic cancer. *Can Res*. **2025**, 85, 3275-3291. DOI: 10.1158/0008-5472.CAN-24-3475

Delle Cave D.; Di Domenico A.; Fantuz M.; Ciotola M.; Mangini M.; Buonaiuto S.; Corrado B.; Corona M.; Saracino F.; Andolfi G.; Di Biase I.; Cucciardi A.; Carrer A.; Sainz B. Jr.; Pirozzi T.; Lo Re D.; Colonna V.; Minchiotti G.; De Luca A.C.; Lonardo E. TGF-β1-Mediated Downregulation of L1CAM in Pancreatic Ductal Adenocarcinoma Drives Upregulation of Collagen 17A1 and MMP2, Facilitating Tumor Invasiveness and Metastasis. *Cell Death Dis*. **2025**, 16, 952. DOI: 10.1038/s41419-025-07859-8

FUNDING:

Comprender los Mecanismos Moleculares que Hacen Funcionar a las Celulas Madre Del Cancer (CSCTICK). PID2024-157515OB-I00. Ministerio de Ciencia Innovación y Universidades (MICIU) - Proyectos de Generación de Conocimiento 2024. 09/2025 - 08/2028. PI: Bruno Sainz

EU TRANSCAN3 - Reversing Epitranscriptomic Alterations for Chemosensitization of Pancreatic Cancer (REACH). AC24/00075. ISCIII. TRNSC-247896SAIN. Asociación Española Contra el Cáncer (AECC). 04/2025-03/2028. Coordinator: Bruno Sainz (IRYCIS, Spain)

Biomodels and Biobanks Platform – IRYCIS. PT23/00098. ISCIII Biomodels and Biobanks Platforms. 01/2024 – 12/2026. PI: Ana María Torres Redondo.

Cancer Associated-Fibroblast and Exosome Biomarkers

TENURED SCIENTISTS

Peña Maroto, Cristina.
(Investigadora Científica, CSIC).

Flores Mauriz, Carmen Lisset.
(Científica Titular, CSIC).

PREDOCTORAL SCIENTIST

Collado Valero, Manuel
León Cabezas, Patricia.

VISITING SCIENTIST

Minotti, Sara.

UNDERGRADUATE STUDENT

Burgos Alemany. Victoria.
Del Blanco Calle, Valeria.
Gonzalez Huerta, Jimena.

KEYWORDS

Colorectal cancer, Tumor Microenvironment, Cancer-Associated Fibroblasts, Biomarkers, Exosomes, Precision Medicine.

RESEARCH LINES:

Overview

In the tumor context, the microenvironment is defined as the ensemble of normal cells, extracellular matrix components, signaling molecules, and blood vessels that surround and support the tumor. Among these, **cancer-associated fibroblasts (CAFs)** are the most abundant cell type. Through dynamic communication with tumor cells and other components of the microenvironment, CAFs actively contribute to tumor progression. A key element in this intercellular cross-talk is **exosomes,** extracellular vesicles that mediate communication between tumor-associated cells and distant organs where metastatic niches develop. Notably, exosomes are present in physiological fluids, such as peripheral blood, providing a valuable opportunity for **disease-specific biomarker identification through liquid biopsies.**

Our research group is primarily focused on **identifying novel biomarkers** associated with the tumor microenvironment, particularly CAF-derived biomarkers, that have **diagnostic, prognostic, or predictive value** in colon cancer patients. Colon cancer remains one of the most prevalent and lethal malignancies internationally. Therefore, identifying biomarkers with **clinical applicability** to support **decision-making and patient management** represents a crucial step toward the advancement of **personalized medicine**.

Specifically, we focus on:

Establishment of primary cultures of CAFs and normal colonic fibroblasts (NFs) from patient samples to investigate their tumorigenic potential.

Characterization and validation of pro-tumorigenic mediators involved in the interaction between primary CAF/NF cultures, extracellular matrices, and colon tumor cells or other tumor microenvironment components.

Analysis of the nucleic acid content of exosomes derived from CAF/NF primary cultures and their functional effects on colon cancer progression.

Identification of exosomal biomarkers in liquid biopsies (peripheral blood) derived from CAFs, correlating their expression with tumorigenic properties, pathological features, and patient survival outcomes.

Development of computational models based on different biomarkers for accurate patient stratification according to relapse risk.

DOCTORAL THESES AND OTHER ACADEMIC WORK:

Valeria del Blanco Calle
Final degree's project. *Respuesta a radioterapia de los fibroblastos asociados a tumores colorrectales.* Universidad Autónoma de Madrid. 2025. Supervisors: Cristina Peña Maroto.

Victoria Burgos Alemany
Final degree's project. *Migración celular inducida por fibroblastos asociados a tumores de colon en respuesta a la radioterapia*. 2025. Universidad Autónoma de Madrid. Supervisors: Cristina Peña Maroto.

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Jimena González Huertas
Final degree's project. *Puesta a punto de la respuesta de células endoteliales a fibroblastos radiados*. Universidad Alfonso X El Sabio. 2025. Supervisors: Cristina Peña Maroto and Manuel Collado Valero.

FUNDING:

"SBRT radioresistance mediated by Cancer Associated Fibroblasts in oligometastatic Colorectal Cancer Patients. CNS2023-144882". AEI. 2024-2026. Cristina Peña Maroto.

"Hacia la medicina de precisión en cáncer de colon: biomarcadores, microambiente tumoral y microbiota. S2022/BMD-7212". Comunidad de Madrid. 2023-2026. José Manuel González Sancho.

"Consorcio Ciber-Area Temática Cáncer. CB16/12/00273". ISCIII. 2017-Indefinido. Alberto Muñoz Terol.

OUTREACH ACTIVITIES

Event. "El CSIC te llama esta noche: La noche de los investigadores". Cuentacuentos: "Serafín y las Emociones". 26-sep-2025

Event "Juegos de Ciencia". Taller "El contagio de las enfermedades". 22-nov-2025

Pharmacogenomics and Tumor Biomarkers

TENURED SCIENTIST

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(Investigador Científico, CSIC)

CONTRACT RESEARCHER

Zaballos Sánchez, Miguel Ángel
(Investigador Contratado Doctor CSIC)

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Arenas Cortés, Alicia

TEMPORAL STAY

de Nicolás Hernández, Javier
Pérez Aparicio, Paula
Valdivia del Rosal, Carlos

MASTER THESIS STUDENT

Dorado Núñez, Iván
Merino Allona, César
Socas Hernández, Nerea

UNDERGADUATED STUDENT

Moreda Baena, Belén

KEYWORDS

Pharmacogenomics, Genitourinary tumors, Renal cell carcinoma, Genomic stratification, Hypoxia inducible factors, Antiangiogenic drugs, Immune checkpoint inhibitors.

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RESEARCH LINES:

Overview

The main interest of our group is understanding how genomic alterations modify drug treatment response, with the ultimate goal of using this knowledge to design more specific drug treatments. Cancer is our priority because therapy failure in oncology is a major clinical problem, and developing safer and more effective anticancer drug strategies is urgently needed. We do this by discovering new mechanisms of anticancer drug sensitivity/resistance, identifying biomarkers predictive of treatment response, and proposing novel cancer therapeutic vulnerabilities. Identifying germline genetic variants that increase drug toxicity risk is also a fundamental part of our objectives. To achieve these goals, we perform translational research that combines multi-omic analyses, cellular and animal models, and clinical knowledge from oncology departments.

Research Lines:

1. Personalization of kidney cancer treatments

In Spain, approximately 9,200 new cases of renal cancer are diagnosed each year, resulting in more than 2,200 deaths. Renal cell carcinoma (RCC) comprises a heterogeneous group of tumors classified into distinct histological subtypes, including clear cell (ccRCC), papillary (pRCC), and chromophobe (chRCC), as well as rarer entities such as FH-deficient and SDH-deficient RCC. These subtypes differ not only morphologically but also molecularly; for example, VHL mutations are characteristic of ccRCC and absent in other subtypes.

Our goal is to define therapeutically relevant molecular subgroups of RCC by identifying molecular drivers and mechanisms of drug response and resistance. We hypothesize that the limited efficacy of current treatments—particularly immune checkpoint inhibitors and antiangiogenic agents—results from broadly applying ccRCC-centered strategies to biologically diverse tumors. Even within ccRCC, substantial heterogeneity driven by secondary alterations influences tumor behavior and treatment response. In non–clear cell subtypes, which lack the genomic drivers characteristic of ccRCC, extrapolating these therapies often lacks a clear molecular rationale and may contribute to poor outcomes. Accordingly, we study subtype-specific mechanisms: in ccRCC, how chromatin remodeler mutations shape the transcriptome and influence drug response, including sex-specific effects; in pRCC, the impact of L-2-HG accumulation on therapy response; and in chRCC, metabolic reprogramming beyond the mTOR pathway.

During this year, we provided novel results for pRCC histology by defining a novel molecular subgroup of tumors with high HIF-activity linked to a unique tumor microenvironment with potential impact on drug response (de Nicolás et al. 2025; Figure 1). Specifically, the transcriptomic analysis of a large series of pRCC cases revealed a clinically distinct HIF-high subgroup (~30% of cases) characterized by high HIF activity, altered mitochondrial metabolism, increased epithelial-mesenchymal transition, and poor prognosis. Mechanistically, we identified L-2-hydroxyglutarate (L-2-HG) accumulation—caused by L2HGDH downregulation—as a novel oncometabolic driver of impaired HIF degradation in pRCC, reinforcing the pivotal role of L-2-HG in cancer metabolism.

HIF-high tumors exhibited an angiogenesis-rich and immune-enriched microenvironment with elevated immunomodulators and increased effector T cell infiltration. Integration of gene expression signatures associated with therapy response and real-world patient treatment outcomes suggests that immunotherapy combinations may provide enhanced benefit in HIF-high pRCC patients. In conclusion, this work establishes a molecular stratification framework for pRCC, revealing a novel tumorigenic mechanism that can explain the variable therapy response of patients to targeted therapies and provides a foundation for personalized precision medicine approaches to improve outcomes in this understudied cancer.

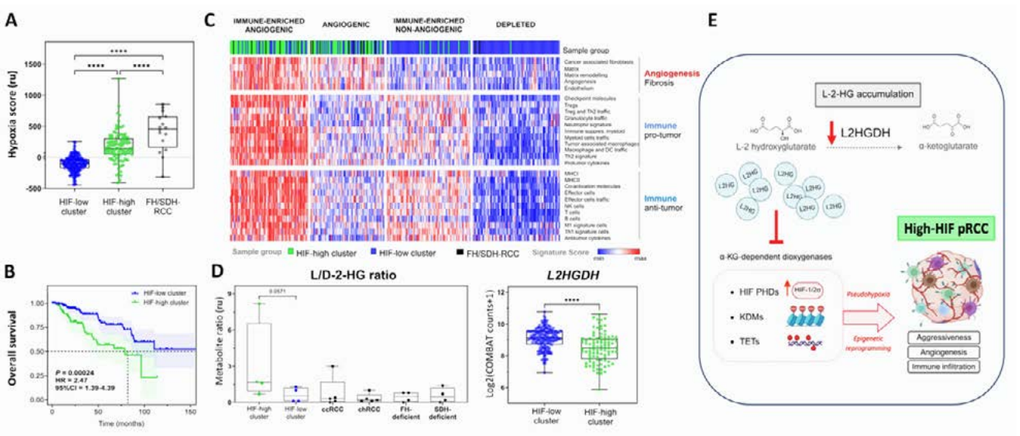

Figure 1. Molecular features of HIF-high versus HIF-low pRCC tumors. Unsupervised clustering of 303 pRCC using 461 HIF-target genes identifies: HIF-low (blue) and HIF-high clusters (green). **(A)** "HIF-high" cluster has increased hypoxia score. Pseudohypoxic RCC with FH and SDHB mutations (black) are used as controls. **(B)** Kaplan-Meier curves showing patient overall survival according to the pRCC clusters. **(C)** HIF-high tumors (green) exhibit a highly angiogenic and immune-infiltrated tumor microenvironment. Conversely, HIF-low are low-angiogenic 'immune desert' tumors. **(D)** HIF-high pRCC are characterized by high L/D-2-HG ratio and low *L2HGDH* mRNA expression. **(E)** Proposed model illustrating the mechanism of L-2-HG accumulation in pRCC and the subsequent effects that define HIF-active pRCC characteristics.

This discovery was the foundation for our recently funded pRCC-TREAT European Project, which aims to personalize pRCC treatment through molecular and metabolic stratification.

Also, this year, we contributed to a study demonstrating that autoencoders—neural networks that compress high-dimensional gene expression data into meaningful latent features—can be applied to ccRCC transcriptomic data to enable effective survival modeling in patients treated with immunotherapy and antiangiogenic therapies (Sanz Ilundain et al., 2025). In addition, we identified a ccRCC gene signature linked to AR overexpression that was associated with better prognosis, longer progression-free survival, and favorable MSKCC risk scores (Osorio et al., 2025).

2. Discovery of cancer susceptibility and prognostic biomarkers

The identification of novel cancer susceptibility and prognostic biomarkers in RCC and other tumor types through "omic" technologies is another long-lasting objective of our group. **This year, we contributed to:**

- The molecular analysis of a phase II trial of neoadjuvant durvalumab plus olaparib in bladder cancer, showing that genomic features did not predict response. Instead, resistance was linked to transcriptional changes—particularly stromal and epithelial–mesenchymal transition signatures—while responder patients exhibited increased circulating CD4+ CD27- CD28- T cells, indicating that adaptive transcriptional reprogramming drives resistance (Rodriguez-Moreno et al. 2025).
- A genomic and transcriptomic profiling analysis of head and neck paragangliomas that revealed novel candidate driver genes and pathways beyond the well-known SDHx mutations, highlighting new mechanisms involved in tumor development (Mellid et al. 2025).
- By being part of the International Consortia OCAC-OTTA we contributed to ovarian cancer research in large multinational collaborative studies (Fu et al. 2025 and Garsed et al. 2025).

3. Germline variation leading to increased risk of adverse drug reactions

An intrinsic part of our pharmacogenomic research is the definition and discovery of germline variation leading to an increased risk of adverse drug reactions. Currently, we are responsible for the design and genomic analysis of the PROCURE Project, a translational research study comprising 26 Spanish institutions that aims to discover genetic variants associated with the risk of pneumonitis in breast cancer patients treated with the novel antibody-drug conjugate trastuzumab-deruxtecan.

This year, our initial results from a Genome Wide Association Study (GWAS) have been presented at ESMO, SEOM, and ASEICA conferences (Sanchez-Bayona et al. In May 2025; Sanchez-Bayona et al. in November 2025; Arenas et al. 2025 in November 2025, respectively).

PUBLICATIONS:

de Nicolás-Hernández, J.; Arenas, A.; Valdivia, C.; Santos, M.; Lanillos, J.; Bechmann, N.; Peitzsch, M.; Gil-Vilariño, E.; Monteagudo, M.; Mellid, S.; Montero-Conde, C.; Cascón, A.; Leandro-García, L.J.; de Velasco, G.; Durán, I.; Puente, J.; García-Donas, J.; Caleiras, E.; Robledo, M.; Rodríguez-Antona, C. HIF pathway activation defines a novel molecular subgroup of aggressive papillary renal cell carcinoma with an angiogenic and immune-enriched tumor microenvironment. de Nicolás- *bioRxiv,* **2025**. DOI: 10.1101/2025.01.24.632989

Sanz Ilundain, I.; Hernández-Lorenzo, L.; Rodríguez-Antona, C.; García-Donas, J.; Ayala, J.L., Autoencoder techniques for survival analysis on renal cell carcinoma. *PLoS One,* **2025**. *20*: p. e0321045. DOI: 10.1371/journal.pone.0321045.

Osorio, L.; Grazioso, T.P.; de Velasco, G.; Etxaniz, O.; Pérez-Gracia, J.L.; Pinto, Á.; Durán, I.; Grande, E.; Garcia, P.B.; Lázaro, M.; Rodriguez, L.; Villalobos, M.L.; García, L.; Cuellar, A.; Solís-Hernández, M.P.; Pernaut, C.; Rodríguez-Moreno, J.F.; Rodriguez-Antona, C.; García-Donas, J., Retrospective study assessing the role of the androgen receptor in clear cell renal cell cancer patients treated with VEGFR inhibitors in monotherapy. *Clin Transl Oncol,* **2025**. *27*: p. 2241-2255. DOI: 10.1007/s12094-024-03652-9.

Rodríguez-Moreno, J.F.; de Velasco, G.; Álvarez-Fernández, C.; Collado, R.; Fernández, R.; Vázquez, S.; Virizuela, J.A.; Gajate, P.; Font, A.; Lainez, N.; Sevillano-Fernández, E.; Graña-Castro, O.; Beltrán, L.; Madurga, R.; Rodríguez-Antona, C.; Berraondo, P.; Ruiz-Llorente, S., and García-Donas, J., Treatment Efficacy and Molecular Dynamics of Neoadjuvant Durvalumab and Olaparib in Resectable Urothelial Bladder Cancer: The NEODURVARIB Trial. *Clin Cancer Res*, **2025**. *31*: p. 1644-1656. DOI: 10.1158/1078-0432.Ccr-24-2890.

Mellid, S.; (...);Rodriguez-Antona, C.; (...); Cascon, A., Molecular profiling unveils genetic complexity and identifies potential new driver mechanisms in head and neck paragangliomas. *Genes Dis*, **2025**. 13: p. 101705. DOI: 10.1016/j.gendis.2025.101705

Fu, Z.; (...); Rodríguez-Antona, C.; (...); Modugno, F., Ovarian cancer risk and survival according to tumor sex hormone receptor expression: An ovarian Cancer association consortium and ovarian tumor tissue analysis consortium pooled analysis. *Gynecol Oncol,* **2025**. *198*: p. 112-129. DOI: 10.1016/j.ygyno.2025.05.013.

Garsed, D.; (...); Rodriguez-Antona, C.; (...); Bowtell, D., Beyond BRCA deficiency: Clinical and molecular predictors of survival in patients with BRCA-deficient tubo-ovarian high-grade serous carcinoma. *Res Sq,* **2025**. DOI: 10.21203/rs.3.rs-7572112/v1.

DOCTORAL THESES AND OTHER ACADEMIC WORKS

César Merino Allona
Master´s thesis. *Role of SART1 in the differential regulation of HIF1α and HIF2α in renal cell carcinoma and its impact on the response to HIF2α inhibitors*. Universidad Autónoma de Madrid. 2025. Supervisors: Cristina Rodríguez-Antona and Miguel A. Zaballos Sánchez. Grade: Sobresaliente.

Paula Pérez Aparicio
Master´s thesis. *The oncometabolite L-2-Hydroxyglutarate is a driver of tumour aggressiveness in papillary renal cell carcinoma.* Universidad Complutense de Madrid. 2025. Supervisors: Cristina Rodríguez-Antona and Miguel A. Zaballos Sánchez.

Belén Moreda Baena
Final degree's project. *Effect of L-2-hydroxyglutarate on gene expression and its association with molecular and clinical characteristics of clear cell renal carcinoma.* Universidad Autónoma de Madrid. 2025. Supervisors: Cristina Rodríguez-Antona and Alicia Arenas Cortés.

FUNDING:

Uncovering therapeutic opportunities for metastatic renal cell carcinomas: genomic definition and molecular mechanisms. PID2024-158237OB-I00. MICIU. 2025-2028. PI: Rodríguez-Antona.

Molecular alterations of metastatic renal cell carcinoma of clinical significance for antitumor drug response. PID2021-128312OB-I00. MICINN. 2022-2026. PI: Rodríguez-Antona.

Caracterización molecular de subpoblaciones de tumores de células germinales avanzados resistentes a cisplatino: diseño de una firma genética. FISEVI/IDIVAL. 2023-2026. PI: Rodríguez-Antona.

OUTREACH ACTIVITIES

1st Update Conference on Kidney Disease, Rey Juan Carlos University.
Title: Molecular Advances in Renal Cancer.
June 19, 2025.

Drivers and Biomarkers of Metastasis

TENURED SCIENTIST

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MASTER THESIS STUDENT

Niles Cholango, Denisse Naomi

UNDERGRADUATE STUDENT

Jaramillo Garcia de Castro, Inés
Velasco López, Claudia
Garcia de Castro, Patricia

KEYWORDS

Metastasis, Lymphovascular Pre-metastatic Niches, Melanoma, Breast Cancer, Therapy Resistance.

RESEARCH LINES:

Overview
Our laboratory is dedicated to elucidating the complex mechanisms underlying cancer metastasis, with a particular focus on Melanoma and Breast Cancer. Our research aims to identify the molecular drivers and biomarkers associated with metastatic progression, as well as to understand the role of the lymphatic system and the intratumoral microbiome in this process.

Molecular Drivers and Biomarkers of Metastasis
We investigate the specific genes, proteins, and metabolic pathways that facilitate the dissemination of cancer cells. By identifying these molecular drivers, we aim to discover biomarkers that can predict metastatic potential and patient prognosis.

Role of the Lymphatic System in Tumor Dissemination
Our research explores how the lymphatic system contributes to tumor cell spread and the establishment of pre-metastatic niches. We study the interactions between tumor cells and the lymphatic vasculature to understand the mechanisms promoting metastasis.

Influence of the Intratumoral Microbiome on Metastasis
We examine the impact of the intratumoral microbiome on cancer progression and metastasis. Our studies aim to determine how microbial communities within tumors affect tumor behavior and response to therapies.

Mechanisms of Therapy Resistance in Metastatic Cancer
We investigate the mechanisms by which metastatic cancer cells develop resistance to current therapies. Our goal is to identify strategies to overcome this resistance and improve treatment efficacy.

Through these research lines, we aim to advance the understanding of metastatic processes and contribute to the development of novel therapeutic strategies to combat cancer metastasis.

FUNDING:

"Antibacterial Stress Responses in Metastasis and Melanoma Therapy Response. PI21/00641". MICINN. 2022-2024

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Melanoma Immunotherapy Modeling

TENURE-TRACK SCIENTIST

Pérez Guijarro, Eva
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Ramos Gómez, Javier
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Albarrán Vilches, Alejandro
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Rincón Álvarez, Ruben
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TECHNICAL SUPPORT PERSONNEL

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Díaz Utrilla, Clara
(Técnico de laboratorio Garantía Juvenil, UAM)

VISITING SCIENTIST

Borroto Alburquerque, Deyanira
(Máster Bioinformática y Biología Computacional, UAM)

KEYWORDS

Immunotherapy, Melanoma, Metastasis, Mouse models, Tumor microenvironment.

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RESEARCH LINES:

Overview

Melanoma is the leading cause of skin-cancer mortality due to its high risk of metastasis and the scarcity of therapeutic options. This aggressiveness is caused by melanoma inherent plasticity that confers adaptability to dynamic tumor microenvironment (TME) conditions. Despite the advances of immunotherapy, over one third of late-stage patients and about 60% of those with brain metastases (BrM) do not respond to current treatments. Our group´s research is centered on understanding the molecular mechanisms driving metastasis and immunotherapy resistance with the ultimate goal of discovering robust biomarkers and developing preventive and therapeutic strategies to improve melanoma patient outcomes.

Our research focus on three aspects:

1. Intratumoral heterogeneity (ITH), with especial interest on melanoma plasticity dynamics and the impact on the TME to understand its role in immune evasion and immunotherapy resistance.

2. The mechanisms of brain colonization, in particular melanoma crosstalk with stromal and immune cells leading to TME remodeling and determining the response to immunotherapy.

3. Discover targetable drivers of immune evasion to develop strategies that prevent brain metastasis and overcome immunotherapy resistance.

Our multidisciplinary approach includes the generation of single-cell multi-omics mouse data sets, development of computation tools and comparative analysis of patient cohorts, gene inactivation and drug screens in co-culture systems and preclinical therapeutic studies. In our studies we employ a unique panel of reliable melanoma mouse models representing human etiology and genetic diversity, single-cell derived clonal sublines and brain metastatic cell lines that exhibit distinct pathological and molecular characteristics, immune infiltrate profiles, and diverse responses to immunotherapy.

Role of melanoma plasticity in immunotherapy resistance

Melanoma plasticity is considered a mayor driver of ITH, which has been demonstrated to be a main cause of chemo- and targeted therapy failure. We aim to understand how melanoma plasticity is modulated by activated TME cells and to identify the specific programs involved in immune evasion in resistant tumors. To determine how the dialogue between melanoma cells and the tumor microenvironment takes place, we employed a

series of clonal cell lines exhibiting distinct molecular and phenotypic characteristics, including various differentiation states, tumor growth kinetics, and responses to immunotherapy. Our work generated multi-omics data sets, emerging as a benchmarking tool to develop computational methods and system biology models by our collaborators. We will perform functional and mechanistic assays both in vitro and in vivo to identify genetic and non-genetic factors involved in the response to immunotherapy.

In addition, we conducted a multilevel study consisting of a longitudinal analysis of immunotherapy-treated melanomas by spatial transcriptomics (ST). Complementing this, we evaluated changes in pro- and anti-inflammatory cytokines in the peripheral blood of mice in the same study. This will allow us to determine the correlation between melanoma phenotypic states and the specific TME compartments that determine immunotherapy efficacy.

Intrinsic melanoma programs driving immune evasion and brain metastasis

Despite the promising advances of immunotherapy to treat brain metastasis, response rates and durability do not reach those obtained systemically. To address current deficiency of brain-metastatic melanoma models, we generated two syngeneic cell lines (M4-BR1 and M4-BR3) by in vivo cycling via intracardiac injection. These models demonstrated high penetrance to the brain, forming numerous pigmented macroscopic metastases in over 90% of injected animals, but displayed diverse histopathology and metastatic potential. Notably, mice implanted with M4-BR1 or M4-BR3 cells responded differently to immune checkpoint blockade (ICB) as monotherapy and in combination. Our molecular and immune analysis uncovered the complexity and heterogeneity of melanoma, immune and stromal cells from each model.

To unravel the mechanisms of brain TME modulation, we focused on the study of soluble factors secreted by melanoma cells. Through proteomic analysis, we have identified a panel of differentially secreted cytokines and chemokines by the two models that could partly explain the observed phenotypes in vivo. Furthermore, a computational analysis of ligand-receptor pair prediction has evidenced distinctive interactions in each model. To carry out the mechanistic assays, we have optimized the isolation of the most relevant brain-stromal populations, microglia and astrocytes, and have established 2D and 3D co-culture conditions. We will functionally validate the soluble factors and ligand-receptor candidates by loss-of-function and gain-of-function assays in vitro and in vivo, utilizing CRISPR/Cas9-based gene editing and blocking antibodies.

PUBLICATIONS:

Hirsch MG, Pal S, Rashidi Mehrabadi F, Malikic S, Gruen C, Sassano A, Pérez-Guijarro E, Merlino G, Sahinalp C, Molloy EK, Day CP, and Przytycka TM. Stochastic modeling of single-cell gene expression adaptation reveals non-genomic contribution to evolution of tumor subclones. *Cell Syst.* **2025** Jan 15;16(1):101156. DOI: 10.1016/j.cels.2024.11.013.

DOCTORAL THESES AND OTHER ACADEMIC WORKS

Javier Ramos Gómez
Master´s thesis. *Modulation of microglia phenotypes by brain-metastatic melanoma cells.* Universidad Autónoma de Madrid. 2025. Supervisor: Eva Pérez Guijarro. Grade: Notable (8.85).

Lucía Suárez Muñiz
Final degree's project. *Modulación de la microglía por el melanoma metastático cerebral.* Universidad Autónoma de Madrid. 2025. Supervisor: Eva Pérez Guijarro. Grade: Sobresaliente (9.7).

FUNDING:

Dissecting the role of melanoma plasticity in metastasis and immunotherapy resistance. RYC2021-034893-I. AEI, MICINN. 2023-2027. PI: Eva Pérez Guijarro.

Dissecting melanoma brain metastasis and response to immunotherapy. MRA-YIA#1037420. Melanoma Research Alliance (MRA). 2023-2026. PI: Eva Pérez Guijarro.

Dissecting the role of melanoma plasticity in metastasis and immunotherapy resistance. PID2022-141113OA-I00. AEI, MICINN. 2023-2026. PI: Eva Pérez Guijarro.

Biomarkers in Colorectal Cancer: Early Diagnosis and Therapeutic Targets

TENURED SCIENTIST

Domínguez Muñoz, Gemma
(Profesora Titular). Group Coordinator

MASTER THESIS STUDENT

San Martín Doblado, Ana
Giribaldi Bello, Fiorella

KEYWORDS

Colorectal cancer, Early diagnosis, Therapeutic markers, Extracellular vesicles, Premalignant lesions, peritoneal carcinomatosis

RESEARCH LINES:

Overview
Our laboratory is interested in understanding the molecular basis of the initiation and progression of colorectal cancer with the aim to identify early diagnosis, prognosis and therapeutical decision-making markers. Our specific objectives are:

Molecular basis of peritoneal metastasis
Characterize, using "omics" approaches, the molecular mechanisms of peritoneal metastasis in order to identify potential therapeutic targets that can be tested in preclinical models. We use in vitro and orthotopic in vivo models and human simples.

ΔNp73 and tumoral progression
ΔNp73 has been described overexpressed in colorectal cancer patients and associated with poor prognosis. We investigate the role of the oncogene ΔNp73 in the different stages of colorectal cancer carcinogenesis and in the response to treatments. We are also focused in the identification of compounds that can downregulate its expression.

Biomarkers for early diagnosis
We are interested in identifying biomarkers for the early diagnosis of the disease through a minimally invasive liquid biopsy. To this end, we explore extracellular vesicles cargo, proteins and metabolites in different fluids in healthy subject, individuals with premalignant colonic lesions and colorectal cancer patients.

Response to neoadjuvant therapy
This line of research started in 2021 and is focused on the identification through "omic" techniques (including radiomics) of molecular biomarkers that can predict the response to the neoadjuvant treatments in patients with locally advanced rectal cancer. Our aim is to provide personalized treatment to these patients.

DOCTORAL THESES AND OTHER ACADEMIC WORKS

Ana San Martín Doblado
Master´s thesis. *Identification of response biomarkers to neoadjuvant treatment in patients with locally advanced rectal cancer.* Universidad Autónoma de Madrid. 2025. Supervisor/s: Gemma Dominguez Muñoz. Grade: Sobresaliente.

Fiorella Giribaldi Bello
Master´s thesis. *Implication of ΔNp73 oncogene and its effector targets in the response to neoadjuvant therapy in patients with locally advanced colorectal cancer.* Universidad Autónoma de Madrid. 2025. Supervisor/s: Gemma Dominguez Muñoz. Grade: Notable.

FUNDING:

Combination of "omics" and imaging techniques for the identification of markers associated with neoadjuvant treatment´s response in rectal cancer patients: Prognosis/therapeutic implications. PI21/01047. FIS-ISCIII. January 2022-June 2026. PI: Gemma Domínguez Muñoz.

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Cancer Molecular Pathology

TENURED SCIENTIST

Moreno Bueno, Gema
(Catedrática, UAM). Group Coordinator

CONTRACT RESEARCHER

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Klett Mingo, Jose Ignacio
Pascual Antón, Lucía
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Alonso Juliá, Gadea

VISITING SCIENTIST

Cassia, Raúl

PREDOCTORAL SCIENTIST

Ballesteros Sánchez, Sandra
Colomo del Pino, Sara
Marina Bueno, Ignacio

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Pompa Prados, Ignacio

TECHNICAL SUPPORT PERSONNEL

Morales Dolores, Saleta

KEYWORDS

Breast Cancer, Gynecological Cancer, Immunotherapy, Molecular Classification, Prognosis, Targeted Therapies.

RESEARCH LINES:

Overview

Our group is focused on the **molecular pathology and translational research of cancer**, mainly (but not limited to) breast, gynecological and colorrectal tumors. One of the most important aims in molecular oncology is to increase the survival of cancer patients through the development of more specific therapies that could also be also effective on metastatic settings. To this end, it is essential the identification and validation of novel prognostic and predictive biomarkers, as well as cancer-specific molecular targets that could be attacked through modern technological approaches. In this sense, our research lines cover from basic investigation on cancer biology and tumor behavior all the way to the pre-clinical validation and therapeutic application of our findings, being our main objectives:

1. Identification of key **molecular alterations and characterization of intratumor heterogeneity** (ITH) and tumor evolution in large series of cancer samples using state-of the-art high throughput genomic analysis technologies.

2. Discovery and **validation of prognostic and predictive markers** using molecular pathology techniques (e.g. immunohistochemistry, mutation analysis) in cancer cohorts (cancer biopsies, patient-derived-xenografts) with clinical information.

3. Definition of the precise **biological and biochemical mechanisms** of key molecular alterations and their impact in the clinical behavior of cancers with the aim of subsequently developing novel targeted treatments using innovative methods (including nanotechnology).

Covering all these three purposes, in the last 10 years our group has pioneered the identification of Gasdermin-B (GSDMB) over-expression as key mediator of tumor aggressiveness, metastasis, poor prognosis and unfavorable clinical response to treatment in HER2-positive breast cancers.

GSDMB, like other Gasdermin family proteins, can mediate either pro-tumor (invasion, metastasis, resistance to therapy) or anti-tumor (including the lytic and inflammatory cell death mechanisms termed pyroptosis) functions depending on the biological context. In this sense, as continuation of this line of research we have generated multiple *in vitro* and *in vivo* pre-clinical models that will be used to investigate the following key aspects:

1. Detailed functional characterization of the **molecular and cellular mechanisms** regulating the diverse GSDMB (pro-tumor and antitumor) activities on healthy and cancer cells.

2. Application of GSDMB over-expression as an innovative therapeutic target in HER2 positive tumors. Further development of improved anti-GSDMB **nanotherapies**.

3. Validation of the GSDMB **biological effects** (such as response to treatment) observed in vitro and in vivo in diverse cohorts of **human tumors** (including PDX).

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PUBLICATIONS:

Lado-Fernández, P; Vilas, JM; Fernandes, T; Carneiro, C; Da Silva-Álvarez, S; Estévez-Souto, V; Pedrosa, P; González-Barcia, M; Abatti, L; Mitchell, JA; Rivas, C; Moreno-Bueno, G; Vidal, A; Collado, M. Transcriptional repression of SOX2 by p53 in cancer cells regulates cell identity and migration. *Int J Cancer*. **2025**, *157(5)*, 980-992. DOI: 10.1002/ijc.35490.

García-Estévez, L; González-Rodríguez, M; Calvo, I; Orta, A; Gión, M; Moreno-Bueno, G; Pérez-García, JM; Cortés, J. Obesity, overweight and breast cancer: new clinical data and implications for practice. *Front Oncol.* **2025**, *15*, 1579876. DOI: 10.3389/fonc.2025.1579876.

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Morillo-Huesca, M; López-Cepero, IG; Conesa-Bakkali, R; Tomé, M; Watts, C; Huertas, P; Moreno-Bueno, G; Durán, RV; Martínez-Fábregas, J. Radiotherapy resistance driven by Asparagine endopeptidase through ATR pathway modulation in breast cancer. *J Exp Clin Cancer Res.* **2025** *44(1),* 74. DOI: 10.1186/s13046-025-03334-6.

FUNDING:

Validation of gene variants implicated in the clinical behavior of breast and endometrial cancer. Reference (Acronym): VarianCancer. Funding organization: Fundación Contigo Contra el Cáncer de la Mujer. 01/01/2025 – 31/12/2027. PI: Gema Moreno-Bueno.

Improving the treatment of HER2 breast cancer brain metastasis with a targeted nanotherapy (nano_HER2BRAIN). Reference: nano_HER2BRAIN. Funding organization: Fundación Contigo Contra el Cáncer de la Mujer. 01/05/2025 - 01/04/2028. PI: Gema Moreno-Bueno.

METABRAIN TOX: Targeting HER2 breast cancer brain metastasis with a novel nanotherapy. Reference: METABRAIN TOX. Funding organization: Asociación Española Cáncer Mama Metastásico. 06/06/2025 - 06/06/2027. PI: Gema Moreno-Bueno.

Impact of the functional crosstalk between GSDMB and GSDMD in HER2 breast cancer genesis, progression, and therapy response (CrossGasdermin). Reference: PID2022-136854OB-I00. Funding organization: Agencia Estatal de Investigación. 01/01/2024 - 31/12/2026. PI: Gema Moreno-Bueno.

Exploring the Feasibility of predictive and pharmacodynamics biomarkers of immunotherapy in solid tumors (Immune4ALL). Reference: PMP22/00054. Funding organization: Instituto de Salud Carlos III (ISCIII). 01/12/2022 - 30/11/2025. CoPI-WP1 & PI-WP6: Gema Moreno-Bueno.

New immunotherapy strategies for the treatment of ovarian cancer (IMCOV). Reference: PMPTA22/00076. Funding organization: ISCIII. 01/01/2023 - 01/07/2025. PI coordinator translational research: Anxo Vidal Figueroa. Collaborator subproject: Gema Moreno-Bueno.

Personalized Clinical Management of Endometrial Cancer using Liquid Biopsy, Genomics and Artificial Intelligence. Reference: ERAPERMED2021-076 ECLAI (Consortium). PERME212426MORE (CIBERONC Group). Funding organization: ISCIII & Fundación Científica de la Asociación Española Cáncer. 01/01/2022 - 31/12/2025. Consortium Coordinator & PI of CIBERONC Group: Gema Moreno-Bueno.

Cell Cycle & Cancer Biomarkers

TENURED SCIENTIST

de Cárcer Díez, Guillermo
(Tenured Staff Scientist). Group Coordinator

AECC POSTDOCTORAL INVESTIGATOR

Sanz Gómez, Natalia

VISITING SCIENTIST

Escribano Cebrián, María
Jaramillo García de Castro, Inés
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UNDERGRADUATE STUDENT

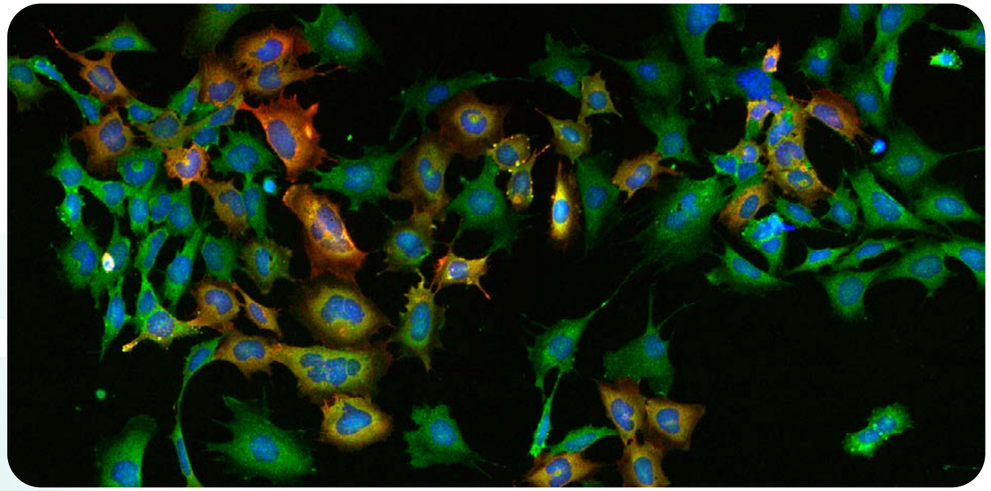
Juan Blazquez, Carla

TECHNICAL SUPPORT PERSONNEL

Montes San Lorenzo, Ángela
Rubio Elbal, Diego

KEYWORDS

Cell Cycle, PLK1, Chromosomal Instability, Aneuploidy, Drug Resistance, CRISPR Screens.



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RESEARCH LINES:

Overview

The main interest of the Cell Cycle & Cancer Biomarkers laboratory (CCCB) is to understand and define oncogenic mechanisms of cell cycle regulators with the ultimate goal of translating this knowledge to the clinic.

One of the main hallmarks of tumoral cells is their limitless proliferation capacity. Deregulation of cell division is a common feature in multiple types of tumors. Tumor cells cancel the checkpoint mechanisms of the cell cycle, resulting in the accumulation of genetic aberrations and Chromosomal Instability (CIN), providing cancer cells with increased genetic plasticity and adaptation capacity. The more aggressive a tumoral cell is, the more expression of cell cycle-related genes, which correlates with increased genomic instability. Indeed, aberrant expression of cell cycle and cell division genes often correlates with tumoral poor prognosis.

Paradoxically, in certain animal tumor models, elevated CIN negatively influences organism fitness, and is poorly tolerated by cancer cells, conferring a good prognosis to the patients. Such an opposing relationship suggests that there may be an optimal level of CIN for tumor progression and that cells need to compensate for highly deleterious CIN through genetic adaptations.

We are using cell cycle regulators and CIN-related genes, as biomarkers for cancer therapy, with the goal to find new therapeutic opportunities. Concomitantly, this will provide us the possibility to understand the mechanisms by which those cell cycle regulators modulate the oncogenic status of tumoral cells.

Identification of Resistance Mechanisms associated with Cell Cycle Drugs.

Researchers Involved: Sanz, Natalia; Montes, Ángela; Escribano, María; Rubio, Diego

A recurring problem with kinase inhibitor therapies is the emergence of drug resistance mechanisms and the resulting loss of efficacy over time. With the recent emergence of a new generation of anticancer drugs, the need to identify novel resistance mechanisms has increased significantly.

To this end, we have established a platform for genome-wide genetic screens using CRISPR-Cas9 technology in breast cancer cell lines and tested a collection of cell cycle and mitotic drugs. We discovered a new molecular mechanism of resistance for the drug rigosertib. This mitotic inhibitor is highly controversial due to the lack of a clear mechanism of action. Rigosertib was once a promising drug, but unfortunately did not reach the clinic due to poor performance in clinical trials. We have found that changes in osmotic stress lead to changes in the response to rigosertib. Interestingly, in recent years it has become

clear that rigosertib is primarily a microtubule-destabilizing agent. We have also found that changes in osmotic balance also result in differential responses to microtubule-associated drugs. More importantly, we described that the kinase WNK1, a master regulator of ion homeostasis, is critical for modulating the response to microtubule-associated drugs, some of them being classical chemotherapeutic agents for the treatment of many tumors.

Analysis of novel molecular mechanisms for whole-genome duplication tolerance in cancer.

Researchers involved: Sanz, Natalia; Escribano, María; Montes, Ángela; Juan, Carla; Jaramillo, Inés; Fuentetaja Marina Victoria; Rubio, Diego

Chromosomal instability (CIN) is a hallmark of cancer that correlates with tumor aggressiveness and poor prognosis. One of the most important sources of CIN is whole genome duplication (WGD), which occurs when cells undergo polyploidization due to severe alterations in cell cycle progression. WGD can have two very different outcomes. On the one hand, it can regulate tissue homeostasis in organs such as the liver, heart, and stratified epithelia, leading to a non-proliferative differentiation program. In other words, WGD hinders cell proliferation and may act as a tumor suppressor. Conversely, WGD has also been described as an early event in tumorigenesis, leading to cancer aggressiveness and poor prognosis.

Therefore, tumor cells that have undergone an early WGD event must overcome its detrimental effects to proliferate. Despite efforts by different research groups in recent years, the question of how cells adapt to WGD in the context of cancer remains unanswered. Using an elegant genetic trick to induce WGD in mouse cells, we identified a genetic signature that may explain this paradoxical shift. We are exploring the possible molecular mechanisms behind it. We found that this genetic signature is particularly important in head and neck squamous cell carcinoma (HNSCC). We are therefore evaluating this tumor context using preclinical models and collaborating with HNSCC clinicians.

PUBLICATIONS:

Monfort-Vengut A; Sanz-Gómez N; Ballesteros-Sánchez S; Ortigosa B; Cambón A; Ramos M; Lorenzo ÁM; Escribano-Cebrián M; Rosa-Rosa JM; Martínez-López J; Sánchez-Prieto R; Sotillo R; de Cárcer G. Osmotic stress influences microtubule drug response via WNK1 kinase signaling. *Drug Resist Updat.* **2025** *Mar;79*:101203. DOI: 10.1016/j.drup.2025.101203.

Flores NG; Fernández-Aroca DM; Garnés-García C; Domínguez-Calvo A; Jiménez-Suárez J; Sabater S; Fernández-Aroca P; Andrés I; Cimas FJ; de Cárcer G; Belandia B; Palmero I; Huertas P; Ruiz-Hidalgo MJ; Sánchez-Prieto R. The CDK12-BRCA1 signaling axis mediates dinaciclib-associated radiosensitivity through p53-mediated cellular senescence. *Mol Oncol*. **2025** *Apr;19(4):*1265-1280. DOI: 10.1002/1878-0261.13773.

López-Sainz, R.; Ayala-Jimenez, E.; Sánchez-Belmonte, A: Ortega, S; Caleiras, E; Zarzuela, E; Isasa, M; Santiveri, CM; Campos-Olivas, R.; de Cárcer, G; Malumbres, M. Cyclin Y Overexpression Drives a Fatal Metabolic Syndrome via Defective Glucose Homeostasis *bioRxiv,* **2025**. DOI: 10.64898/2025.12.15.694124

DOCTORAL THESES AND OTHER WORKS:

Carla Juan Blázquez

Final degree's project. *Study of the genetic determinants of tolerance to chromosomal instability*. Universidad Autónoma de Madrid. 2015. Supervisor/s: Guillermo de Cárcer and Natalia Sanz. Grade: Excellent

FUNDING:

Analysis of novel molecular mechanisms for whole-genome duplication tolerance in cancer. PID2024-159104OB-I00. MICINN. PI: Guillermo de Cárcer Díez

Study of novel physiological and tumor biomarkers associated with the therapeutic target PLK1. PID2021-125705OB-I00. MICINN. PI: Guillermo de Cárcer Díez

Cellular Senescence and Tumour Suppression

TENURED SCIENTIST

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Group Coordinator

PREDOCTORAL SCIENTIST

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Hernández Ruiz de Azcárate, Paula

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Grosso, Chiara,
Lobo Aldezabal, Ana

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UNDERGRADUATE STUDENT

Mena Rodríguez, Laura

TECHNICAL SUPPORT PERSONNEL

Santarén Alvarez, Alicia

KEYWORDS

Senescence, Cancer, Development, Plasticity.

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RESEARCH LINES:

Overview

Cellular senescence is a complex biological program characterized by a stable cell cycle arrest and an exacerbated secretory phenotype, which plays a key role in diverse physiological and pathological settings. Senescence can act as a stress response triggered by different forms of cellular damage such as genotoxic stress, oncogene activation or mitochondrial dysfunction, among others. In addition, senescence can also participate in the control of cell balance and tissue homeostasis in the context of normal physiology and embryonic development.

A growing list of pathologies, many of them age-related, have been associated to senescence dysfunction. These include cancer, atherosclerosis, fibrosis or diabetes among others. Strikingly, the role of senescence in disease is highly context dependent, with examples of both protective and pathogenic effects. In the context of cancer, senescence can display a dual role. On one hand, it acts as an effective tumour suppressor barrier that prevents tumour initiation, by blocking the proliferation of cells with potentially oncogenic alterations. Conversely, the accumulation of senescent cells in tumours, due to therapeutical interventions or other factors, is usually detrimental, being associated to increased tumour growth, aggressiveness and dissemination.

In our lab, we are interested in understanding the mechanisms that control cell senescence, its crosstalk with other essential cell processes, and how the disruption of the physiological program of senescence may contribute to cancer and other adult and developmental diseases. To address these questions, we use a combination of experimental approaches that include cell biology, transcriptomics and mouse models.

Senescence in physiological and pathological development

We are interested in understanding the role of cellular senescence in embryonic development and developmental disorders. Our work has focused on the human Branchio-Oto-Renal (BOR) syndrome, a rare developmental disorder (OMIM 113650, ORPHA 107) characterized by hearing loss, renal anomalies and defects in branchial arches closure, which is linked to mutations in the SIX/EYA regulatory pathway. Notably, we have previously shown the key role of SIX1 in adult cellular senescence (Adrados, Oncogene, 2016, PMID 26500063, De Lope, Sci Rep, 2019, PMID 30723235). With this background, we have used Six1-deficient mice, an animal model of the BOR syndrome, to show that inner ear defects linked to this syndrome are connected to the disruption of the physiological senescence program

in this organ (De Lope, Development, 2023, PMID 37017267). During this period, we have continued the study of normal and aberrant developmental senescence, using a combination of histological and advanced transcriptomics approaches.

Senescence in cancer: Heterogeneity, SASP and cell communication

A defining feature of senescent cells is the production and release of a complex secretome, known as the Senescence-Associated Secretory Phenotype (SASP). The SASP plays a central role in the communication of senescent cells with neighbouring cells and tissue microenvironment, mediating local and systemic cell-extrinsic effects of senescence. Accumulating evidence indicates that the SASP, along with other core features of senescence, is highly heterogeneous, due to the combinatorial effect of multiple factors, including cell type, senescence trigger, and spatiotemporal context. Importantly, substantial cell-to-cell variability is observed even within a single senescence setting. We have previously shown that dynamic changes in the SASP mediate critical effects of senescence on fibroblast cell plasticity (Lopez-Antona, Aging Cell, 2022, PMID 35266275). Building on this, our work during this period has focused on studying senescence heterogeneity and its impact on cell communication in the context of cancer, with particular attention to time-resolved changes and single-cell variability, aiming to deepen our understanding of senescence heterogeneity and its functional consequences.

PUBLICATIONS:

El Motiam, A.; Bouzaher, Y. H.; Chen, H.; Seoane, R.; Vidal, S.; Blanquer, M.; Tolosa, R. M.; Rodriguez-Lemus, B.; Herrera-Gavilan, J. A.; Vidal, A.; Palmero, I.; Rodriguez, M. S.; Sutherland, J. D.; Barrio, R.; Xirodimas, D.; Collado, M.; Bremner, R.; Rivas, C., SUMOylation of the lysine-less tumor suppressor p14ARF counters ubiquitylation-dependent degradation. *Cell Death Dis* **2025**, *16* (1), 519. DOI: 10.1038/s41419-025-07854-z

DOCTORAL THESES AND OTHER WORKS:

Laura Arranz Ortega

Master´s thesis. *Heterogeneidad en modelos celulares de senescencia inducida por terapia.* Universidad Autónoma de Madrid. 2025. Supervisor: Ignacio Palmero Rodríguez. Grade: Matrícula de Honor.

Laura Mena Rodríguez

Final degree's project. *Senescence and cellular plasticity in cancer.* Universidad Autónoma de Madrid. 2025. Supervisor: Ignacio Palmero Rodríguez. Grade: Sobresaliente

FUNDING:

Análisis del papel de la senescencia celular en fisiología y enfermedad. PID2024-155945OB-I00. AEI. 2025-2027. PI: Ignacio Palmero Rodríguez.

Senescencia celular en fisiología y enfermedad. P2022/BMD-7393. Comunidad de Madrid. 2023-2027. PI: Ignacio Palmero Rodríguez (PI of participating group in consortium of 5 groups).

OUTREACH ACTIVITIES:

Photo exhibition "Azul" on prostate cancer. Nuria Casa (Asociación Fotográfica Solidaria "lovely_photo"). With the support of AECC. Madrid, Fuenlabrada, Toledo, Talavera de la Reina. March-November 2025.

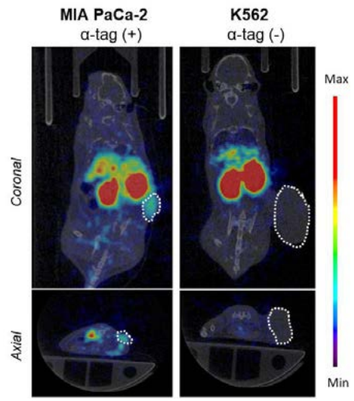
Radiopharmaceuticals for Molecular Diagnosis and Therapy

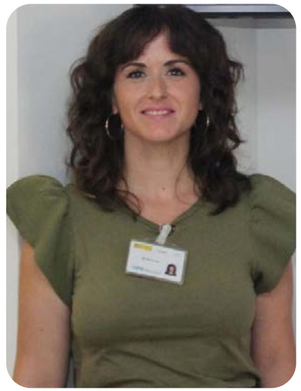
TENURED-TRACK SCIENTIST

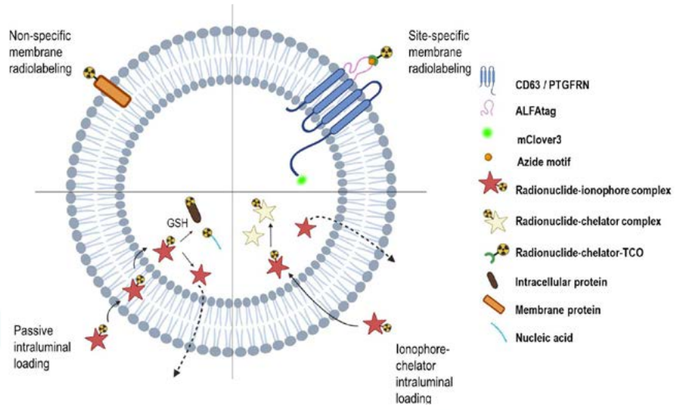
Baguña Torres, Júlia
(Investigadora Ramón y Cajal, UAM);
Coordinadora de grupo

KEYWORDS

Nuclear medicine, Molecular imaging, Radiotheranostics, Gastrointestinal cancers







RESEARCH LINES:

Overview

Our research focuses on the development and translational application of radiopharmaceuticals for molecular diagnosis and targeted therapy in oncology. Nuclear medicine provides a unique platform for the non-invasive visualization and quantitative assessment of biological processes in vivo, enabling the characterization of disease at the molecular level across the entire body. By administering radiolabeled compounds at sub-pharmacological doses, nuclear imaging allows real-time evaluation of tumor-associated biomarkers and pathways, thereby complementing and extending conventional anatomical imaging with functional and molecular information. Radiopharmaceuticals are uniquely positioned at the interface of diagnosis and therapy. In oncology, they not only support early tumor detection but also enable disease phenotyping, patient stratification, therapy selection, and treatment response monitoring. Moreover, the same molecular targeting principles can be leveraged for targeted radionuclide therapy, delivering cytotoxic radiation selectively to cancer cells while sparing healthy tissues. This theragnostic paradigm, integrating diagnostic imaging and therapy within a single molecular framework, represents a central pillar of precision oncology.

The Radiopharmaceuticals for Molecular Diagnosis and Therapy group (RMDT) is dedicated to the design, synthesis and biological evaluation of novel radiotracers and radiotherapeutic probes, with particular emphasis on malignancies of the gastrointestinal tract. Our compounds are engineered to selectively recognize cancer-specific biomarkers, enabling imaging readouts that approximate histopathological characterization without the need for invasive biopsies. Through quantitative in vivo mapping of radiopharmaceutical biodistribution, we aim to capture the spatial and temporal heterogeneity of tumor biology while preserving the integrity of underlying biological systems.

Our research is inherently interdisciplinary, integrating radiochemistry, molecular and cell biology, medical physics, pharmacology and translational oncology. We combine advanced radiolabeling strategies, in vitro mechanistic studies, nuclear imaging, dosimetry and quantitative image analysis to establish robust pipelines from target identification to preclinical validation to ensure direct clinical applicability. Ultimately, our goal is to contribute to more precise, personalized, and effective cancer management strategies by expanding the repertoire of clinically relevant radiopharmaceuticals and advancing the implementation of theranostic approaches in gastrointestinal and other solid tumors.

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Identification and validation of imaging biomarkers for the early diagnosis and prognosis of gastrointestinal cancers

Researchers involved: Baguña Torres, Júlia

Gastrointestinal cancers, and especially pancreatic ductal adenocarcinoma (PDAC), remain among the most lethal cancers due to late diagnosis, rapid progression and limited therapeutic options. Conventional imaging techniques primarily provide anatomical information and often fail to detect early-stage disease or to accurately capture tumour heterogeneity. Molecular imaging offers the opportunity to non-invasively visualize specific biological processes associated with tumour initiation, progression and therapeutic resistance.

This research line focuses on the identification, biological validation and translational evaluation of novel imaging biomarkers relevant to gastrointestinal cancers. We investigate tumour-associated receptors, metabolic pathways, stromal components and microenvironmental features that can serve as selective molecular targets for radiopharmaceutical development. Special emphasis is placed on pancreatic cancer, where early detection and improved patient stratification are urgent unmet clinical needs.

To achieve these goals, we integrate target discovery approaches (immunohistochemical analysis of patient samples), in vitro validation in relevant cellular models and in vivo assessment using relevant preclinical imaging systems. Quantitative imaging, biodistribution studies and correlation with histopathology are used to determine the diagnostic performance and prognostic value of candidate radiotracers. Ultimately, this line aims to enable earlier detection, refine risk stratification and support personalized therapeutic decision-making in gastrointestinal oncology.

Development of innovative radiolabeling strategies for biomolecules and nanoconstructs

Researchers involved: Baguña Torres, Júlia; Glynn, Thomas

This research line is dedicated to the design and implementation of innovative radiochemistry approaches for the labelling of complex biomolecules and nanoscale platforms with diagnostic and therapeutic radionuclides. We explore novel chelators, prosthetic groups, click-chemistry strategies and site-specific conjugation techniques to improve labelling efficiency and in vivo stability. Attention is given to maintaining the functional integrity of the targeting moiety and minimizing off-target accumulation.

In parallel, we investigate multifunctional nanoconstructs that enable multivalent targeting, controlled pharmacokinetics and potentially combined therapeutic payload delivery. Radiochemical optimization is coupled with physicochemical characterization, stability testing, and biological evaluation to establish standardized synthesis protocols compatible with clinical translation.

Nuclear imaging-based tracking of specific immune cell subpopulations for immunotherapy monitoring

Researchers involved: Baguña Torres, Júlia

Immunotherapy has transformed the treatment landscape of several cancers. However, therapeutic response remains highly variable and difficult to predict. The dynamic behaviour, spatial distribution and functional state of immune cell subpopulations within the tumor microenvironment are critical determinants of treatment efficacy. Non-invasive tools capable of monitoring immune responses in real time in vivo are therefore essential for response monitoring, patient stratification and immunotherapy development and optimization.

This research line aims to develop nuclear imaging strategies to track specific immune cell subsets involved in anti-tumour immunity, such as cytotoxic T lymphocytes, T helper cells, macrophages and natural killer cells. We design and validate radiotracers that selectively bind to immune-related markers or directly label immune cells ex vivo for in vivo tracking. Quantitative imaging is used to assess immune cell infiltration and activation status during immunotherapy, or trafficking patterns of adoptive cell therapies.

By correlating imaging findings with therapeutic outcomes and immunological profiling, this work seeks to identify predictive imaging biomarkers of response and resistance. Ultimately, this approach will support treatment monitoring, early response assessment, drug development and optimization of combination therapies in the context of precision immuno-oncology.

Design of radiotheranostic agents integrating diagnostic imaging and targeted radiotherapy

Researchers involved: Baguña Torres, Júlia; Glynn, Thomas

Radiotheranostic agents enable the use of the same molecular target for both diagnostic imaging and therapeutic intervention, facilitating patient selection, treatment planning and response monitoring within a coherent framework. Our work focuses on the design and preclinical validation of radiotheranostic agents directed against clinically relevant cancer biomarkers, with particular emphasis on gastrointestinal tumours. We develop radioconjugates using peptide ligands, antibody fragments or tumour-derived extracellular vesicles as targeting vectors. These platforms are engineered and structurally optimized to achieve favorable pharmacokinetics, enhanced in vivo stability and maximal tumour accumulation, thereby increasing radiation dose deposition at the tumour site while minimizing off-target exposure.

Our approach includes systematic modification of linker chemistry, chelator selection, molecular size, charge, and hydrophilicity to fine-tune biodistribution profiles and tumour-to-background ratios. Diagnostic radio-

nuclides are used to quantify target expression and perform dosimetric calculations, which guide the selection and optimization of corresponding therapeutic radionuclides. Comprehensive in vivo evaluation encompasses pharmacokinetic studies, quantitative imaging, radiation dosimetry and assessment of therapeutic efficacy and toxicity.

FUNDING:

New immuno-PET tools for personalised cancer diagnosis and immunotherapy management. RYC2023-043944-I. Agencia Española de Investigación. 01/09/2025-01/09/2030. PI: Julia Baguña Torres.

RadioSomes: Leveraging Cancer Communication for High-Precision Radiotherapeutic Intervention in Pancreatic Cancer. PRDMA258696GLYN. Asociación Española Contra el Cáncer. 01/12/2025-01/12/2029. PI: Julia Baguña Torres.

AWARDS:

"RadioSomes: Leverging Cancer Communication for High Precision Radiotherapeutic Intervention in Pancreatic Ductal Adenocarcinoma". Best poster in 19th Scientific Day and 29th Annual Conference of Vall d'Hebron. Barcelona. 04/12/2025.

Cytoskeleton And Metastasis

TENURED-TRACK SCIENTIST

Orgaz Bueno, Jose Luis
Investigador Talento CAM, CSIC).
Group Coordinator

CONTRACT RESEARCHER

Durán Renieblas, Marta
García Pérez, Andrea
Sánchez García, Lucía

UNDERGRADUATE STUDENT

Delgado López, Ángel

KEYWORDS

Cytoskeleton, Melanoma, Metastasis, Myosin, Therapy Resistance

RESEARCH LINES:

Overview

Cell migration and invasion are essential processes in physiology (development, immune system function, wound healing, angiogenesis) and also in pathologies such as cancer. Some tumour cells are able to move away from the primary tumour mass, invade into surrounding tissue, intravasate into the vasculature and eventually colonize other organ(s), developing new tumours (metastases).

Rho GTPase signalling controls the cell cytoskeleton through regulation of actin polymerization and actomyosin contractility; both machineries are essential for cell movement to take place. Non-muscle Myosin II (NMII hereafter) is a holoenzyme with actin cross-linking and contractile properties. NMII activity is controlled by several kinases. In particular, Rho-kinase (ROCK) promotes phosphorylation of myosin light chain (p-MLC2) that activates the NMII complex, which drives contractile forces required for migration, invasion and metastasis. Importantly, high NMII activity (p-MLC2) is found in the invasive edge of cutaneous melanomas, suggesting that these cells with high NMII activity are the ones that will most likely disseminate and eventually metastasise. Therefore, efforts should be focused on targeting them by blocking NMII activity.

Cutaneous melanoma is a highly aggressive and metastatic skin cancer with poor prognosis if diagnosed late. Cutaneous melanomas arise from melanocytes, which are cells responsible for producing the pigment melanin in the skin. Most melanomas carry mutations in the mitogen activated protein kinase (MAPK) pathway (RAS-BRAF-MEK-ERK), in particular in BRAF ($BRAF^{V600E}$ being the most common, 50% patients) and RAS (20% patients). Mutant BRAF constitutively activates ERK signalling that drives cancer cell proliferation and tumour progression. Targeted therapies using $BRAF^{V600E}$ inhibitors (BRAFi) and also in combination with MEK inhibitors increase survival of BRAFV600E melanoma patients. Unfortunately, responses are temporary and patients relapse due to acquired drug resistance in less than a year.

Importantly, resistance to MAPKi in melanoma involves extensive cytoskeletal remodeling and NMII hyperactivation. This renders MAPKi-resistant cells very dependent on NMII for their survival, thus NMII inhibition using ROCK inhibitors overcomes resistance to MAPKi in vitro and in vivo. Importantly, ROCK-NMII also contributes to resistance to immune checkpoint inhibitors by establishing an immunosuppressive microenvironment.

The aim of the Group is to understand how the cytoskeleton, in particular NMII, is regulated during cancer progression, especially in cutaneous melanoma. The elucidation of these mechanisms of regulation could yield potential actionable targets. Importantly, these findings could also be translated to other mutant MAPK-driven cancers (thyroid, lung, pancreatic, colorectal, ovarian, etc.) and also in fibrosis-related diseases that curse with aberrant contractility.

Regulation of NMII during melanoma progression

Researchers involved: Durán,M.; Sánchez, L.; Orgaz, J.

This research line is focused on studying how NMII is regulated during transformation and malignant progression of normal melanocytes towards melanoma, and also within different melanoma phenotypes.

Regulation of NMII during adaptation to anti-MAPK therapies

Researchers involved: García, A.; Delgado, A.; Orgaz, J.

In this research line we are investigating how NMII is regulated during adaptation and resistance to MAPK-targeted therapy.

Identifying vulnerabilities to eradicate cutaneous melanomas

Researchers involved: Sánchez, L.; Orgaz, J.

This research line is focused on studying the different outcomes and vulnerabilities after perturbation of the cytoskeleton in cutaneous melanoma, and how this could be used to implement second-line and/or combination therapies to eradicate melanomas.

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PUBLICATIONS:

Maiques, O.; Sallan, M.C.; Laddach, R.; Pandya, P.; Varela, A.; Crosas-Molist, E.; Barcelo, J.; Courbot, O.; Liu, Y.; Graziani, V.; Arafat, Y.; Sewell, J.; Rodriguez-Hernandez, I.; Fanshawe, B.; Jung-Garcia, Y.; Imbert, P.R.C.; Grasset, E.M.; Albrengues, J.; Santacana, M.; Macià, A.; Tarragona, J.; Matias-Guiu, X.; Marti, R.M.; Tsoka, S.; Gaggioli, C.; Orgaz, J.L.; Fruhwirth, G.O.; Wallberg, F.; Betteridge, K.; Reyes-Aldasoro, C.C.; Haider, S.; Braun, A.; Karagiannis, S.N.; Elosegui-Artola, A.; Sanz-Moreno, V. Matrix mechano-sensing at the invasive front induces a cytoskeletal and transcriptional memory supporting metastasis. *Nat Commun.* **2025**, *16:1394,* 1-23. DOI: 10.1038/s41467-025-56299-7.

DOCTORAL THESES AND OTHER WORKS:

Ángel Delgado López

Final degree's project. *Regulación del citoesqueleto de miosina durante la adaptación a terapias dirigidas en melanoma.* Universidad Autónoma de Madrid. 2025. Supervisor: Jose Luis Orgaz Bueno. Grade: 7,8

FUNDING:

"Identifying strategies after targeting the cytoskeleton to eradicate cutaneous melanomas. CNS2023-143636". Ministerio de Ciencia, Innovación y Universidades. 2024-2026

"Finding new vulnerabilities after inhibition of non-muscle myosin II in melanoma. 2023-5A-BMD-28922". Comunidad de Madrid. 2024-2025

"Understanding the regulation of non-muscle myosin II in melanoma progression. PID2021-122306OB-I00". Ministerio de Ciencia e Innovación. 2022-2025

OUTREACH ACTIVITIES:

Interview by five 4º ESO students from Padre Maryanet School about my career and our research for their Science Project. 24/10/25.

Genomic Biomarkers and Precision Oncology

TENURED SCIENTIST
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(Científico Titular, CSIC). Group Coordinator

PREDOCTORAL SCIENTIST
Gordillo Gallo, Clara

CONTRACT RESEARCHER
Sánchez Núñez, Noelia

KEYWORDS
Cancer genomics, Genetic susceptibility, Mechanisms of tumour aggressiveness, Ovarian cancer, Therapeutic vulnerabilities

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RESEARCH LINES:

Overview
The Genomic Biomarkers and Precision Oncology group focuses on the identification and functional characterization of prognostic and predictive biomarkers, as well as the discovery of cancer susceptibility genes, with a primary emphasis on ovarian cancer (OC). The group maintains a strong translational approach, bridging large-scale genomic data and clinical decision-making. Key strengths include access to unique, well-curated ovarian cancer cohorts—encompassing familial cases and rare histological subtypes—combined with strong expertise in advanced genomic technologies. Participation in major international consortia such as OTTA and OCAC also provides access to unique resources and datasets and international visibility. Epithelial ovarian cancer remains the leading cause of gynecological cancer-related death in developed countries, with a five-year survival rate of approximately 45%. Identifying biomarkers that predict disease evolution and treatment response is crucial. As for familial ovarian cancer, although several risk genes have been identified, over half of hereditary susceptibility remains unexplained. Defining the causal gene in each family is essential for prevention, early detection, and personalized therapy, including the use of PARP inhibitors in BRCA-mutated patients. With these challenges in mind, our main research lines include:

Definition of prognostic and predictive biomarkers in endometrioid and clear cell ovarian cancer
Current molecular prognostic classifiers do not accurately stratify outcomes for most endometrioid (EOC) and clear cell (CCOC) ovarian cancer patients, and chemotherapy resistance greatly limits treatment options in advanced disease. Given the prevailing “one-size-fits-all” approach for these subtypes, our research aims to: i) develop an improved molecular prognostic classifier with sufficient resolution for effective clinical implementation, and ii) identify actionable molecular vulnerabilities to guide targeted therapies. Our recent results indicate that EOC and CCOC tumors previously categorized as having a Non-Specific Molecular Profile (NSMP) can be further stratified into clinically distinct subgroups based on levels of chromosomal instability (CIN). By using CIN signatures (Fig. 1) we are currently dissecting the biological mechanisms underlying CIN-driven differential prognosis, which may also reveal actionable predictive biomarkers.

Identification of novel ovarian cancer susceptibility genes
TIn this second line of research, we aim to identify novel moderate-risk genes in non-BRCA (BRCAX) ovarian cancer families to reduce the unresolved inherited OC risk and improve ge-

netic counselling, early detection, prevention, and precision treatment strategies. We are applying an innovative, agnostic approach based on CIN signatures, which are closely linked to tumour biology and may reveal novel driving mechanisms in BRCAX tumours. Unlike traditional homologous recombination–focused strategies, we propose that this pathway-independent framework will enable unbiased discovery of moderate-risk variants.

Prediction of mismatch repair deficiency in ovarian cancer

Mismatch repair deficiency (MMRd) occurs in ~15% of EOC and CCOC and identifies patients eligible for immune checkpoint inhibitors, yet current diagnostic assays, originally designed for colorectal cancer, have lower sensitivity and specificity in OC. We have identified the combined activity of two specific indel mutational signatures as the most robust genomic marker of MMRd across ovarian, colorectal, and endometrial cancers. With our CNIO collaborators, we have subsequently developed a machine-learning–based predictor that outperforms gold-standard assays and most existing NGS methods, while remaining compatible with small routine clinical gene panels, thereby improving immunotherapy stratification and Lynch syndrome detection.

Identification of KMT2C- and PTEN-driven vulnerabilities in clear cell ovarian cancer

This line of research aims to establish a molecular rationale for the use of PARP inhibitors in CCOC, where clinical evidence remains limited. Using CRISPR-Cas–based functional assays in CCOC cell lines, we are assessing the predictive value of *KMT2C* and/or *PTEN loss*—alone and in combination—and examine the modulatory role of co-altered ARID1A. In parallel, we are investigating how genomic and clinical features relate to antibody–drug conjugate (ADC) target expression. Altogether, we propose that this research line will allow the identification actionable vulnerabilities and enable biomarker-driven personalized therapies for this aggressive ovarian cancer subtype.

PUBLICATIONS:

Fu, Z.; Borho L.; Taylor, S.E.; Kelemen, L.E.; DeFazio, A.; Webb, P.M.; Köbel, M.; Meagher, N.S.; Na, R.; Antoniou, A.C.; Brand, A.H.; Kennedy, C.J.; Nevins, N.; Pharoah, P.D.P.; Shvetsov, Y.B.; Winham, S.J.; Alsop, J.; Beckmann, M.W.; Bolithon, A.; Boros, J.; Bowtell, D.D.L.; Brenton, J.D.; Carney, M.E.; Chudecka-Głaz, A.; Cook, L.S.; Cybulski, C.; Fasching, P.A.; Fereday, S.; Fortner, R.T.; García, M.J.; Goode, E.L.; Goodman, M.T.; Gronwald, J.; Hartmann, A.; Hernandez, B.Y.; Høgdall, E.; Huntsman, D.G.; Jensen, A.; Jimenez-Linan, M.; Joseph, J.M.; Karlan, B.Y.; Kaznowska, E.; Kjaer, S.K.; Kluz, T.; Koziak, J.M.; Lester, J.; Longacre, T.A.; Lycke, M.; McGuire, V.; Moysich, K.B.; Murphy, R.A.; Orsulic, S.; Ramus, S.J.; Rodríguez-Antona, C.; Rothstein, JH.; Samra, S.; Sieh, W.; Steed, H.; Sundfeldt, K.; Talhouk, A.; Uciński, J.; Wang, C.; Wentzensen, N.; Whittemore, A.S.; Wilkens, L.R.; Songer, T.; Brooks, M.M.; Tang, L.; Modugno, F. Ovarian cancer risk and survival according to tumor sex hormone receptor expression: An ovarian Cancer association consortium and ovarian tumor tissue analysis consortium pooled analysis. *Gynecol Oncol.* **2025**, *198*:112-129. DOI: 10.1016/j.ygyno.2025.05.013.

DOCTORAL THESES AND OTHER WORKS:

Clara Gordillo Gayo

Master´s thesis. *Characterization of pathogenic mutations in tumor samples from high-risk ovarian cancer patients using a targeted next- generation sequencing multigene panel.* Universidad Autónoma de Madrid. 2025. Supervisor/s: María J. García Pérez.

FUNDING:

"Chromosomal Instability (CIN) signatures, an INnovative approach to identify susceptibility genes and prognostic factors in Non-BRCA Ovarian cancer patients (CIN2 Non-BRCA Ovarian). PID2023-151298OB-I00". MICIU. 2024-2027

OUTREACH ACTIVITIES:

Science Week. Proteins, the machines of life: Talk and workshop for primary school children during Science Week at Rabindranath Tagore Public School. 26/11/2025.

Chromosome Instability & Tumorogenesis

TENURED SCIENTIST

Sánchez Pérez, Mª Isabel.
(Catedrática, UAM). Group Coordinator

Calés Bordet, Carmela
(Catedrática, UAM)

PREDOCTORAL SCIENTIST

Melones Herrero, Jorge

CONTRACT RESEARCHER

Delgado Aliseda, Patricia

VISITING SCIENTIST

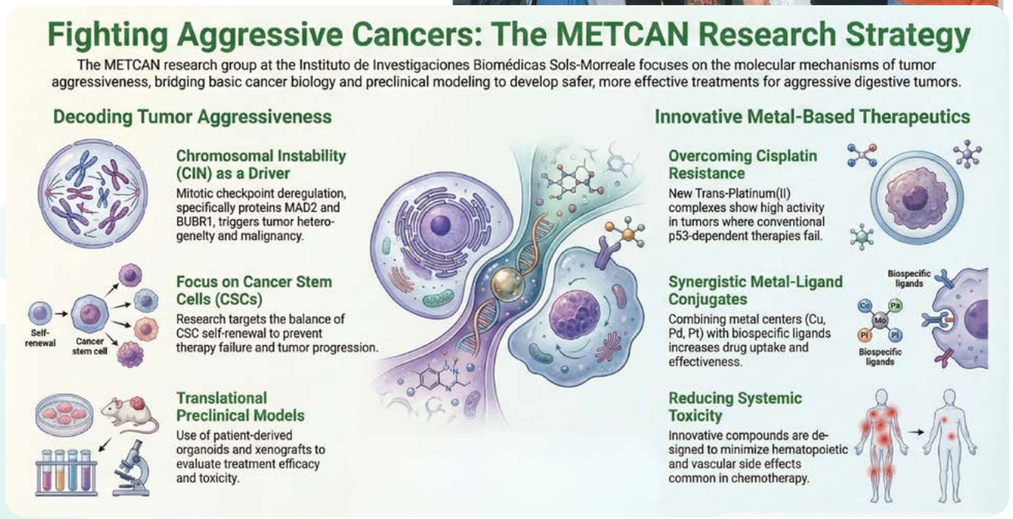
González Herrera, Fabiola Lorena
Spagnoli, Sofía

MASTER THESIS STUDENT

Priego Gutierrez, Susana
Rivera Cabrera, Mª Pilar

KEYWORDS

Cancer stem cells, Chromosomal instability, Metal-based anticancer therapies, Preclinical cancer research.



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RESEARCH LINES:

Overview

Our group focuses on understanding the molecular mechanisms underlying tumor aggressiveness and therapy resistance in digestive cancers, with a particular emphasis on gastric cancer and pancreatic ductal adenocarcinoma. Our research is centered on the role of chromosomal instability as a major driver of tumor heterogeneity, cancer stem cell maintenance and treatment failure.

A central objective of the group is to identify vulnerabilities associated with chromosomal instability and cancer stem cell biology that can be exploited for therapeutic intervention. To achieve this goal, we combine mechanistic studies with the development and preclinical evaluation of innovative metal-based anticancer compounds, including copper- and platinum-derived complexes, aimed at overcoming chemoresistance and eliminating cancer stem cell populations.

Our approach integrates advanced cellular and patient-derived models, such as organoids and xenograft-based systems, with multi-omics analyses and functional assays to assess therapeutic efficacy as well as treatment-associated side effects. In particular, the group places strong emphasis on the evaluation of endothelial dysfunction and hematopoietic toxicity, contributing to the development of safer and more effective anticancer strategies.

Through national and international collaborations, the group adopts a translational perspective that bridges basic cancer biology, preclinical modeling and biomarker discovery, with the ultimate goal of supporting personalized therapeutic approaches in aggressive digestive tumors.

Research Lines

Building on the integrative approach described above, the group develops its research through the following interconnected lines:

1. Chromosomal Instability and Cancer Stem Cell Biology in Digestive Tumors

This research line focuses on dissecting the molecular mechanisms by which chromosomal instability promotes cancer stem cell maintenance, tumor heterogeneity and therapeutic resistance in aggressive digestive cancers, particularly gastric cancer and pancreatic ductal adenocarcinoma. The group investigates alterations in mitotic control, stress-response pathways and metabolic adaptations that sustain stemness and tumor progression.

Researchers involved: M. I. Sánchez-Pérez, C. Calés, P. Delgado, MP. Rivera

2. Development of Metal-Based Therapeutic Strategies Targeting Cancer Stem Cells
This line is dedicated to the design, characterization and preclinical evaluation of innovative metal-based anticancer compounds, including copper-, palladium- and platinum-derived complexes. The aim is to develop therapeutic strategies capable of selectively targeting cancer stem cell populations, overcoming resistance to conventional chemotherapy and inducing tumor cell death while minimizing systemic toxicity.
Researchers involved: M. I. Sánchez-Pérez, C. Calés, P. Delgado, MP. Rivera

3. Translational Preclinical Models, Biomarkers and Treatment Safety
This research line integrates advanced preclinical models with multi-omics and functional analyses to evaluate therapeutic efficacy and identify predictive biomarkers of response. A key component of this line is the assessment of treatment-related side effects, with particular emphasis on endothelial dysfunction and hematopoietic toxicity, in order to support the development of safer and more personalized therapeutic approaches.
Researchers involved: M. I. Sánchez-Pérez, C. Calés, J. Melones, S. Priego, F. González, S. Spagnoli.

PUBLICATIONS:

Melones-Herrero, Jorge, Aguilar-Rico Francisco,. Matesanz Ana I , Calés Carmela, Sánchez-Pérez Isabel, G. Quiroga Adoracion. Antiproliferative activity in breast cancer cells of PtL2: A steroid-thiosemicarbazone platinum(II) complex. *J Inorg Biochem.***2025**, *V- 270:* 112923 DOI: 10.1016/j.jinorgbio.2025.112923.

Guerrero-López, R , Manguán-García, C , Carrascoso-Rubio, C , Lozano, ML, Toldos-Torres, M, García-Castro, L, Sánchez-Dominguez, R, Alberquilla, O, Sánchez-Pérez, I, Molina-Molina, M, Bueren, JA, Guenechea, G, Perona, R , Sastre, L . Premature ageing of lung alveoli and bone marrow cells from Terc deficient mice with different telomere lengths. *Sci Rep*. **2025**, *V-15 (1):* 6102. DOI :10.1038/s41598-025-90246-2

DOCTORAL THESES AND OTHER WORKS:

Jorge Melones Herrero
Ph.D. thesis. *Caracterización Biomolecular y Evaluación del Efecto Antitumoral de los Yodocomplejos de Platino (II): trans y cis-[PtI2(isopropilamina)2]*: Universidad Autónoma de Madrid.2025 Supervisor/s: Adoración. Gomez Quiroga e Isabel Sanchez Pérez. Grade: Sobresaliente Cum Laude. International Doctoral Mention.

Susana Priego Gutierrez
Master´s thesis: *In vitro study of the Unfolded Protein Response triggered by cis-[PtI$_2$(isopropylamine)$_2$] and its correlation with cell death in gastrointestinal tumor cell cultures* Universidad Autónoma de Madrid. 2025. Supervisor/s: Isabel Sánchez Pérez. Grade: Sobresaliente

Mª del Pilar Rivera Cabrera
Master´s thesis: *Efecto de nuevos compuestos metálicos con Pt in vitro.* Universidad Autónoma de Madrid. 2025. Supervisor/s: Isabel Sánchez Pérez. Grade: Notable

FUNDING:

New metal complexes as molecular targets and preclinical cancer models. 2023-2026 PID2022-137373OB-I00. Ministry of Science and Innovation (MICINN). PI: A. Gómez Quiroga and M Isabel Sánchez Pérez

PATENTS:

"Nucleotide and peptide sequence GSE 24.2 of dyskerin inducing telomerase activity, its procedure, therapeutic compositions, and applications". R. Perona, I. Sánchez Pérez, R. Machado, L. Sastre, and J.R. Murguía. International/National Patent Reference number. 2019 PCT/ES2006/070152. España. European EP06849419.4, Japan 5560398, USA US9.074,194B2 Licenciada ALODIA Farmacéutica S.L.

OUTREACH ACTIVITIES:

VENTUCIENCIA 2025. Discovering DNA. May 2025
4th ESO+Empresa Program of the Community of Madrid – SIES AFRICA, MORALEJA DE EN MEDIO. March 2025
European Researchers' Night 2025. Extract DNA from a strawberry. September 2025

Emerging Genes in Thyroid Cancer

TENURED SCIENTIST
Santisteban Sanz, Pilar

KEYWORDS
Oncogenes, Thyroid cancer, Signalling, Sodium Iodide Symporter.

RESEARCH LINES:

Overview

Over the years, our laboratory has been interested in understanding the molecular mechanisms that regulate thyroid function and how these are altered in thyroid cancer. We have identified the main molecular mechanisms that lead to the transformation of normal thyroid follicular cells into invasive thyroid carcinoma.

Thyroid differentiation depends on the expression of genes involved in iodine metabolism, which are essential for the biosynthesis of thyroid hormones and constitute the basis for radioactive iodine treatment in thyroid tumors. However, some tumors progress to a metastatic disease that is refractory to radioactive iodine therapy due to loss of differentiation. Understanding the molecular basis underlying this progression toward more aggressive and metastatic forms of the disease has been the focus of our research in recent years.

One of the most important genes involved in iodine metabolism is the Sodium/Iodide Symporter, known as NIS. Our most recent work on NIS has been conducted in collaboration with Dr. Antonio de la Vieja from Endocrine Tumors Unit (UFIEC), Instituto de Salud Carlos III.

1. Radioiodine therapy for the treatment of ovarian cancer

Researchers involved: Santisteban, Pilar

This study provides new preclinical evidence suggesting that radioiodine (I-131) therapy—commonly used for thyroid cancers—could be a promising alternative treatment for ovarian cancer by targeting a novel molecular marker: the NIS protein. Using animal models, the research explores exploiting the tumor's expression of NIS (sodium/iodide symporter), which can transport and accumulate iodine in cells, to selectively direct the effects of radioiodine to the cancer cells.

Ovarian cancer is the gynecological malignancy with the highest mortality rate. Despite the availability of several therapeutic options, overall survival remains low, and recurrence rates are high, with no significant improvements in treatment outcomes. In this context, a targeted therapeutic strategy based on the NIS protein has been explored. This approach has the potential not only to treat ovarian cancer but also to monitor disease progression using non-invasive imaging techniques.

The most relevant finding of the study is that, in murine models of ovarian cancer, the administration of a therapeutic dose of radioiodine I-131 resulted in a significant reduction in tumor volume and, in some cases, complete tumor disappearance after the follow-up period. Furthermore, when compared with commonly used chemotherapeutic agents such as cisplatin and paclitaxel, radioiodine demonstrated greater antitumor efficacy and fewer adverse effects in treated animals. Since NIS expression was not completely lost after chemotherapy, these findings open the possibility of investigating combined or sequential therapeutic approaches in the future.

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PUBLICATIONS:

Mielu, L.M., Rodal-Bravo L.; Diego-Hernández, C.; Varona, S.; Fajardo-Delgado, D.; Hortiguela, R.; Torres-Ruiz, M.; Cuesta, I.; Pallares, P.; Santisteban, P.; García-Jiménez, C.; Martín-Duque, P.; De la Vieja, A.Targeted radioiodine therapy of ovarian cancer via the sodium/iodide symporter (NIS). *Biomed Pharmacother*. **2025** *Dec;193*: 118873. DOI: 10.1016/j.biopha.2025.118873. Epub 2025 Dec 10.

FUNDING:

“Estudio de la heterogeneidad celular y del entorno inmunológico en las patologías tiroideas: cáncer y enfermedad autoinmune. Entidad Financiadora: Programa de Biomedicina Comunidad de Madrid 2023-2022 IP coordinator: Mercedes Robledo (CNIO), Pilar Santisteban, Associated Researcher.

“Efectos de la Vitamina D sobre las células troncales y el microambiente tumoral en cáncer de colon: diferenciación, metabolismo y comunicación intercelular” Financiado por MICIN, 2023-2026. IPs, Alberto Muñoz, Maria Jesus Larriba. Pilar Santisteban. Team researcher.

Bioinformatics and Computational Biology of Cancer Evolution

TENURED SCIENTIST

Díaz Uriarte, Ramón
(Catedrático de Universidad, UAM).
Group Coordinator

CONTRACT RESEARCHER

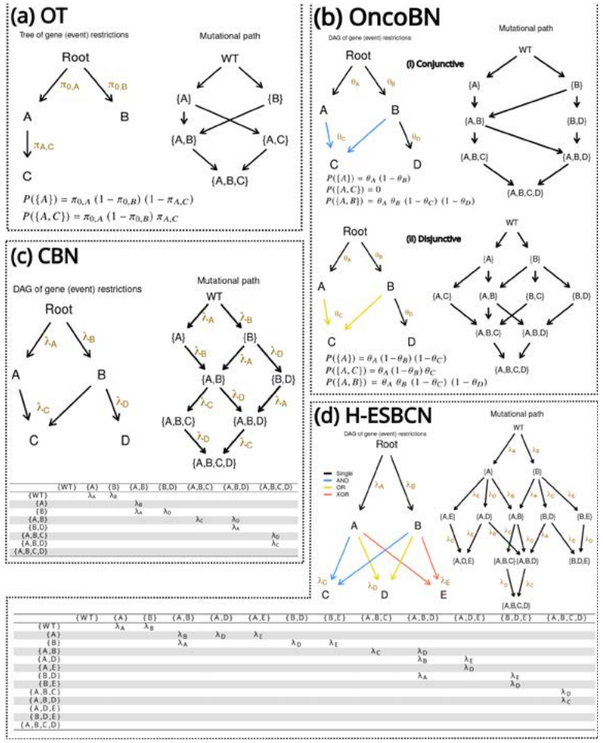
Idmbarek, Asmaa

UNDERGRADUATE STUDENT

Barreiro Fernández, Marco

KEYWORDS

Cancer progression models, Computer simulation, Evolution, Evolutionary accumulation models, Statistics



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RESEARCH LINES:

Overview

We are a computational biology group that focuses mainly on evolutionary models of cancer. Specifically, most of our work is centered on trying to infer the sequence of driver genetic events and predict tumor evolution using computational models of cancer progression using cross-sectional data. We try to understand the kinds of statistical inferences we can perform from this data with a family of methods often called “cancer progression models” (most of them related to probabilistic graphical models). The main questions we try to address are: a) the effects of different evolutionary and sampling scenarios (e.g., different evolutionary regimes, whole-tumor vs. single-cell sampling) on the performance of these methods; b) whether these types of methods can be used to estimzate tumor predictability and to make predictions about tumor evolution (both overall evolutionary paths and conditional predictions of next stages conditioned on the current observation); c) whether these methods could guide the choice of therapeutic targets and the application of adaptive therapy; d) addressing the above problems using simulation-based inference. As part of this work, we devote considerable effort to software implementation; in particular, forward genetic simulation of clonal evolution (which is crucial for the assessment of the statistical performance of the methods studies and for simulation-based inference), and the development of unified interfaces for the analysis of cross-sectional data with cancer progression models (essential to compare different methods and to allow other researchers to use state-of-the art approaches).

In addition to the above main research line, we also work on other problems in computational biology and bioinformatics, in particular the use of statistical methods for high-dimensional problems.

PUBLICATIONS:

Renz, J.; Dauda, K. A.; Aga, O. N. L.; Diaz-Uriarte, R.; Löhr Iren H.; Blomberg, B.; Johnston, I. G. Evolutionary accumulation modeling in AMR: machine learning to infer and predict evolutionary dynamics of multi-drug resistance. *mBio*. **2025**, *16*, 1-13. DOI:10.1128/mbio.00488-25

Diaz-Uriarte, R.; Johnston, I. G. A picture guide to cancer progression and evolutionary accumulation models: systematic critique, plausible interpretations, and alternative uses. *IEEE Access*. **2025**, *13*, 62306-62340. DOI: 10.1109/ACCESS.2025.3558392

Johnston, I. G.; Diaz-Uriarte, R. A hypercubic Mk model framework for capturing reversibility in disease, cancer, and evolutionary accumulation modelling. *Bioinformatics*. **2025**, *41*, 1-10, DOI: 10.1093/bioinformatics/btae737.

DOCTORAL THESES AND OTHER WORKS:

Danilo Ben Jakovenko
Final degree's project. *Mutual Hazard Networks for cancer progression models:differences between implementations.* Universidad Autónoma de Madrid. 2025. Supervisor/s: Ramón Díaz Uriarte. Grade: Matrícula de Honor

Eduardo Iglesias Santa Cruz
Final degree's project. *Aptitud dependiente de la frecuencia en la evolución del cáncer: modelos de acumulación de mutaciones y progresión.* Universidad Autónoma de Madrid. 2025. Supervisor/s: Ramón Díaz Uriarte. Grade: Sobresaliente

FUNDING:

Terapia antitumoral adaptativa usando datos transversales y predicciones de modelos de progresión tumoral. PID2019-111256RB-I00. AEI. 2020-2025. PI: Ramón Díaz Uriarte.

Inferencia causal, filogenias, y software: de datos transversales a cohortes de filogenias intra-paciente en cáncer y resistencia antimicrobiana.. PID2024-156888OB-I00. AEI. 2025-2029. PI: Ramón Díaz Uriarte.

Oncogenic Signaling and Therapy Resistance

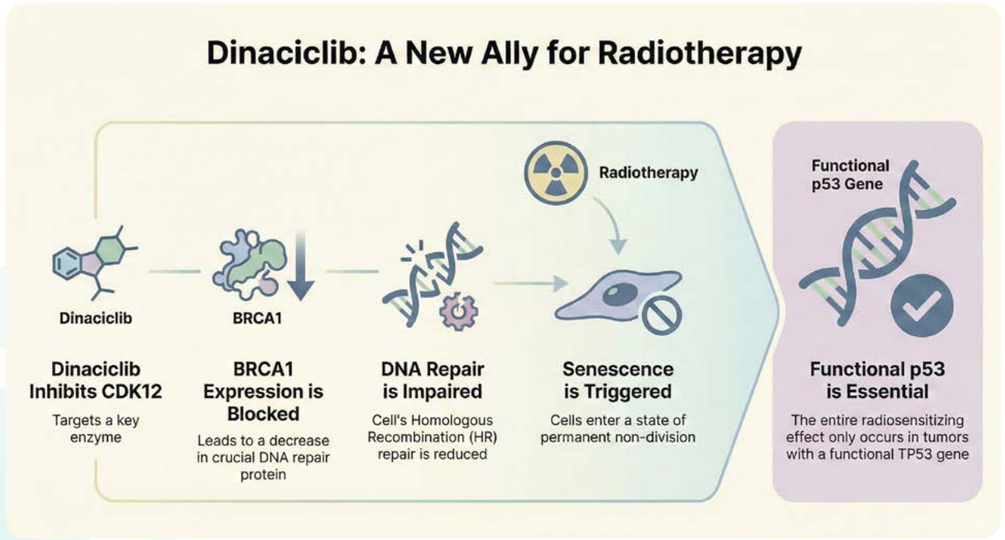
TENURED SCIENTIST

Sánchez Prieto, Ricardo.
(Científico titular). Group Coordinator

Belandia Gómez, Borja.
(Científico titular)

KEYWORDS

Chemotherapy, MAPK, Radiotherapy, Resistance, Sarcoma



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RESEARCH LINES:

Overview

This laboratory investigates the molecular mechanisms of cellular transformation and their impact on therapeutic efficacy in cancer. Our research integrates cell culture models, genetically engineered animal studies, and clinical tumor sample analysis. Methodologically, we employ a combination of molecular and cellular biology techniques, precision gene perturbation (e.g., CRISPR/Cas, shRNA), and multi-omics profiling (including RNA-seq and whole-exome sequencing). The ultimate objective of this work is to advance diagnostic paradigms and inform the development of more effective, mechanism-based therapeutic interventions in oncology.

Molecular Determinants of Radio- and Chemoresistance

Researchers involved: Sanchez Prieto Ricardo, Belandia Gomez

Our research group has more than three decades of experience investigating the molecular mechanisms underlying resistance to chemotherapy and radiotherapy. In recent years, our work has focused specifically on elucidating the molecular basis of radioresistance and on developing strategies to enhance the therapeutic efficacy of radiotherapy.

Radiotherapy represents a cornerstone of cancer treatment, benefiting over 50% of cancer patients and serving as the treatment of choice in numerous clinical contexts. Nevertheless, the emergence of radioresistance—either intrinsic or acquired following exposure to ionizing radiation—constitutes a major clinical challenge, frequently resulting in tumor recurrence and treatment failure.

To address this limitation, we are currently generating radioresistant cellular models through repeated exposure to ionizing radiation, thereby recapitulating clinically relevant scenarios of acquired radioresistance. These models will be comprehensively characterized using whole-exome sequencing (WES) and transcriptomic profiling (RNA-seq). In parallel, genome-wide CRISPR/Cas9 screening approaches will be applied to identify genes that are essential for the survival and maintenance of the radioresistant phenotype. Together, these complementary strategies aim to identify novel biomarkers of radioresistance as well as previously unrecognized therapeutic vulnerabilities, ultimately contributing to the development of more effective and personalized radiotherapy-based cancer treatments.

As a complementary research line, we are evaluating novel targeted therapies as potential radiosensitizing agents. Cellular responses to ionizing radiation are assessed across multiple experimental models, with particular emphasis on the DNA damage response

and molecular targets implicated in radiosensitization. Our approach integrates in silico, in vitro, and in vivo studies with radiobiological and omics-based analyses to identify new radiosensitizing compounds capable of improving the precision and personalization of radiotherapy strategies.

Cell Signalling Pathways in Sarcoma Pathogenesis and Treatment

Researchers involved: Sanchez Prieto Ricardo, Belandia Gomez

Sarcomas comprise a highly heterogeneous group of malignancies originating from connective tissues that provide structural and functional support throughout the body. To date, more than 150 sarcoma subtypes have been described, arising from fibrous, muscular, adipose, cartilaginous, osseous, vascular, or lymphatic tissues. As a result, sarcomas can develop in virtually any anatomical location. Clinically, they are classified into soft tissue sarcomas (STS) and bone sarcomas (osteosarcomas). Gastrointestinal stromal tumors (GISTs) represent a distinct subset of soft tissue sarcomas with unique diagnostic and therapeutic characteristics.

Although sarcomas account for approximately 1% of adult malignancies, they represent nearly 15% of cancers diagnosed in pediatric patients. Despite their clinical relevance, the molecular mechanisms driving sarcoma initiation and progression remain poorly understood, and therapeutic options are limited. Our previous studies have demonstrated a critical role for the ERK5 signaling pathway in sarcomagenesis induced by the chemical carcinogen 3-methylcholanthrene (3MC) in murine models. Transcriptomic analyses (RNA-seq) revealed more than 500 differentially expressed genes in ERK5-deficient tumors, many of which are involved in key oncogenic processes, including angiogenesis, cell motility, cell adhesion, genomic stability, and transcriptional regulation. These findings support an oncogenic role for ERK5 and underscore its relevance in sarcoma diagnosis and therapy.

We have functionally validated several ERK5-regulated genes, including *KLF2* and *VCAN*, as critical mediators of tumorigenesis. Notably, we have recently demonstrated that *VCAN* is a direct transcriptional target of the ERK5 pathway in soft tissue sarcomas and accounts for approximately 30% of the ERK5-regulated gene program, particularly those associated with cell motility and vascular development. Clinical validation using human tumor samples revealed significant overexpression of *VCAN* and a strong correlation with ERK5 expression in aggressive sarcoma subtypes, such as leiomyosarcoma and undifferentiated pleomorphic sarcoma. These findings identify the ERK5–VCAN axis as a promising novel therapeutic target in soft tissue sarcomas.

The primary objective of this research line is to establish human cellular models deficient in ERK5 and/or ERK5-regulated genes using gene interference approaches (CRISPRi, shRNA) and gene-editing technologies (CRISPR/Cas9). These models will enable the identification of ERK5-dependent gene signatures through comprehensive transcriptomic profiling. In parallel, analogous analyses will be conducted using selective pharmacological inhibitors of ERK5 to distinguish genes regulated by its kinase-dependent activity from those regulated through kinase-independent mechanisms.

This integrated strategy aims to identify novel therapeutic targets suitable for pharmacological intervention. Candidate gene signatures will be validated in the context of tumor biology, diagnostic relevance, and therapeutic response, including chemo-, radio-, and immunotherapy. Finally, the expression patterns of selected candidate genes will be evaluated in human sarcoma biopsy samples.

Collectively, these studies will facilitate the identification of new biomarkers and therapeutic targets, thereby advancing the development of personalized diagnostic and treatment strategies for sarcomas—an underexplored and clinically challenging group of malignancies.

PUBLICATIONS:

Jiménez-Suárez J; Garnés-García C; Fernández-Aroca P; Aparicio-Canada J; Belandia B, Ruiz-Hidalgo MJ; Sánchez-Prieto R; Cimas FJ. Specific transcriptional signature dataset of extracellular signal-regulated kinase 5 in leiomyosarcoma derived cell lines. *BMC Res Notes.* **2025** *Nov 13;18(1)*:477. DOI: 10.1186/s13104-025-07551-2.

Flores, NG; Fernández-Aroca, DM; Garnés-García, C; Domínguez-Calvo,A; Jiménez-Suárez, J; Sabater, S; Fernández-Aroca, P; Andrés, I; Cimas,FJ; de Cárcer,; Belandia, B; Palmero, I; Huertas, P; Ruiz-Hidalgo, MJ; Sánchez-Prieto, R. The CDK12-BRCA1 signaling axis mediates dinaciclib-associated radiosensitivity through p53-mediated cellular senescence. *Mol Oncol*. **2025**, *April; 19(4):*1265-1280. DOI: 10.1002/1878-0261.13773

Monfort-Vengut A; Sanz-Gómez N; Ballesteros-Sánchez S; Ortigosa B; Cambón A; Ramos M; Lorenzo ÁM; Escribano-Cebrián M; Rosa-Rosa JM; Martínez-López J; Sánchez-Prieto R; Sotillo R; de Cárcer G. Os-

motic stress influences microtubule drug response via WNK1 kinase signaling. *Drug Resist Updat.* **2025** *Mar;79*:101203. DOI: 10.1016/j.drup.2025.101203.

DOCTORAL THESES AND OTHER WORKS:

Juan Jesus Gómez Martínez
Master´s thesis. *Molecular mechanisms of radiosensitivity associated with dubermatinib in lung cancer cell models.* Universidad de Castilla la Mancha .2025. Supervisor/s: Ricardo Sánchez Prieto. Grade:9,2.

Juan Alonso Mas Cazorla
Final degree's project. *Axl, una aproximación in silico al estudio pronóstico de su papel en el carcinoma renal y vesical.* Universidad de Castilla la Mancha. 2025. Supervisor/s: Ricardo Sánchez Prieto. Grade: 8,7

FUNDING:

Biología de la radiorresistencia; implicaciones en cáncer. PID2024-155601OB-100. Agencia Estatal de Investigación. 2025-2028. PI: Ricardo Sánchez Prieto.

Estudio de nuevos genes dependientes de la ruta erk5 en patología sarcomatoide: implicaciones en biología tumoral y terapia. PID2021-122222OB-100. Agencia Estatal de Investigación.2022-2025. PI: Ricardo Sánchez Prieto.

Búsqueda de Nuevas Dianas y Marcadores en Patología Sarcomatoide: Implicaciones en Quimio, Radio e Inmunoterapia. SBPLY/23/180225/000007. Agencia de Investigación e Innovación, Junta de comunidades de Castilla-La Mancha. 2024-2027. PI: Ricardo Sánchez Prieto.

Papel de la señalización celular mediada por MAPK en infecciones por patógenos emergentes. Universidad de Castilla La Mancha. 2023-2025. PI: Ricardo Sánchez Prieto

Campaña de micro mecenazgo "IRRADIANDO Esperanza". 2022-2026. PI: Ricardo Sánchez Prieto.

Nuevos determinantes de radiorresistencia Research Contracts. Asociación Comarcal contra el Cáncer de Motilla del Palancar. 2025. PI: Ricardo Sánchez Prieto.

Nuevos determinantes de radiorresistencia Research Contracts. Asociación Taller Solidario " El Árbol de la Vida (Pedroñeras). 2025. PI: Ricardo Sánchez Prieto.

Nuevos determinantes de radiorresistencia Research Contracts. Asociación Jareña Contra el Cáncer. 2025. PI: Ricardo Sánchez Prieto.

OUTREACH ACTIVITIES:

Semana de la Ciencia Universidad de castilla la Mancha). Title: Tinción con cristal violeta para cuantificar la viabilidad celular en respuesta al fármaco cis-platino Date. 9/11/2025

Talk in Centro Cultural de Zafra de Záncara Title: ¿Qué es y como se trata el cáncer?. Date. 25/04/2025

Talk in Centro Cultural de Motilla del Palancar. Title: "Irradiando salud" Date: 28/03/2025

Molecular Mechanisms of Aging and Cancer

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Ben Aribi, Taysir
Garcia-Perrote Agra, Natalia
Minaya Solera, Alejandra

KEYWORDS
Cancer, Aging, FOXO3, TRIB2, Nuclear export, CRM, Drug development

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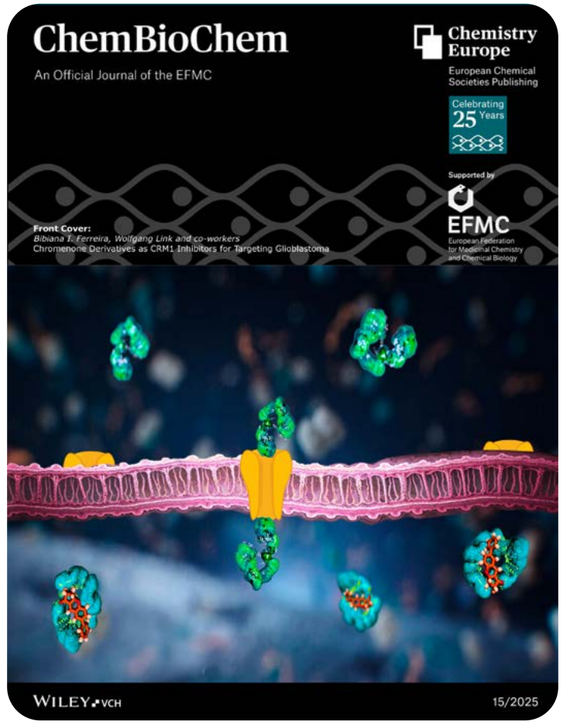

RESEARCH LINES:

Overview
Over the past decade, our research has been dedicated to understanding the functions of FOXO transcription factors in the contexts of cancer and longevity, with the aim of targeting their activities for therapeutic purposes. FOXO factors are pivotal in maintaining cellular homeostasis and fortifying the body's defense mechanisms against cellular stress. Intriguingly, FOXO3 stands out as the second most replicated gene associated with extreme human longevity.

In our laboratory, we've developed cutting-edge screening technologies to monitor the activity of the PI3K/AKT/FOXO signaling pathway. Utilizing these tools, we've identified and characterized numerous genetic and pharmacological approaches to manipulate the activity of FOXO proteins. One of our most significant breakthroughs came with the discovery of the FOXO repressor protein TRIB2 as a novel oncogene in melanoma. TRIB2 belongs to the Tribbles family of pseudokinases and plays a critical role in conferring resistance to anticancer drugs through direct interaction with AKT. Notably, TRIB2 is often overexpressed in melanoma and is associated with a poor response to treatment.

Our research uniquely positions us to translate our insights into TRIB2 biology into practical tools that can enhance the clinical outcomes for melanoma patients and those with other solid cancers. We also explore the pharmacological modulation of FOXO proteins as a strategy for treating cancer and age-related diseases. Our research group possesses an exceptional resource, with a collection of over 200 small chemical compounds capable of activating FOXO factors. From this collection, several potential anti-cancer drug candidates have been developed.

In an effort to harness the therapeutic potential of these small molecule FOXO modulating compounds, we founded Refoxy Pharma, a biotech company with locations in Berlin and Boston (www.refoxy.com). Additionally, we're working on developing Nuclear Export Inhibitors as potential anti-cancer drugs. Our team has devised a multiplexed high-content screening platform for the systematic evaluation of small molecule inhibitors of nuclear export."

Research lines:

1. Understanding and targeting of FOXO transcription factors in cancer and aging

2. Role of TRIB2 protein in solid cancers

3. Development of CRM1 inhibitors for anticancer and antiviral therapy

Understanding and targeting of FOXO transcription factors in cancer and aging

Researchers involved: Link Wolfgang, Jiménez Gómez Lucía, Sastre Arcones, Marta

FOXO3 is a transcription factor responsible for coordinating gene expression programs essential for cellular responses to stimuli such as oxidative, metabolic, and genotoxic stress. As a result, FOXO3 has gained recognition as a potential target for drug development and geroprotectors. It is one of the two human genes consistently linked to extreme longevity in various populations. In many cases, FOXO3 is inactivated in human cancers due to cytoplasmic retention. Our goal is to comprehend the isoform-specific regulation of FOXO3 and explore methods for pharmacologically activating FOXO3.

Role of TRIB2 protein in solid cancers

Researchers involved: Link Wolfgang, Mayoral Varo, de la Rocha Muñoz, Andrés, Amenábar Blázquez, Carlos, Mansi Mashhour, Neveen, Ruiz Martí, Alicia, Kyriakou, Panagiota

Together with TRIB1 and TRIB3, TRIB2 is part of the well-conserved mammalian Tribbles family of proteins. Our group initially identified TRIB2 in a genetic screen with the goal of discovering inhibitory proteins for FOXO transcription factors. We characterized TRIB2 as an oncogene in melanoma and as a biomarker to both diagnose and evaluate melanoma progression and to predict clinical responses to cancer therapies. Our research focuses on comprehending the role of Tribbles proteins, especially TRIB2, in the progression of melanoma and other solid cancers, and in the development of TRIB2 inhibitors.

Development of CRM1 inhibitors for anticancer and antiviral therapy

Researchers involved: Link, Wolfgang, Jiménez Gómez Lucía, Mourato Paulo, Cristiana

Nuclear export receptor CRM1 binds nuclear export signals (NESs) present in many cellular and viral proteins. The novelty of our approach consists in attacking CRM1 that helps cancer cells to inactivate tumor-suppressive proteins by transporting them from the cell nucleus to the cytoplasm. We develop a technology to identify new chemical compounds capable of blocking the activity of CRM1 in a manner that produces less toxicity than available inhibitors which poison the CRM1 protein. This approach is also relevant for the development of antiviral drugs.

PUBLICATIONS:

Masci D, Ling L, Yang L, Puxeddu M, Colla C, Coluccia A, Santelli M, Sciò P, Cuřínová P, Ansari MSZ, Naro C, Sette C, Jimenez L, Link W, Bigogno C, Dondio G, Hamel E, Liu T, Silvestri R, La Regina G. Ferroptosis Induction by a New Pyrrole Derivative in Triple Negative Breast Cancer and Colorectal Cancer. *J. Med. Chem.*, **2025**, *68, 16,* 17840–17858. DOI: 10.1021/acs.jmedchem.5c01561.

Princiotto S., Jiménez L., Domínguez L., Sequeira J.G.N., Mourato C., Orea-Soufi A., Santos B, Dallavalle S., Machuqueiro M., Ferreira B.I. and Link W. Chromenone derivatives as CRM1 Inhibitors for Targeting Glioblastoma. *Chembiochem*. **2025**, e202500195. DOI: 10.1002/cbic.202500195.

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Santos B.F., Maia A.T., Ferreira B.I. and Link W. Combined expression of FOXO and Tribbles proteins predicts survival of glioma patients *BioRxiv.* **2025**, DOI: https://www.biorxiv.org/content/10.1101/2025.01.20.633940v1.

Ferreira B.I. and Link W. FOXO Transcription Factors: A Brief Overview. *Methods Mol Biol.* **2025**, *2871*, 1-8. DOI: 10.1007/978-1-0716-4217-7_1.

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Cancela M.L., Alouane A., Bertelli PM., Camacho A., Derudder R, Forlino A., Harris MP., Jacinto M., Lengyel I., Link W., Murshed M., Pasch A., Kaliya-Perumal A.K., Quaglino D., Qin Z., Sabbagh Y., Seminari E., Villar M.M., Winkler C and Vanakker O.M. 2024 Annual Meeting of the International Network on Ectopic Calcification (INTEC) *Gout Urate Cryst. Depos. Dis*. **2025**, *3*, 14. doi.org/10.3390/gucdd3030014

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Amenabar C., Jimenez L., Mourato C., Mayoral-Varo V., Megías D., Ferreira B.I. and Link W. Multiplexed Dual-Color Fluorescence-Based Distinction between Nuclear Trapping and Translocation of FOXO3. *Methods Mol Biol.* **2025**, *2871*, 163-170. DOI: 10.1007/978-1-0716-4217-7_15.

Jimenez L., Domínguez L., Amenabar C., Serrão G., Link W., Ferreira B.I., and Santos B. Immunocytochemistry-Based Detection of FOXO Isoforms in Human Cancer and Fibroblasts. *Methods Mol Biol.* **2025**, *2871*, 171-176. DOI: 10.1007/978-1-0716-4217-7_16.

DOCTORAL THESES AND OTHER WORKS:

Neveen Mansi Mashhour
Master´s thesis. *Characterization and Modulation of the TRIBbles/AKT/FOXO Axis in Melanoma (TAFmelanoma)*. Universidad Autónoma de Madrid. 2025. Supervisor: Wolfgang Alexander Link Grösslinger

Alicia Ruiz Martí
Master´s thesis. *Caracterización de la regulación de TRIB2 y FOXO3 sobre la vía Wnt/beta-catenina en melanoma.* Universidad Complutense de Madrid. 2025. Supervisors: Lucía Jiménez Gómez and Wolfgang Alexander Link Grösslinger

Sara de la Calle Santiago
Final degree's project. *Papel de la regulación de SFRP1 mediada por TRIB2 sobre las propiedades oncogénicas del melanoma.* Universidad Autónoma de Madrid. 2025. Supervisors: Lucía Jiménez Gómez and Wolfgang Alexander Link Grösslinger

FUNDING:

Characterizing and modulating the Tribbles/ AKT/FOXO axis in melanoma (TAFmelanoma) Financing entity: Ministerio de Ciencia e Innovación Reference: PID2022-136654OB-I00. 2023 – 2026 Principal Investigator: Wolfgang Alexander Link Grösslinger.

OUTREACH ACTIVITIES:

https://www.csic.es/en/node/167858

https://iib.uam.es/en/iibm-society/-/asset_publisher/5om71ijE6EQi/content/refoxy-pharma-co-founded-by-wolfgang-link-secures-%E2%82%AC9.1-million/227928

department of Metabolic & Immune Diseases

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MicroRNAs in immune tolerance, autoimmunity and cancer

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KEYWORDS
B cell tolerance, Tumor immunology, Autoimmune diseases, microRNAs, T cell responses.

RESEARCH LINES:

Overview

Our laboratory investigates the cellular and molecular mechanisms underlying autoimmune diseases and cancer, with a central focus on immune tolerance. In particular, we study the role of microRNAs (miRNAs) and their target genes in regulating immune responses. These small regulatory RNAs orchestrate gene networks that are essential for maintaining self-tolerance, preventing autoimmunity, and enabling effective antitumor immunity. By identifying and characterizing novel regulatory mechanisms, we aim to advance our current understanding of adaptive immunity and contribute to the development of new biomarkers and therapeutic strategies for autoimmunity and cancer.

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Regulatory mechanisms of B cell tolerance and autoimmunity

Researchers involved: González-Martín, Alicia; Lacadena Díaz-Varela, Jaime; Gámez Reche, Laura; Tapia Narváez, Juan Carlos; Herrero Fernández, Beatriz; Moreno Jerez, Alba; Gallego Alonso, Lucía.

This research line focuses on deciphering how miRNAs regulate B cell tolerance. Over the past months, we have investigated miRNA networks controlling B cell tolerance. We identified a novel miRNA that regulates this process and elucidated its mechanism of action. Through integrative analysis of downstream effectors within the miRNA network, we also uncovered a previously unknown regulatory mechanism that modulates B cell tolerance in a sex-dependent manner. In addition, we conducted a systematic analysis of a specific miRNA targetome, which showed that it regulates B cell tolerance through a remarkably small number of target genes, a fundamental question largely debated in the field with relevant therapeutic implications.

MicroRNA control of adaptive immunity in cancer

Researchers involved: González-Martín, Alicia; Mendieta Homs, Marina; Cordero Pedrero, Carla; Jiménez Sánchez, Ana; Calderón Sacristán, Rocío; Burgos Díaz, Lucía.

The objective of this line of research is to establish how miRNAs regulate adaptive immunity to tumors and harness their activity to improve cancer immunotherapy. Over the past period, we have identified a specific miRNA that suppresses tumor progression in preclinical models of lung cancer and melanoma through its effects on T lymphocytes, and characterized its cellular and molecular mechanisms of action. Notably, we have also established that elevated levels of this miRNA at the tumor site correlate with a more favorable prognosis in patients with these malignancies, through bioinformatic analyses of public datasets. Furthermore, we conducted an in vivo functional screen that identified a different miRNA that promotes tumor growth, currently under investigation.

PUBLICATIONS:

Mastrangelo, A.; Robles-Vera, I.; Mañanes, D.; Galán, M.; Femenía-Muiña, M.; Redondo-Urzainqui, A.; Barrero-Rodríguez, R.; Papaioannou, E.; Amores-Iniesta, J.; Devesa, A.; Lobo-González, M.; Carreras, A.; Beck, K.R.; Ivarsson, S.; Gummesson, A.; Georgiopoulos, G.; Rodrigo-Tapias, M.; Martínez-Cano, S.; Fernández-López, I.; Nuñez, V.; Ferrarini, A.; Inohara, N.; Stamatelopoulos, K.; Benguría, A.; Cibrian, D.; Sánchez-Ma-

drid, F.; Alonso-Herranz, V.; Dopazo, A.; Barbas, C.; Vázquez, J.; López, J.A.; González-Martín, A.; Nuñez, G.; Stellos, K.; Bergström, G.; Bäckhed, F.; Fuster, V.; Ibañez, B.; Sancho, D. Imidazole propionate is a driver and therapeutic target in atherosclerosis. *Nature*. **2025**, *645(8079)*:254-261. https://doi.org/10.1038/s41586-025-09263-w

DOCTORAL THESES AND OTHER WORKS:

Juan Carlos Tapia Narváez
Master´s thesis. *The role of microRNAs in the regulation of immune tolerance and autoimmunity.* Universidad Autónoma de Madrid. 2025. Supervisor: Alicia González Martín.

Rocío Calderón Sacristán
Final degree's project. *Caracterización funcional de miRNAs y sus genes diana en inmunidad antitumoral.* Universidad Autónoma de Madrid. 2025. Supervisor: Alicia González Martín. Grade: Sobresaliente (9.8/10).

FUNDING

Systematic analysis of tumor-specific B cell immunity. CNS2022-136069. Ministry of Science and Innovation. 2023-2025. PI: Alicia González Martín.

Use of miRNAs for cancer immunotherapy. XXI National Call for Research Grants in Life Sciences. Ramón Areces Foundation. 2023-2026. PI: Alicia González Martín.

MicroRNA regulatory networks in B cell tolerance and autoimmunity. PID2021-128244OB-I00. Ministry of Science and Innovation. 2022-2026. PI: Alicia González Martín.

Harnessing microRNAs for lung cancer immunotherapy. XXIII Beca FERO. FERO Foundation. 2022-2025. PI: Alicia González Martín.

Achieving Long-Term Humoral Protection Against HIV and other Antibody Resistant Pathogens. Bill and Melinda Gates Foundation. 2019-2025. PI: Alicia González Martín.

PATENTS:

MicroRNAs for use in the stimulation of T cell antitumor responses. González-Martín A, González-Molina MdP. International Patent PCT/EP2024/064900; EP24728247. 2025.

Physiopathology and Molecular Mechanisms of Obesity and Comorbidities

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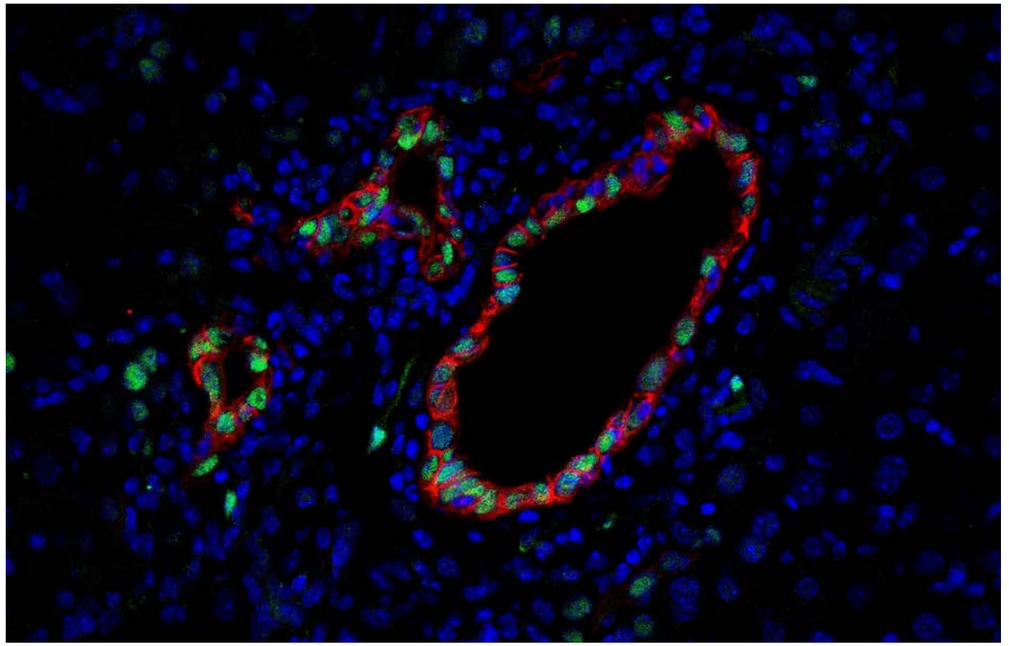
KEYWORDS

Obesity, Type 2 diabetes, Metabolic dysfunction-associated steatotic liver disease, Vascular risk, Extracellular vesicles, Novel Food, products, Second generation antipsychotics



RESEARCH LINES:

Overview

Obesity is a global problem: over 40% of the worldwide population is currently affected by overweight or obesity, and forecasts estimate that this prevalence will continue to increase in the coming years. The close relationship between metabolism and the immune system (immunometabolism) plays an essential role in the development of obesity and related comorbidities, including type 2 diabetes mellitus (T2D), metabolic dysfunction-associated steatotic liver disease (MASLD) and Obstructive Sleep Apnea (OSA). Among the tissues involved in metabolic control, the liver is a target organ for the proinflammatory mediators from the gut (endotoxins) and/or adipose tissue (cytokines, adipokines, free fatty acids and reactive lipid species) and, furthermore, this organ capable of recruiting circulating monocytes that, together with the resident macrophages (Kupffer cells), contribute to exacerbate the intrahepatic inflammatory response. These conditions determine

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the progression of MASLD, a disease with a high incidence in the obese and insulin-resistant population that begins with accumulation of fat in the liver (steatosis) and progresses to steatohepatitis (MASH), fibrosis, cirrhosis, and ultimately, hepatocellular carcinoma (HCC). Our group investigates the molecular bases of the development of obesity and comorbidities, with a major focus in the liver, adipose tissues (white and brown) and pancreas. To achieve this, we use cellular models (i.e. hepatocytes, Kupffer cells, stellate cells, liver progenitor cells, endothelial cells, pancreatic islets), as well as preclinical experimental models that recapitulate obesity and the different stages of MASLD. In this context, we are also studying therapeutic approaches with peptides multiagonists of incretin receptors and inhibitors of IL1β, to prevent or reverse obesity and MASLD. Other pharmacological targets of interest are the protein kinase D family, the BMP (bone morphogenetic factors) family, hypoxia-inducible factors (HIFs), as well as extracellular vesicles. We are also investigating metabolic changes induced by chronic treatment with widely used drugs (antipsychotics) or the benefit of novel foods (insect meal) on incretin secretion and glycemic control. Regarding liver pathophysiology, our group aims to decipher new mediators and mechanisms involved in acute liver failure (ALF), as well as in biliary diseases including primary biliary cholangitis (PBC) and primary sclerosing cholangitis (PSC).

Novel regulatory nodes of acute and chronic liver diseases: protein kinase D2 (PKD2)

Researchers involved: Elena Carceller, Águeda González Rodríguez, Teresa Iglesias, Ángela Martínez Valverde, Patricia Rada,

Protein kinase D family (PKD1, PKD2, PKD3) is emerging as relevant regulator of metabolic homeostasis in a tissue-dependent manner. However, the precise role of PKD2 in hepatic diseases has not been fully elucidated and is the focus of this research line. The general objective of this research line is to unravel an unknown role of PKD2 as a key player in the inflammatory processes associated with acute or chronic liver damage linked to pathologies of high incidence in humans. Taking into account our previous data, we hypothesize that modulation of PKD2 may impact on the inflammatory processes in the liver upon acute or chronic damage. To address this challenge, mice with specific PKD2 deficiency in immune cells of the myeloid compartment (PKD2ΔLysM) have been generated to be used in the following objectives: 1. Investigate the role of PKD2 in inflammatory responses associated with acute drug-induced liver injury induced by APAP in PKD2ΔLysM mice and their corresponding PKD2fl/fl control mice. 2. Evaluate the progression of MASLD in an experimental model of non-alcoholic steatohepatitis (NASH) in PKD2ΔLysM mice and their corresponding PKD2fl/fl control mice. 3. Investigate the involvement of PKD2 in liver inflammation associated to biliary damage in a preclinical model that resembles PSC features. 4. Conduct in vitro mechanistic studies on the effect of PKD2 deletion in myeloid cells on hepatocyte survival in the aforementioned pathological contexts.

Emerging role of the liver progenitor cells (LPCs) in chronic liver diseases

Researchers involved: Silvia Calero, M. Pilar Valdecantos, Laura Villamayor, Elena del Fresno, Ana B. Hitos, Águeda González-Rodríguez, Ángela Martínez Valverde

Activation of LPCs has been implicated in a regenerative response of the liver during chronic liver diseases (CLDs), including steatohepatitis (MASH) and cholangiophaties such as primary sclerosing cholangitis (PSC). In this study our goal was to analyze the benefits of deleting the protein tyrosine phosphatase 1B (PTP1B) in favoring oxidative metabolism and survival in LPCs, as well as in expanding the LPC niche in mice with MASH or PSC. Transcriptomic analysis revealed changes in several molecular pathways in PTP1B -/- LPCs, including upregulation of oxidative metabolism and hepatocyte-related genes and downregulation of apoptosis-related genes. Ptpn1 deletion in LPCs enhanced survival signaling, induced NRF2 nuclear translocation and its antioxidant targets, improved mitochondrial bioenergetics and reduced apoptosis upon treatment with palmitic acid or macrophage-derived lipo-inflammatory conditioned medium (CM). In a preclinical model of MASH, mice transplanted with PTP1B+/+ or PTP1B-/- LPCs had more hepatic glycogen. Importantly, mice transplanted with PTP1B-/- LPCs showed better engraftment, reduced transaminase activity and higher serum albumin levels compared with mice receiving transplantation of PTP1B+/+ LPCs. On the other hand, stimulation of LPCs with a PSC-like CM from PTP1B -/- macrophages increased survival signaling and proliferation. In mice with PSC, PTP1B deletion in the immune compartment promoted LPC proliferation, favored the recruitment of anti-inflammatory immune cells in the liver, decreased pro-inflammatory populations and protected mice against biliary injury. Therefore, targeting PTP1B may open new therapeutic perspectives to enhance liver regeneration in CLDs by improving LPC plasticity.

Small extracellular vesicles (sEV): new messengers of the paracrine and endocrine intreactome inMASLD and T2D with diagnostic potential

Researchers involved: Irma García-Martínez, Héctor Cañeque, Sofía Rodríguez, Manuel Izquierdo-Pastor, Ángela Martínez Valverde

MASLD is a common feature of obesity and type 2 diabetes. Under lipotoxic stress, hepatocytes release small extracellular vesicles (sEV) which act locally and contribute to MASLD progression, but their role in beta cell function and development of type

2 diabetes mellitus (T2DM) remains largely unexplored. We aim to examine whether hepatocyte-derived sEVs (Hep-sEV) under lipotoxic conditions impact on liver and pancreas inflammation and subsequent effects in beta cell function. Our results revealed that lipotoxic Hep-sEV targeted pancreatic islet macrophages and induced Toll-like receptor 4 (TLR4)-mediated inflammation. The subsequent inflammatory response down-regulated beta cell identity genes and impaired glucose-stimulated insulin secretion (GSIS) in both INS-1 beta cells and isolated pancreatic islets. Specific deletion of TLR4 in macrophages protected pancreatic islets against inflammation and the impairment of GSIS induced by lipotoxic Hep-sEV. Chronic administration of lipotoxic Hep-sEV in lean mice induced liver and pancreas inflammation through the recruitment of immune cells. This intervention induced hepatocyte injury and fibrotic damage together with detrimental immunometabolic systemic effects. Insulin resistance in hepatocytes and a compensatory insulin secretion that prevented glucose intolerance was also observed in mice treated with lipotoxic Hep-sEV. This study has provided evidence of liver and pancreas inflammation and beta cell dysfunction induced by lipotoxic Hep-sEV. Our data also envision TLR4-mediated signaling in islet macrophages as a key mediator of the effects of lipotoxic Hep-sEV on beta cell function.

Therapies based on incretin receptor multiagonism to combat obesity and co-morbidities.

Researchers involved: Beatriz Herrero, M. Pilar Valdecantos, Patricia Rada, Karol M. Artunduaga, Ángela Martínez Valverde.

Bariatric surgery is effective for the treatment and remission of obesity and type 2 diabetes, but pharmacological approaches which exert similar metabolic adaptations are needed to avoid post-surgical complications. This research line is devoted to investigate the effects of peptides multiagonists of incretin receptors in preventing diet-induced obesity and its underlying molecular mechanisms. We are particularly focused in the spatio-temporal metabolic rewiring in response to these molecules that involves an inter-organ crosstalk between relevant metabolic tissues. We have demonstrated how G49, an oxyntomodulin (OXM) analog and dual glucagon/glucagon-like peptide-1 receptor (GCGR/GLP-1R) agonist, triggers an inter-organ crosstalk between white adipose tissue (WAT), pancreas, and liver which is initiated by a rapid lipolytic response of WAT in a GCGR-dependent manner. This interactome leads to elevations in adiponectin and fibroblast growth factor 21 (FGF21), causing WAT beiging, brown adipose tissue (BAT) activation, increased energy expenditure and weight loss. We are now investigating the sustainability of G49 treatment in maintaining weight loss and its effect in thermogenic brown adipocyte differentiation. Additionally, in collaboration with Pep2Tango Therapeutics (MD, USA) we are currently investigating the efficacy of next generation incretin receptor multiagonists in reducing obesity without altering muscle mass and the tissue-specific actions.

Metabolic side-effects of long treatment with second generation antipsychotics: impact of the hypothalamic-liver crosstalk in insulin sensitivity.

Researchers involved: Vitor Ferreira, Ana B. Hitos, Ángela Montes, Patricia Rada, Ángela Martínez Valverde.

This research line evaluates the metabolic side effects of second generation antipsychotics (SGAs), particularly olanzapine (OLA), in energy balance and glucose/lipid metabolism in mice treated with this drug. Recently, we demonstrated that the oral treatment of male mice with OLA induces weight gain and hepatic steatosis. However, mice receiving OLA via intraperitoneal (i.p.) lost weight, an effect related to higher OLA levels reaching the hypothalamus and activation of brown adipose tissue. Since clinical studies have shown insulin resistance in individuals under OLA treatment, this research line investigates the impact of the i.p. treatment on insulin sensitivity, particularly in the crosstalk between liver and skeletal muscle. OLA i.p. treatment induces systemic insulin resistance, pyruvate intolerance and attenuates insulin signaling in both liver and skeletal muscle. These effects concurred with enhanced JNK phosphorylation and IRS1 serine phosphorylation in the liver. Similar diminished peripheral insulin response was found 48 h after a single OLA intrahypothalamic injection, that was also preceded by activation of hepatic JNK and elevation of IRS1 serine phosphorylation. Deletion of either hypothalamic or hepatic JNK1, as well as surgical vagotomy, prevented OLA-induced impairments in insulin signaling in liver and skeletal muscle. Importantly, downregulation of hepatic Fgf21 was found in mice treated with OLA via i.p. or receiving an intrahypothalamic injection. This decrease was prevented by deletion of hypothalamic JNK1. A step further, hepatic FGF21 overexpression abolished OLA-induced insulin resistance in skeletal muscle, but not in liver, pointing OLA-induced impairment in skeletal muscle insulin signaling secondary to the hepatic alterations. This interplay between the hypothalamus-liver-skeletal muscle was absent in OLA-treated mice with global deletion of PTP1B that were protected against insulin resistance. Despite of the beneficial effect of OLA administered via i.p. in avoiding weight gain, it reduces peripheral insulin sensitivity through a hypothalamus-liver axis controlled by hypothalamic JNK1 activation which, in turn, activates hepatic JNK via the vagus nerve. This axis results in hepatic insulin resistance and a reduction of both hepatic and circulating FGF21 levels that ultimately

impairs insulin signaling in the skeletal muscle. Importantly, the protection conferred by PTP1B deficiency against OLA-induced insulin resistance strongly suggests that targeting PTP1B might prevent metabolic comorbidities in patients under OLA treatment in a personalized manner.

Effect of novel food products derived from insects in incretin and insulin sensitivity: DiBaN EIC-Pathfinder

Researchers involved: Silvia Calero, Ángela Montes-San Lorenzo, Ángela Martínez Valverde.

Poor nutritional regimes are main drivers of metabolic diseases and intestinal dysbiosis. Type 2 diabetes (T2D) prevalence is expected to reach 7% of the global population by 2023, and to curb its rise is an urgent public health need. Intestinal dysbiosis is a determinant factor in the progression of insulin resistance to T2D, shifting host metabolism though undefined mechanisms whose understanding would be crucial to allow personalized interventions. Advances have been limited by microbiome complexity and inter-individual variance. DiBaN EIC-Pathfinder project binds together the necessary combination of expertise to address this question, based on the concept that the initial driver of the nutritional effects is the metabolic shift that takes place in the intestinal bacteria which is transduced to the host. In this context, technological breakthrough tools for novel food development,that ensure the promotion of a healthy microbiome-host metabolic interface, are an urgent need to prevent dysbiosis and T2D. DiBaN project aims to overcome current limitations in nutrient-testing by developing advanced ex-vivo platforms that fully recapitulate the in vivo setting of dysbiosis and insulin resistance that will be validated with in vivo omic data. To warrantee the health promoting effects of novel foods we will test a new concept in the emerging field of insect food technology, that an adequate insect's metabolic-intestinal health ensures the healthy properties of its derived products. The validation of this idea will also serve to identify biomarkers for the monitorization of the insect's health status. Accheta domesticus will be the testing model of choice, due to its excellent nutritional profile, that will be boosted by complementing the insect's diet with microalgae bioactive-rich extracts. Our particular contribution to this project will be focused on deciphering the impact of A. domesticus-derived products in modulating insulin sensitivity in metabolic tissues, whole-body glucose homeostasis, energy balance and incretin secretion.

Impact of Bone Morphogenetic Proteins (BMPs) on the progression of MASLD and its associated vascular risk.

Researchers involved: Raluca Alexandra Pirvan, Patricia Rada, Ángela Martínez Valverde, Águeda González-Rodríguez

Metabolic dysfunction-associated steatotic liver disease (MASLD) is considered the hepatic manifestation of metabolic syndrome and encompasses a series of liver lesions of progressive severity. It is also an important risk factor for the development of cardiovascular disease (CVD).

Recently, bone morphogenetic protein 2 (BMP2) has been identified as a potential biomarker for the non-invasive diagnosis of MASLD, and, as it is crucial to understand the mechanisms involved in the development of this disease and the associated vascular complicationsassociated with it, the objective of this study is to investigate the role of this BMP2 protein in vascular damage associated with MASLD.

Results from a clinical study suggest that BMP2 is a potential serum biomarker for the diagnosis of both hepatic steatosis and CVD, and results from the experimental study reveal that this BMP exerts proatherosclerotic effects on vascular smoot muscle cells (VSMCs). In fact, the conditioned medium generated by hepatocytes treated with palmitic acid (CM-PA) also induces the migration capacity of these vascular cells. When analyzing the expression of BMP2 in

PA-treated hepatocytes, an increase was observed, in parallel with its release into CM-PA. In addition, the BMP2 protein produced by hepatocytes is conveyed by exosomes. Taken together, these results suggest that this BMP protein might be key in hepato-vascular communication, so currently, we are evaluating BMP2 effects on an in vivo experimental model.mouse model (mice fed with high fat choline-deficient diet, CDAA).

Modulation of IL1β synthesis as a potential therapeutic target for MASLD progression.

Researchers involved: Ángela Berlana San Segundo,Carolina Pérez Utrera, Elena del Fresno, Águeda González-Rodríguez

The mechanism underlying MASLD progression is influenced by a wide variety of factor. One of them is inflammation, which appears in the early stages of the disease, and its reversal is crucial, as prolonged persistence of this inflammatory state leads to chronic tissue damage and fibrosis. Regarding proinflammatory signals involved in the disease progression, IL1β is involved in different stages of the disease, including the promotion of hepatic steatosis, inflammation, and fibrosis. Given its central role in MASLD progression, IL1β represents a potential therapeutic target. AIK3a305 (AIK), a novel allosteric inhibitor of JNK, is a potent selective inhibitor of IL1β production. Thus, the aim of this research line is to evaluate the effect of the pharmacological inhibition of this cytokine triggered by this compound on MASLD progression. By using different cellular systems and a mouse model of MASLD, we have demonstrated that

AIK selectively reduces LPS and palmitate (PA)-induced IL1β synthesis in macrophages, blocking their proinflammatory activation. Moreover, AIK protects against PA-induced lipotoxicity in hepatocytes by inhibiting JNK signalling induced by this fatty acid, and, selectively, IL1β synthesis. In addition, treatment with this compound reduced hepatic stellate cell activation. In addition, the results of the preclinical study revealed that AIK ameliorates MASLD progression, reducing intrahepatic lipid accumulation and fibrosis in a MASLD mouse model (mice fed with high fat choline-deficient diet, CDAA).

Evaluation of metabolic and cardiovascular complications associated with chronic respiratory diseases.

Researchers involved: Polet Barrionuevo Carmilema, Carolina Pérez Utrera, Águeda González-Rodríguez

Intermittent hypoxia, a main characteristic of Obstructive Sleep Apnea (OSA), has been implicated in the pathogenesis of MASLD, although its molecular influence is not completely understood. This research line aims to investigate the relationship between intermittent hypoxia (IH) and MASLD. This study comprised a clinical and an experimental analysis. In the clinical study, several parameters were evaluated in control subjects and in OSA patients, revealing that OSA patients were older and men often, had higher levels of fasting glucose, HOMA-IR and triglycerides. The prevalence of hepatosteatosis, according to the Fatty Liver Index (FLI) and metabolomic test, was significantly higher in OSA patients than in controls. In the histological study, the livers of mice subjected to an intermittent hypoxia protocol exhibited lipid accumulation, oxidative stress, and inflammation, along with alterations in lipid metabolism at the molecular level: induction of lipogenesis and impairment of free fatty acid oxidation. Currently, the impact of normalization of oxygen levels on OSA-associated comorbidities, including, MASLD is being studied.

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Platelet C3G protects from liver fibrosis, while enhancing tumor growth through regulation of the immune response. *J Pathol.* **2025**, *265(4):*502-517. DOI: 10.1002/path.6403.

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DOCTORAL THESES AND OTHER WORKS:

Silvia Calero Pérez.
Ph.D. thesis. *Role of the protein tyrosine phosphatase 1B in the interactome between liver progenitor cells and the immune compartment in chronic liver diseases.* Universidad Autónoma de Madrid. 2025. Supervisors: Ángela Martínez Valverde, M. Pilar Valdecantos Jiménez de Andrade. Grade: Sobresaliente Cum Laude.

Stephania Isaza Chávez.
Ph.D. *Impact of Bone Morphogenetic Proteins on the progression of comorbidities associated with Metabolic Syndrome.* Universidad Autónoma de Madrid. 2025. Supervisor: Águeda González Rodríguez. Grade: Sobresaliente Cum Laude.

Polet Barrionuevo Carmilema.
Master´s thesis. I*mpact of extracellular vesicles on vascular atherosclerotic damage associated with metabolic dysfunction-associated steatohepatitis (MASLD).* Universidad Complutense de Madrid. 2025. Supervisor: Águeda González Rodríguez. Grade: Sobresaliente.

Nerea Ugarte Hurtado.
Final degree's project. *Functional analysis of protein phosphatase 1B inhibition in MASLD metafolic context: an in vitro study*. Universidad Francisco de Vitoria. 2025. Supervisor/s: Ángela Martínez Valverde, Silvia Calero Pérez. Grade: Sobresaliente.

Raluca Alexandra Pirvan.
Final degree's project. *Search for new molecular targets related to MASLD and associated vascular damage.* Universidad Autónoma de Madrid. 2025. Supervisor: Águeda González Rodríguez. Grade: Sobresaliente.

FUNDING:

Linking Intestinal Bacteria and Host Metabolism to Tackle Type 2 Diabetes with Novel Food (DIBAN). Horizon-EIC-Pathfinder-Challenge-2023. Grant Agreement number 101162517. 2024-2028. PIs (coordinators): Ángela Martínez Valverde, María Monsalve Pérez.

Role of Protein Kinase D2 in the inflammatory response of the liver (PKD2-INFLIVE). PID2023-150994OB-I00. AEI (MICIU). 2024-2026. PI Patricia Rada Llano.

Avanzando en el conocimiento de nuevos mediadores, mecanismos e interactoma tisular en situaciones de resistencia a la insulina (MOIR-ACTOME-CM). P2022/BMD-7227. Comunidad de Madrid. 2023-2026. PI: Ángela Martínez Valverde.

Obesity and comorbidities: advances in the research on tissue/cell interactomes in its development and therapeutic interventions (OBE-INT). PID2021-122766OB-I00. AEI (MICIU). 2022-2026. PI: Ángela Martínez Valverde,

Assessment of progression risk to type 2 diabetes in obese patients. Novel inflammatory molecules and extracellular vesicles as biomarkers in wellphenotypically characterized subjects using an innovative digital monitoring platform (RENDITIONS). CIBER de Diabetes y Enfermedades Metabólicas Asociadas (CIBERdem,ISCIII). 2024-2026. PI: Ángela Martínez Valverde.

Experimental design for studies on multiagonist peptides in obesity and metabolic diseases. Pep2Tango Therapeutics. 2021-2025. PI: Ángela Martínez Valverde.

Impact of bone morphogenetic protein 12 on NAFLD progression and the associated-vascular atherosclerotic damage. ISCIII. 2023-2025. PI: Águeda González Rodríguez.

Evaluation of the inhibitory capacity of Allinky compounds in non-alcoholic fatty liver disease (NAFLD). Allinky Biopharma. From 2025. PI: Águeda González Rodríguez.

Assessment of precision medicine-based algorithms for prediction of NASH and advanced liver fibrosis (NIT NASH) Pfizer, S.A. 2024-2025. PI: Águeda González Rodríguez.

AWARDS:

Best communication presented by a young researcher in Molecular Basis of Pathology in SEBBM Congress. Cáceres, September 2025.

OUTREACH ACTIVITIES:

Día Internacional de la Mujer y la Niña en la Ciencia “Como investigar en obesidad y sus complicaciones en diferentes etapas de la carrera investigadora: el apasionante día a día”. Colegio Internacional Altair (Madrid). February, 2025.

Participation in the “Científicos en Prácticas” program (CSIC), June 2025.

Ruta científica en el IIBM. Bioimagen aplicada en biomedicina. November, 2025.

Semana de la Ciencia. Taller de Investigación Traslacional: línea directa entre el laboratorio y el paciente. November, 2025.

Beta Cell Mass and Pancreatic Islet Development

TENURED SCIENTIST

Bartolomé Herranz, Alberto
(Científico Titular, CSIC). Group Coordinator

PRE-DOCTORAL SCIENTIST

Matas Aguado, Diego
Hernanz Martín, Mario
García de la Fuente, Marta

MASTER THESIS STUDENT

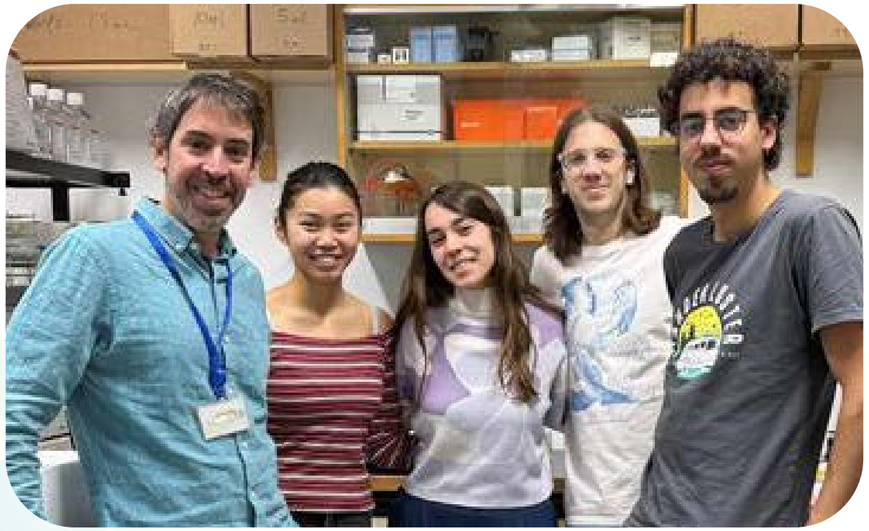
López Poveda, Elia He

UNDERGRADUATE STUDENT

Sopranis Pérez, Carmen
Gonzalo Rodríguez, Cristina María

KEYWORDS

Diabetes, Pluripotent stem cells, Differentiation protocols, Pancreatic beta cells, Insulin.



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RESEARCH LINES:

Overview

β cell mass plays a pivotal role in type 2 diabetes progression, with decreased mass linked to reduced insulinaemia, glucose intolerance, and diabetes onset. Notably, β cell mass exhibits considerable heterogeneity across individuals, but current clinical tools fall short of effectively measuring it. Predictive genetic information could serve as a valuable tool for efficient diabetes diagnosis, treatment, and prevention, and aid in patient stratification in this era of personalized medicine. In our quest to understand diabetes better, we probe genetic variants that escalate diabetes risk in both its monogenic and polygenic forms. Given that animal models often fail to faithfully represent human diabetes phenotypes linked with these genetic modifications, we rely on the use of alternative models to explore human genetics further.

Our lab specializes in modeling human endocrine pancreas development through the use of **pluripotent stem cells** and differentiation protocols steered towards the endocrine lineage. By merging this approach with the genome-editing power of **CRISPR/Cas9** technology and comprehensive "omics" methodologies, we can decode the molecular characteristics of human genetic variants in pancreatic development. Our ultimate goal is to unveil the influence of these variants, a step that could significantly aid in patient stratification and preemptive diagnosis. Furthermore, understanding novel disease effectors may open doors to innovative therapies for both rare and common forms of diabetes.

Unraveling the genetic basis of human β cell mass by the study of diabetes risk loci

Adult beta cell mass is determined by the size and proliferation of the pancreas progenitor pool. Our focus lies in examining type 2 diabetes risk loci and discerning the influence of specific genes on the proliferation of pancreatic progenitors and the trajectory of endocrine differentiation. Utilizing "loss-of-function" approaches, we investigate these genes. After genetic perturbation in pluripotent stem cells, we apply differentiation protocols that emulate human development. Our primary aim is to elucidate the molecular link between diabetes risk and single-nucleotide polymorphisms by integrating GWAS, eQTL databases, and "omics" data from differentiation protocols. The ultimate objective is clinical translation of our findings, connecting genetic data to pathophysiological events and accelerating the advent of personalized medicine.

Study of novel genes and mutations putatively associated with monogenic diabetes

Monogenic diabetes, accounting for 1-5% of all diabetes cases, is often underdiagnosed

and under-researched. Recognized monogenic variants are predominantly linked with genes vital for endocrine pancreas development. Given that animal models fall short of replicating most human diabetes phenotypes linked with these genetic modifications, we need alternative models for probing human genetics and deepening our understanding of monogenic diabetes.

We study clinically relevant genetic alterations putatively linked with monogenic diabetes. Our proposed studies can help determine if observed clinical phenotypes arise from defective endocrine development or abnormal mature β cell function. Furthermore, they shed light on associated molecular mechanisms – invaluable insights for improving diagnosis and treatment modalities for these patients.

PUBLICATIONS:

Mathieu, C.; Meireles, M.; Pagotto, U.; Wabitsch, M.; Banerjee, I.; Bartolomé, A.; Battelino, T.; Beck, J.; Chiarelli, F.; De Leon, D. D.; Dovč, K.; El Ghoch, M.; Galderisi, A.; Gevers, E.; Gillard, P.; Haliloglu, B.; Hoermann, H.; Mankovsky, B.; Mertens, J.; Mohnike, K.; Oram, R.; Pasquini, T.; Pearson, E.; Pieber, T. R.; Polovina, S.; Raskin, J.; Roeper, M.; Ruck, L.; Salomon Estebanez, M.; Tankova, T.; Thornton, P.; Van Rossum, E. F. C.; Vukovic, R.; Worth, C.; Zachurzok, A. EndoCompass Project: Research Roadmap for Diabetes, Obesity, and Metabolism. *European Journal of Endocrinology* **2025**, *193* (Supplement_2), ii47–ii71. https://doi.org/10.1093/ejendo/lvaf065.

DOCTORAL THESES AND OTHER WORKS:

Carmen Sopranis Pérez
Final degree's project. *Differentiation of hPSCs into the pancreatic lineage and CRISPR/Cas9-mediated gene editing for the study of diabetes-associated gene JAG1.* Universidad Autónoma de Madrid. 2025. Supervisor: Alberto Bartolomé Herranz.

Cristina María Gonzalo Rodríguez
Final degree's project. *Caracterización del gen PTF1A asociado a la diabetes monogénica en un sistema de diferenciación basado en células madre pluripotentes*. Universidad Autónoma de Madrid. 2025. Supervisor: Alberto Bartolomé Herranz.

FUNDING:

"Bases genéticas de la masa de célula beta. PID2021-122284NA-I00". AEI, MICIU. 2022-2025.PI: Alberto Bartolomé Herranz.

"Modelado del desarrollo pancreático en diabetes monogénicas mediante el uso de células madre humanas". Ayudas a Proyectos de Investigación en Diabetes dirigidos por jóvenes investigadores.Sociedad Española de Diabetes. 2024-2025.PI: Alberto Bartolomé Herranz.

"Impacto de las variantes comunes y raras de NOTCH2 en la masa de célula beta y riesgo de diabetes. CNS2023-145179." Consolidación Investigadora 2023. AEI, MICIU. 2024-2026. PI: Alberto Bartolomé Herranz.

OUTREACH ACTIVITIES:

Publicación en la revista Diabetes (Sociedad Española de Diabetes). Modelos de células madre como herramienta para investigar la genética de la diabetes. M. Hernanz, A. Bartolomé

Conferencias del CSIC a los centros educativos
de la Comunidad de Madrid.
- Colegio Santa María del Bosque. Febrero 2025
- Colegio Anna Frank. Octubre 2025

Inmunobiology of Platelets

TENURED-TRACK SCIENTIST

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KEYWORDS

Plaquetas, microambiente tumoral, inmunoterapia, enfermedades metabolicas

RESEARCH LINES:

Overview
How do chronic diseases impact in the tumor development and the response to cancer therapies.

The growth and progression of cancer are strongly influenced by the tumor microenvironment (TME), a complex ecosystem composed of tumor cells, immune cells, stromal components, and circulating factors. Increasing evidence indicates that platelets—classically recognized for their role in hemostasis—are active regulators within this environment. Upon activation, platelets interact with tumor and immune cells and release a wide range of bioactive mediators that can promote tumor survival, immune evasion, and metastatic dissemination. As a result, platelet-targeted strategies are emerging as promising adjuvants to conventional cancer therapies, including chemotherapy and immunotherapy.

Beyond cancer, platelets play a central role in chronic metabolic diseases characterized by systemic inflammation and vascular dysfunction, such as diabetes, obesity, and atherosclerosis. In these conditions, platelets exhibit a persistent hyperactivated state that precedes and accompanies disease progression. This chronic platelet activation alters immune responses and tissue homeostasis, potentially reshaping the TME when cancer develops in metabolically diseased individuals.

The focus of our laboratory is to understand how metabolic diseases influence cancer growth and progression by modulating platelet function and platelet–immune–tumor interactions. We hypothesize that metabolically driven platelet reprogramming contributes to an immunosuppressive tumor microenvironment, thereby supporting tumor progression and reducing the efficacy of anti-cancer therapies. By dissecting the mechanisms through which metabolic dysfunction alters platelet behavior, our work aims to identify novel therapeutic strategies to restore anti-tumor immunity and improve cancer treatment outcomes across a broad spectrum of metabolic diseases.

Researchers involved: Guadalupe Ortiz Muñoz, Maria Fernandez Fernandez, Mario Rucio, Lucia Redondo

Impact of Activated Platelets on NET Formation: Effects on Tumor Immunophenotype and Cancer Immunotherapy Response.

Platelets may promote tumor progression through the induction of neutrophil extracellular traps (NETs), DNA-based structures released by neutrophils that exert potent pro-tumorigenic effects. Within the tumor microenvironment (TME), NETs enhance tumor growth, invasion, and metabolic adaptation, while simultaneously promoting immune evasion by shielding tumor cells from cytotoxic lymphocytes and delivering immunosuppressive signals such as PD-L1.

Evidence from inflammatory disease models shows that NET formation depends on interactions between activated platelets and neutrophils, and that platelet inhibition markedly reduces NET generation and tissue damage. We therefore hypothesize that excessive platelet activation in cancer similarly drives NET formation within the TME, creating a self-sustaining inflammatory and immunosuppressive loop that limits the efficacy of cancer immunotherapy.

Tumor immune phenotypes—immune desert, excluded, or inflamed—strongly predict immunotherapy response, yet the mechanisms underlying their emergence remain poorly understood. Using the STAMP tumor model, which uniquely enables high-resolution in vivo tracking of immune dynamics, we will investigate whether platelet-driven NET formation shapes tumor immune phenotypes and whether targeting the platelet–NET axis can reprogram the TME toward an immune-inflamed state and enhance immunotherapy efficacy.

Researchers involved: Guadalupe Ortiz Muñoz, Alicia ALvarez Alvarez

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PUBLICATIONS:

Alvarez-Alvarez, Alicia, Fernandez-Fernandez, Maria and Ortiz-Muñoz, Guadalupe. Systemic Lipid Metabolic Alterations and the Tumor Microenvironment: A Hidden Driver of Immune Dysfunction. *Seminars in Cancer Biology* **2016** (acepted Manuscript Number: YSCBI-D-25-00072).

DOCTORAL THESES AND OTHER WORKS:

Federico Etchechurry
Final degree's project. *Estudio de la activación plaquetaria frente a líneas tumorales y sus implicaciones.* Universidad Europea. 2015. Supervisor/s: Guadalupe Ortiz Muñoz. Grade: sobresaliente.

FUNDING:

Impact of Activated Platelets on NET Formation: Effects on Tumor Immunophenotype and Cancer Immunotherapy Response. Ref: PID2024-161000OB-I00 By MICIN Period: 2025-2029. Investigador responsable: Guadalupe Ortiz-Munoz.

T Cell Modulation by plateltes inactivation: Exploring the Effects of Diabetes and Metabolic Disorders on Cancer and cancer response to Immunotherapy. Ref: CNS2024-154308 By MICIN Period: 2025-2027. Investigador responsable: Guadalupe Ortiz-Munoz

Aterosclerosis en la progresion y respuesta del cancer a inmunoteapias. Ref: PID2021-126811OB-I00 By MICIN Period: 2022-2026. Investigador responsable: Guadalupe Ortiz-Munoz

Associated Chronic inflammation and response to immunotherapy. Ref: 2020-T1/BMD-20365 By CAM Period: 2022-2027. Investigador responsable: Guadalupe Ortiz-Munoz

OUTREACH ACTIVITIES:

Conferences

Alvarez-Alvarez A, Fernandez-Fernandez Maria and Ortiz-Muñoz Guadalupe. (March, 2025). The Role of Activated Platelets in NET Formation and Its Implications for Cancer Progression. Cell Biology of Megakaryocytes and Platelets Gordon Research Conference. Ventura (California). Poster.

María Fernández-Fernández, Alicia Álvarez-Álvarez, Carmen Gomez-Guerrero, Maria Kavanagh, Raquel Nieto, María Beatriz Álvarez and Guadalupe Ortiz-Muñoz. (March, 2025). Atherosclerosis Associated Chronic inflammation in the progression and response of cancer to immunotherapies: MDSC Modulation by platelets. Cell Biology of Megakaryocytes and Platelets Gordon Research Conference. Ventura (California). Poster.

María Fernández-Fernández, Alicia Álvarez-Álvarez, Carmen Gomez-Guerrero, Maria Kavanagh, Raquel Nieto, María Beatriz Álvarez and Guadalupe Ortiz-Muñoz. (November 2025) Preconditioned Platelets in Atherosclerosis: A Key Link to Tumor Progression. IIBM–UAM Retreat, La Cristalera (Miraflores, Madrid). Awarded the Best Poster Prize.

María, Fernández Fernández (January 2026). Preconditioned Platelets in Atherosclerosis: A Key Link to Tumor Progression. IIBM PhDay 1st edition, Instituto de Investigaciones Biomédicas Alberto Sols- Gabriela Morreale. Awarded the Best oral presentation Prize.

NANOimmunology of T Lymphocyte Activation and Apoptosis

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Izquierdo Pastor, Manuel
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Group Coordinator

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Villar Castelló, Mencía
García Silverio, Marta

KEYWORDS

Actin cytoskeleton, CAR-T cells, Exosomes, FMNL1, Immune synapse, T lymphocytes

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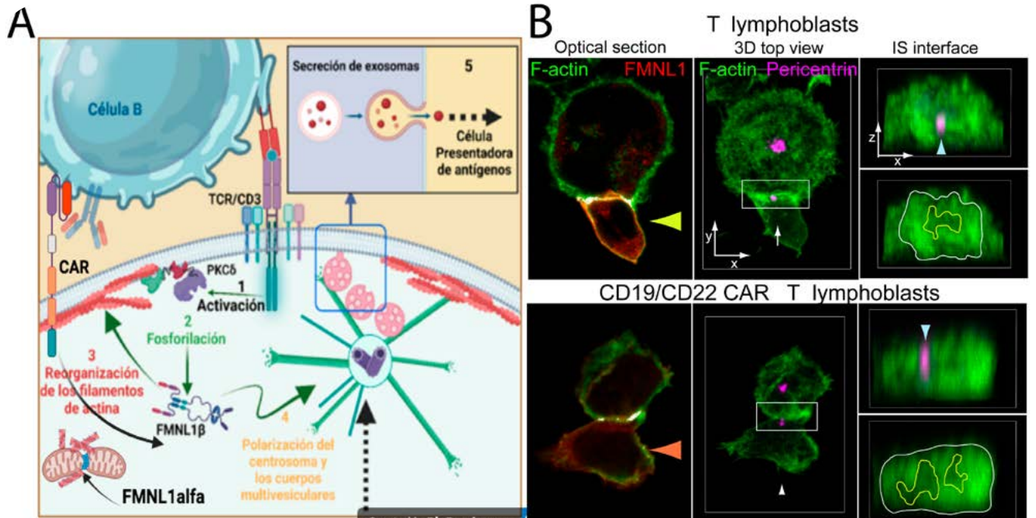

FMNL1β phosphorylation regulates exosome secretion at the immune synapse
A) After IS formation in T cells, FMNL1β is transiently recruited to the IS independently of PKCδ. However, its phosphorylation at S1086 residue is mediated by PKCδ, and despite not being sufficient, is indispensable for MTOC/MVB polarization towards the IS. In addition, S1086 phosphorylation in FMNL1β is also required for cortical F-actin rearrangement at the IS and subsequent exosome secretion. FMNL1β phosphorylation at S1086 emerges as a crucial event in the control of polarized T cell trafficking in the IS.
B) In the left column, confocal optical section of a Tlymphoblast (yellow arrow) forming immune synapse with a superantigen loaded Raji cell (top), and a CD19/CD22 CAR (red arrow)formin synapse with a $CD19^{+}CD22^{+}$ Raji cell. The top view is shown on the middle column, while the view on the right, after a 90° rotation, shows the immune synapse interface (white rectangle). The F-actin architecture at the SI interface and the centrosome position are visible (clear blue arrow). The image demonstrates the multifocal nature of the synapse developed by tandem CAR-T cells and the centrosome's position at one of the foci. F-actin is labelled in green, MTOC in magenta and FMNL1 in red, whereas colocalization between F-actin and FMNL1 is shown in white. FMNL1 accumulates with F-actin at the distal SMAC (dSMAC).

RESEARCH LINES:

Overview

Our general interest is to decipher the molecular mechanisms that participate in the formation of the immune synapse (IS) and to study some of the T lymphocyte effector responses that derive from IS formation. T-cell receptor (TCR) stimulation by antigen bound to the major histocompatibility complex (MHC) on an antigen-presenting cell (APC) induces the formation of the IS and accumulation of filamentous actin (F-actin) at the IS, followed by depletion of F-actin at the central region of the IS (cIS) and the polarization of lytic granules/multivesicular bodies (MVB) and the microtubule-organizing center (MTOC) to the IS. Among several T lymphocyte effector responses, the fusion of MVBs with the plasma membrane at the IS produce the secretion of MVB intraluminal vesicles as exosomes, leading to

polarized exosome secretion at the IS cleft (Fig 1). The exosomes are involved in several crucial immune responses, including the cytolytic activity of cytotoxic T lymphocytes (CTLs) against target cells such as tumor cells, and activation-induced autocrine apoptosis (AICD) of T lymphocytes, which is involved in controlling autoimmunity. Overall, a better understanding of the signals involved in MVB maturation and traffic will allow designing strategies to modulate exosome secretion by CTL and hence modify their function. With this knowledge in the hand it will be possible to modify some biological and pathological consequences derived from T lymphocyte secretion, including cytotoxic activity against tumor cells and autoimmunity. Thus, it will be feasible to design new therapeutic approaches against cancer and certain autoimmune diseases.

Our research comprises oriented, basic research directed to enhance our knowledge of the relationship among cytoskeleton, MVB secretory traffic and IS, which are fundamental components necessary to develop and appropriate adaptive immune response. By investigating these interactions within the context of human chimeric antigen receptor (CAR)-T cell IS in collaboration with clinicians (Fig. 1), it is expected that the results might eventually lead to strategies to genetically manipulate T lymphocytes and CAR-T cells used during T cell adoptive therapy protocols, in order to improve persistence and avoid exhaustion of effector CAR-T lymphocytes.

Role of FMNL1 formin in polarized secretory traffic towards the TCR and CAR-evoked immune synapse

Researchers involved: Ruiz-Navarro, J.; Fernández-Hermira, S.; Sanz-Fernández, I.; Barbeito, P.; Navarro-Zapata, A.; Pérez-Martínez, A.; Garcia-Gonzalo, F.R.; Calvo, V.;, Izquierdo, M.

This research line focuses on the contribution of FMNL1 formin-regulated actin cytoskeleton on IS architecture and function as well as its effects to MVB secretory traffic. We analysed here how formin-like 1 β (FMNL1β), an actin cytoskeleton-regulatory protein, regulates microtubule-organizing center (MTOC) and multivesicular bodies (MVB) polarization and exosome secretion at an immune synapse (IS) model in a phosphorylation-dependent manner. IS formation was associated with transient recruitment of FMNL1β to the IS, which was independent of protein kinase C_δ (PKC_δ). Simultaneous RNA interference of all FMNL1 isoforms prevented MTOC/MVB polarization and exosome secretion, which were restored by FMNL1βWT expression. However, expression of the non-phosphorylatable mutant FMNL1βS1086A did not restore neither MTOC/MVB polarization nor exosome secretion to control levels, supporting the crucial role of S1086 phosphorylation in MTOC/MVB polarization and exosome secretion. In contrast, the phosphomimetic mutant, FMNL1βS1086D, restored MTOC/MVB polarization and exosome secretion. Conversely, FMNL1βS1086D mutant did not recover the deficient MTOC/MVB polarization occurring in PKC_δ-interfered clones, indicating that S1086 FMNL1β phosphorylation alone is not sufficient for MTOC/MVB polarization and exosome secretion. FMNL1 interference inhibited the depletion of F-actin at the cIS, which is necessary for MTOC/MVB polarization. FMNL1βWT and FMNL1βS1086D, but not FMNL1βS1086A expression, restored F-actin depletion at the cIS. Thus, actin cytoskeleton reorganization at the IS underlies the effects of all these FMNL1β variants on polarized secretory traffic. FMNL1 was found in the IS made by primary T lymphocytes, both in TCR and chimeric antigen receptor (CAR)-evoked synapses (Fig 1). Taken together, these results point out a crucial role of S1086 phosphorylation in FMNL1β activation, leading to cortical actin reorganization and subsequent control of MTOC/MVB polarization and exosome secretion. Further experiments are in progress to characterize the role of actin cytoskeleton and FMNL1 in polarized secretion at the immune synapse evoked by CAR-T cells. Our findings may allow to design strategies to improve the persistence and effector functions of CAR-T cells during T cell adoptive therapy protocols, opening new venues in therapeutic approaches against cancer.

Imaging the immune synapse: three-dimensional analysis of the TCR and CAR-evoked immune synapse.

Researchers involved: Ruiz-Navarro, J.; Blázquez-Cucharero, S.; Calvo, V.;, Izquierdo, M.

T cell receptor (TCR) stimulation of T lymphocytes by antigen bound to the major histocompatibility complex (MHC) of an antigen-presenting cell (APC), together with the interaction of accessory molecules, induces the formation of the immunological synapse (IS), the convergence of secretion vesicles towards the centrosome, and the polarization of the centrosome to the IS. Upon IS formation, an initial increase in cortical filamentous actin (F-actin) at the IS takes place, followed by a decrease in F-actin density at the central region of the IS, which contains the secretory domain (Fig. 1). These reversible, cortical actin cytoskeleton reorganization processes that characterize a mature IS occur during lytic granule secretion in cytotoxic T lymphocytes (CTL) and Natural Killer (NK) cells and cytokine-containing vesicle secretion in T-helper (Th) lymphocytes, but also characterize the IS-evoked by CAR-T cells. However, apart of this “classical” bullseye IS structure, non–classical IS including multifocal IS are developed by double-positive thymocytes, Th2 cells and CAR-T cells. Besides, IS formation constitutes the basis of a signalling platform that integrates signals and coordinates molecular interactions that are necessary for an appropriate antigen-specific immune response. In this line we deal with the three-dimensional

(3D) analysis of the synaptic interface architecture, as well as the analysis of the localization of different markers at the IS. This approach allows the simultaneous assessment of the sub-synaptic architecture and colocalization of several different markers in the three dimensions of the IS. This method can be used to assess cortical F-actin in different types of IS, including the synapses made by NK cells and B lymphocytes and CAR-T cells. The crucial importance of the synaptic structure in processes such as centrosome polarization or secretory vesicles traffic and degranulation has stimulated the development of accurate analysis methods of the synaptic interface. Moreover, considering that actual biological interactions occur in three-dimensional space, following this approach, we can study the architecture of the IS and the three-dimensional colocalization and potential interactions between different markers in this region, thus overcoming the limitations of two-dimensional analyses.

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Ruiz-Navarro, J., Blázquez-Cucharero, S., Calvo, V., & Izquierdo, M.. Imaging the immune synapse: Three-dimensional analysis of the immune synapse. *Methods Cell Biol*, **2025**, *193*, 15-37. DOI: 10.1016/bs.mcb.2023.04.003

DOCTORAL THESES AND OTHER WORKS:

David Rodríguez Hernández
Final degree's Project. *Estudio del control de tráfico polarizado en la sinapsis inmunitaria: función de FMNL1.* Universidad Autónoma de Madrid. Facultad de Medicina. 2025. Supervisor/s: Manuel Izquierdo, Javier Ruiz-Navarro. Grade: Sobresaliente 9,13.

Mencía Villar Castelló
Final degree's Project. *Estudio del tráfico polarizado en la sinapsis inmune: función de FMNL1.* 2025. Universidad de Alcalá de Henares. Supervisor/s: Manuel Izquierdo, Javier Ruiz-Navarro. Grade: Pending.

Marta García Silverio
Final degree's Project. *Estudio del control polarizado en la sinapsis inmunitaria: papel de la formina FMNL1.* Universidad Politécnica de Madrid. Escuela Técnica Superior De Ingeniería Agronómica, Alimentaria y de Biosistemas. 2025. Supervisor/s: Manuel Izquierdo, Javier Ruiz-Navarro. Grade: Notable 8,5.

FUNDING:

"Estudio de las bases moleculares de la secrecion polarizada de exosomas por los linfocitos T: papel de la formina FMNL1 y del citoesqueleto de actina (linfoexosomas). PID2020-114148RB-I00". Ministerio de Ciencia e Innovación. 2021-2025.

OUTREACH ACTIVITIES:

Trabajo de divulgación. "¿Qué papel pueden tener los exosomas en terapias contra el cáncer metastásico?". 11/11/2025
https://www.fundacionmuyinteresante.org/
exosomas-inmunoterapia-cancer-metastasico.html

Semana de la Ciencia. “Explorando la Biología Celular: Detección de Mutaciones y Marcaje Celular”. 27/11/2025.

Olimpiada de Biología. “Olimpiada Española de Biología en el #IIBM, Malen Bustamante”.
23/06/2025.

Programa 4º ESO EMPRESA. “Alumnos/as de IES MONTSERRAT CABALLÉ y SEVERO OCHOA, Madrid, dentro del Programa 4º ESO + Empresa visitan el Centro de Investigación”. 10/04/2025.

X Festival de Nanociencia y de Nanotecnología. ¨La nanotecnología llega al Instituto: Zoombando en la Nanoinmunología”. IES JUANA DE CASTILLA. 24/04/2025.

X Festival de Nanociencia y de Nanotecnología. ¨La nanotecnología llega al Instituto: Zoombando en la Nanoinmunología”. IES Ana Frank. 18/03/2025.

X Festival de Nanociencia y de Nanotecnología. ¨La nanotecnología llega al Instituto: Zoombando en la Nanoinmunología”. IES ALAMEDA DE OSUNA. 27/02/2025.

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Precision Medicine in Diseases Caused by Alterations in Lipid Metabolism

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KEYWORDS
Phospholipids metabolism, Choline kinase α, Cancer, Obesity,

RESEARCH LINES:

Overview

Choline kinase α (ChoKα) is a critical enzyme involved in the regulation of phosphatidylcholine metabolism, the major phospholipid in all eukaryotic membranes. Overexpression of ChoKα is oncogenic and modulates the expression of genes directly involved in the regulation of cell proliferation and apoptosis, promoting the progression of tumours. ChoKα affects signalling pathways including ERK, AKT, PI3K, c-Src and EGFR. Inhibition of ChoKα induces endoplasmic reticulum stress (ERS) and Unfold Protein Response (UPR), leading to a drastic reduction in the levels of the G1->S phase checkpoint mediators pRB and E2F1α. These effects results in a potent antitumor activity, promoting a variety of effects as increased ceramides production and the subsequent activation of cell death, with an exquisite specificity towards cancer cells and a reversible, non-toxic effect on normal, non-tumorigenic cells.

A better knowledge of the mechanisms by which ChoKα contributes to cancer onset and progression and those involved in sensitivity and resistance to drugs targeting enzymes involved in lipid metabolism may facilitate the design of more specific and effective therapies. Indeed, due to their unique mechanism of action, ChoKα inhibitors (ChoKαIs) could be used in many combinatorial regimes against a broad spectrum of human cancers. ChoKαIs show potent antitumor activity, and one of our drugs, RSM-932A, has completed the first Phase I clinical trial in humans. However, and as expected for any chemotherapeutic approach, resistance to ChoKαIs has also been found. This makes imperative the search for tools that discriminate sensitive from resistant tumours to ChoKαIs.

Precision oncology requires the development of adequate tools and protocols for the selection of patients suffering from each specific type of cancer to optimize their clinical management. Especially relevant is the case of PDAC and NSCLC tumours, with dreadful prognosis that can benefit from a targeted personalised treatment. These protocols would result from the combination of studies using appropriate biological reagents such as Patient-Derived Xenografts (PDX), Patient-Derived Organoids (PDOs) and the use of omic analysis. We are using this strategy to identify genomic, proteomic and lipidomic alterations induced by modulation of ChoKα activity to identify specific biomarkers in both sensitive and resistant tumours to inhibitors of ChoKα. These results will allow the selection of candidates that may benefit from targeted, personalized, precision therapeutic approaches. Our group is focused in the study of PDAC and NSCLC models to identify Response Predictive Signature (RPS) for each pathology in response to ChoKα inhibitors (ChoKαIs).

In addition to this role in cancer onset and progression, we and others have demonstrated the relevance of ChoKα as a therapeutic target in diseases produced by parasites as malaria, caused by *Plasmodium falciparum,* and Leishmaniasis, caused by *Leishmania infantum*. This is further expanded to the successful use of ChoKαIs against bacterial infections produced by Gram-positive *S. pneumoniae* and Gram-negative *H. influenza,* responsible for pneumonia, otitis and bronchitis.

Using several animal models, ChoKαIs has demonstrated to be a potent therapeutic tool in even other diseases. These include rheumatoid arthritis (RA), LPS-induced septic shock model, Muckle–Wells syndrome (MWS), familial cold auto inflammatory syndrome (FCAS) and neonatal-onset multisystem inflammatory disease (NOMID). The last three syndromes are a consequence of mutations in the NLRP3 gene that cause chronic activation of the inflammasome, suggesting that targeting ChoKα has the potential to be an efficient approach to also treat inflammatory diseases.

Therefore, ChoKα inhibition may play an important role in the treatment of a large diversity of human diseases. Further research on the clinical application of our ChoKαIs in this plethora of human illnesses will disclose and clarify whether its tremendous potential as broad-spectrum therapeutics can be a reality. We are working to resolve this challenging enterprise.

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Díaz-Riascos ZV, Llaguno-Munive M, Lafuente-Gómez N, Luengo Y, Holmes S, Volatron J, Ibarrola O, Mancilla S, Sarno F, Aguirre JJ, Razafindrakoto S, Southern P, Terán FJ, Keogh A, Salas G, Prina-Mello A, Lacal JC, Del Pozo A, Pankhurst QA, Hidalgo M, Gazeau F, Somoza Á, Schwartz S Jr, Abasolo I. Preclinical Development of Magnetic Nanoparticles for Hyperthermia Treatment of Pancreatic Cancer. *ACS Appl Mater Interfaces.* **2025** *Jan 15;17(2)*:2924-2939. DOI: 10.1021/acsami.4c16129. Epub 2025 Jan 2. PMID: 39745145.

FUNDING:

"El metabolismo lipídico como nueva diana terapéutica en oncología de precision en cancer de páncreas y de pulmón (ONCOLIPIDS). PID2020-116165RB-C21". PI: Juan Carlos Lacal. Grant: 169.000 € (2021-2025). Miembros del Equipo de Trabajo: Dr. Juan Casado Vela, Dr. Francesca Sarno, Yolanda Durán Jiménez (FP Grado Superior), Juan Antonio Quintana Fernández (FP Grado Superior).

MATERIAL TRANSFER AGREEMENT between CONSEJO SUPERIOR DE INVESTIGACIONES CIENTÍFICAS, M.P. and Gossamer Bio Services, Inc. 3013 Science Park Road, Suite 200, San Diego, CA 92121. PI: Juan Carlos Lacal. Grant: 6,000€ (2022-2025)

2024 LLAV 00109- IP: Carolina Arfmengol. Fundació Institut d'Investigació en Ciències de la Salut Germans Trias i Pujol G60805462 Targeting choline metabolism in high-risk hepatoblastoma with a novel release-controlled drug. Grant: 20.000€ (2024-2025).

PATENTS:

1. INVENTORS: T. Zimmerman, S. Ibrahim, JC Lacal. PATENT NUMBER: PCT/ES2025/070147

2. INVENTORS: JC Lacal, T. Zimmerman. PATENT NUMBER: EP25382266.2/2025

Hypoxia and Angiogenesis

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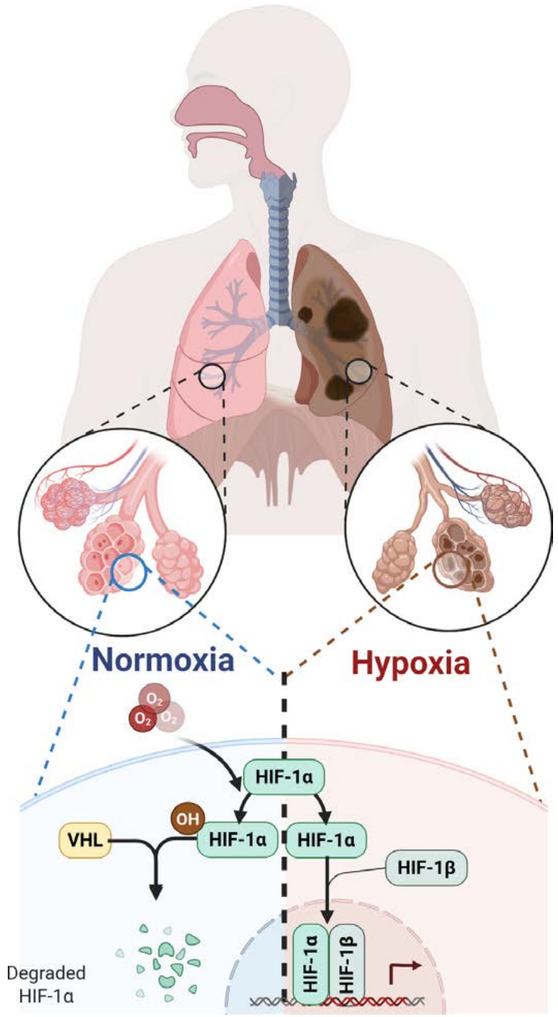
KEYWORDS

Bioinformatics, Cardiopulmonary diseases, Hypoxia, Transcription



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RESEARCH LINES:

Overview

The investigation into cellular and molecular adaptive responses to hypoxia holds significant importance, given its relevance to physiological processes and the development of prevalent pathologies such as cancer and cardiovascular diseases. Hypoxia Inducible Transcription Factors (HIFs) play a central role in orchestrating these responses by regulating the expression of a multitude of genes involved in adapting to hypoxic conditions. Our research group is dedicated to advancing our understanding of the transcriptional response to hypoxia and the underlying cellular and molecular mechanisms governing crucial adaptive processes like angiogenesis. Our goal is to leverage this knowledge to enhance the clinical management of conditions where tissue hypoxia is a common feature.

Transcriptional Response to Hypoxia

Researchers involved: Esteve Pastor, Pilar; Berrouayel Dahour, Yosra; del Peso Ovalle, Luis.

While the transcriptional response to hypoxia has been extensively studied in vitro, much less is known about the gene expression programs induced in vivo. To address this critical gap, we are investigating the early transcriptional response to sustained and intermittent hypoxia as models of chronic obstructive pulmonary disease (COPD) and obstructive sleep apnea (OSA), respectively.

Role Brown Adipose Tissue and Bhlhe40 in Metabolic Syndrome Associated with Obstructive Sleep Apnea

Researchers involved: Pescador Sánchez, Nuria; Fernández Cañizares, María,Sánchez Peña, Claudia; Bernabé Pérez, Rocío; Berrouayel Dahour, Yosra; del Peso Ovalle, Luis.

OSA is a highly prevalent condition characterized by intermittent obstruction of the upper respiratory tract during sleep, leading to cyclic hypoxia. OSA patients face an increased risk of cancer and cardiovascular diseases, yet the mechanisms linking these conditions remain poorly understood. Using a combination of animal models and primary cell cultures, we found that intermittent hypoxia strongly affects brown adipocyte function, leading to brown adipose tissue dysfunction. This alteration may contribute to the increased risk of metabolic syndrome and cardiovascular diseases observed in obstructive respiratory disorders.

Computational approaches to analyze transcriptional responses

Researchers involved: Berrouayel Dahour, Yosra; del Peso Ovalle, Luis.

Gene expression profiling analyses often yield sets of genes that are differentially expressed in a given context. To identify the transcriptional regulators responsible for the coordinated regulation of these genes, we

developed the tool TFEA.ChIP. Our current research in this area focuses on further improving this tool by incorporating recent advances in enhancer–gene assignment methods.

Understanding the Role of Bhlhe40 in Endothelial Cell Differentiation and Proliferation during Hypoxia-Induced Angiogenesis

Researchers involved: Jiménez Cuenca, Benilde.

Angiogenesis, the primary mechanism driving vascular expansion, is a fundamental adaptive response to hypoxia. However, our understanding of how HIFs regulate angiogenesis remains incomplete. Given Bhlhe40's prominent role in the hypoxic transcriptional response, our research focuses on elucidating the role of the HIF/Bhlhe40 axis on endothelial cell proliferation and differentiation during hypoxia-induced angiogenesis. To this end, we utilize stem cell-based angiogenesis models and CRISPR-mediated gene editing approaches. Our findings reveal a novel role for Bhlhe40 in regulating proliferation and angiogenesis in mouse embryoid bodies under hypoxic conditions.

PUBLICATIONS:

Rodríguez-Pérez J.; Andreu-Martínez R.; Daza R.; Fernández-Arroyo L.; Hernández-García A.; Díaz-García E.; Cubillos-Zapata C.; Lozano-Diez A.; Morales A.; Ramos D., Aragonés J.; Cogolludo Á.; Del Peso L.; García-Río F.; Calzada MJ. Oxidative Stress and Inflammation in Hypoxemic Respiratory Diseases and Their Comorbidities: Molecular Insights and Diagnostic Advances in Chronic Obstructive Pulmonary Disease and Sleep Apnea. Antioxidants (Basel). 2025, 14(7), 839-850. doi.org/10.3390/antiox14070839.

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DOCTORAL THESES AND OTHER WORKS:

María Fernández Cañizares

Master's thesis. *Exposure to Sustained and Intermittent Hypoxia mimicking Chronic Obstructive Respiratory Disorders: effects on Brown Adipose Tissue.* Universidad Autónoma de Madrid. 2025. Supervisor/s: Nuria Pescador and Luis del Peso. Grade: Matrícula de Honor.

Claudia Sánchez Peña

Final degree's project. *Papel de la ruta HIF/BHLHE40 en la homeostasis metabólica y adaptación a hipoxia.* Universidad Autónoma de Madrid. 2025. Supervisor/s: Nuria Pescador and Luis del Peso. Grade: Matrícula de Honor.

FUNDING:

Beyond Cell Culture: Characterizing Early In Vivo Transcriptional Adaptations to Hypoxia. PID2024-159980OB-I00. MICINN. 2025-2029. PI: Luis del Peso.

Identification of Mechanisms, Biomarkers, and Interventions in Comorbidity in Hypoxemic Respiratory Diseases through Preclinical, Clinical, and Computational Approaches. P2022/BMD7224. CAM. 2023-2026. PI: Luis del Peso.

Contribution of BHLHE40 to the transcriptional response to hypoxia and its implication in metabolic and respiratory diseases. PID2020-118821RB-I00. MICINN. 2021-2025. PI: Luis del Peso and Benilde Jiménez.

Cardiovascular Physiopathology

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KEYWORDS

Cardiac remodeling, Channelosome, Heart failure, Kv1.5, Immunometabolism, Inflammation

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RESEARCH LINES:

Overview

Cardiovascular function

Heart failure (HF) is a complex clinical syndrome that constitutes a major health problem, and treatment remains ineffective in more than half of patients despite the continuous introduction of new therapeutic interventions. Almost all cardiovascular diseases eventually progress to heart failure if not effectively treated or alleviated. During the progression of HF, cardiac remodeling occurs. This remodeling is a process of regional or global structural and functional changes in the heart as a consequence of stressors, such as myocardial infarction, pressure overload (aortic stenosis, hypertension), myocarditis, idiopathic dilated cardiomyopathy, or volume overload (valvular regurgitation), and constitutes an important determinant of the clinical outcome of HF, related to disease progression and poor prognosis. The remodeling process is characterized by abundant inflammatory and profibrotic responses, neurohormonal activation, and enlargement of the heart to manage increased hemodynamic demand. Therefore, the search for better treatments for HF is one of the great challenges of cardiology. In this area, we have been working on the evaluation of the role of the nuclear receptor AhR in protection against HF. Our data suggest that AhR activation may ameliorate HF dysfunction by regulating ferroptosis, a relevant event leading to HF. The study of the pathways involved in the cardioprotective role of AhR activation is the subject of our current work in improving HF.

Immunometabolism and COVID-19

The cytokine storm observed in patients with COVID-19 has emerged as a key contributor to disease severity and associated mortality. Our study aimed to elucidate the metabolic alterations macrophages undergo in response to the COVID-19 cytokine storm, thereby shedding light on the mechanisms driving the exacerbated immune response. By integrating genomic-scale data and experimental metabolic measurements, we developed a comprehensive workflow. We applied this workflow to identify the metabolic alterations that occur in human macrophages during the SARS-CoV-2-induced cytokine storm. The methods used include genome-scale metabolic models (GSMM) to represent metabolic pathways, flux balance analysis (FBA) and flux variability analysis to predict flux distributions, and the metabolism-moderated gene deletion algorithm (GIM3E) to model the complex interaction between gene expression and metabolism. The data obtained offer insights into macrophage metabolic reprogramming, providing a deeper understanding of their role in the pathogenesis of the COVID-19 cytokine storm.

Kv1.5 and Kv4.3 channelosomes

Ion channels are responsible, among other functions, for muscle contraction, cardiac rhythm, and synaptic transmission. We focus our research on voltage-dependent potassium channels present in the human myocardium, mainly Kv1.5 (which generates IKur) and Kv4.3 (which generates Ito), which are the most important potassium channels responsible for atrial repolarization and thus serve as pharmacological targets for antiarrhythmic drugs used in the treatment of cardiac arrhythmias. Ion channels form signaling complexes, or channelosomes, which are essential for optimal, fast, and efficient signal transmission. Therefore, knowledge of the interactors of these channelosomes is essential for the validation of new proteins that may constitute therapeutic targets, as well as for the design and synthesis of new chemical agents that may be useful drug candidates. We have focused on Lgi3-4 and KChIP2.

Channelopathies

One of our research lines is the electrophysiological characterization of new mutations on ion channels or some of their regulatory subunits. These mutations found in the clinics can induce cardiac arrhythmias such as Long QT, Short QT, or Brugada Syndromes.

Resolution of inflammation in cardiovascular pathologies

The resolution phase following an inflammatory process is required for its completion to prevent chronic inflammation and, therefore, chronic disease. During the transition from inflammation to resolution, there is an increase in the enzymatic synthesis of SPMs. Several cardiovascular diseases, including atrial fibrillation (AF), myocardial infarction, heart failure, and hypertension, involve inflammation. Therefore, understanding the possible effects of SPMs on different cardiac ion channels may help in the treatment and/or prevention of cardiac diseases.

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Sánchez-García S, Jaén RI, Lozano-Rodríguez R, Avendaño-Ortiz J, Pascual-Iglesias A, Hurtado-Navarro L, López-Collazo E, Boscá L, Prieto P. Lipoxin A4 levels correlate with severity in a Spanish COVID-19 cohort: potential use of endogenous pro-resolving mediators as biomarkers. *Front Immunol.* **2025** Jan 23;15:1509188. DOI: 10.3389/fimmu.2024.1509188. PMID: 39916956; PMCID: PMC11798798.

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lism and absolute contraindication to systemic thrombolysis. *Cardiovasc Revasc Med*. **2025** *Jan;70*:56-61. DOI: 10.1016/j.carrev.2024.06.020.

Baena-Nuevo M, Vera-Zambrano A, Martinez-Laperche C, Buño I, Muñoz-Calleja C, Valenzuela C, Zapata JM, Perez-Chacon G. Indole-3-carbinol is an inhibitor of Kv1.3 potassium voltage-gated channel activity in chronic lymphocytic leukemia cells. *Eur J Pharmacol.* **2025** Nov 15;1007:178281. DOI:10.1016/j.ejphar.2025.178281.

Cruz A, Ronchi C, Bartolucci C, Socuéllamos PG, Benito-Bueno A, Severi S, Zaza A, Valenzuela C. RvD1 and LXA₄ inhibitory effects on cardiac voltage-gated potassium channels. *Biomed Pharmacother*. **2025** Jun;187:118083. DOI: 10.1016/j.biopha.2025.118083.

DOCTORAL THESES AND OTHER WORKS:

Sergio Sánchez García
Ph.D. thesis. *Nitric oxide and pro-resolving mediators in COVID-19 pathogenesis.* Universidad Autónoma de Madrid. 2025. Supervisor/s: Lisardo Boscá Gomar, Patricia Prieto Chinchilla. Grade: Sobresaliente Cum Laude.

María Elegido Vanrespraille
Final degree's project. *Regulación funcional y metabólica de cultivos primarios de linfocitos y macrófagos humanos por la acción de ecteinascidinas.* Universidad Autónoma de Madrid. 2025. Supervisor/s: Adrián Povo Retana, Rodrigo Landauro Vera. Grade: Sobresaliente

Mateo Iriarte Martín
Final degree's project. *Efectos inmunometabólicos de la oxitiamina (O-B1) en linfocitos y macrófagos humanos.* Universidad Autónoma de Madrid. 2025. Supervisor/s: Adrián Povo Retana. Grade: Sobresaliente

FUNDING:

Assessment of the protective role of AHR in heart failure mechanisms of inhibition of ferroptosis by AHR activation in the failing heart. PID2023-148933OB-I00. AEI. 2024-2028. Lisardo Boscá Gomar; Carmen Delgado Canencia

Consorcio para el estudio del fracaso renal y su impacto en la patología cardiovascular. S2022/BMD-7223". Comunidad de Madrid. 2023-2026. Lisardo Boscá Gomar.

Mechanisms underlying the cardioptotective effects of nuclear vitamin D receptor activation in heart failure .CSIC (Ref:UCRAN20050). Programa CSIC de cooperación científica con Ucrania. 2022- 2026. Carmen Delgado Canencia.

Characterization of the immunometabolic actions of benfo-oxythiamine (B-OT) and analogs in human macrophages: An approach to understanding the role of these drugs on tumor-associated macrophages (TAM). Benfovir AG. Lisardo Boscá Gomar

Papel de los canalosomas Kv1.5 y Kv4.3 en la fibrilación auricular. Búsqueda de nuevas dianas terapéuticas y herramientas moleculares. PID2022-137214OB-C21". AEI. 2023-2026. Carmen Valenzuela Miranda.

Red Española de Canales Iónicos. RED2022-134420-T". AEI. 2023-2025. Carmen Valenzuela Miranda

"Attila Ziegelhoffer Poster Award in the 10th meeting European Section of the International Academy of Cardiovascular Sciences. Bratislava (Slovak Republic)". October 28-30, 2024.

Cellular Compartmentalization, Homeostasis and Inflammation

TENURED-TRACK SCIENTIST
Sánchez Álvarez, Miguel
(Ramón y Cajal contract)

VISITING SCIENTIST
García López, Álvaro

TECHNICAL SUPPORT PERSONNEL
Agüera Gómez, Lucía
Valdés Vaquero, Lucía

UNDERGRADUATE STUDENT
Rodríguez Álvarez, Sofía

KEYWORDS
Organelles, Secretory Apparatus, Cell Stress, Systems Biology, Cell Defense

RESEARCH LINES:

Overview

The relevance of regulated secretion and its tight coordination with other cell functions in multicellular organisms is difficult to overstate: distinct cell types engage in communication through secreted factors; exchange nutrients such as lipids through secreted transport particles; and layer and remodel the extracellular matrix (ECM), essential for tissue morphogenesis, homeostasis and repair through the coordinated synthesis, maturation, trafficking and secretion of large proteins such as collagens. These activities, which in specialized cell types can represent a major share of the total expenditure of energy and resources, need to fit tightly with the cell functional state to ensure appropriate responses to different stimuli and conditions. The dysfunction of components of this complex cell system is at the core of a very large number of diseases not only because of its impact on primary secretion, but also because of that pervasive reciprocal communication with other cell structures and functions. For example, mutations of proteins regulating the shape, dynamics and recycling of the endoplasmic reticulum (ER) are frequently associated with altered morphogenesis of neurons and motor control. Our laboratory aims to contribute to the better understanding of how the dynamics and function of specific cell compartments of the secretory apparatus are regulated and coordinated with cell state at systems-level, and how the disruption of these mechanisms underpins disease. An integral aspect of our research is the application and integration of unbiased molecular profiling techniques and high-content microscopy screening, which we also conduct in collaborative research.

The control of endoplasmic reticulum (ER) remodeling

The ER is an intricate system of intracellular membrane domains delimiting a single luminal space, continuous with the outer nuclear envelope. The architecture of the ER and its dynamics contribute to the several essential functions of this organelle, including calcium and redox homeostasis, complex lipid metabolism, management of other endomembrane systems, and the maturation and assisted folding of ~30% of the proteome. ER membrane subdomains can adopt discrete shapes (including ER 'tubules' (peripheral, reticular tubes of ER, with rather low densities of associated ribosomes) and ER 'sheets' (flat enlargements or "cisternae" of peripheral ER, usually rich in bound polysomes); this model may oversimplify a more complex variety of ER architectures. The specific functional relevance of ER architectural remodeling are still incompletely understood, but beyond adding to the net functional capacity of the ER, it is essential for the appropriate configuration of specific cell states. A major example is embodied by the particular structure of neurons, whereby the interplay between the ER and the microtubule cytoskeleton stabilizes developing axons and neuronal spines.

We have studied, using high-content microscopy approaches, how cells expand their ER when undergoing ER stress. Surprisingly, the eIF2alpha kinase EIF2AK3/PERK is required for this process: translation regulation controls the anchoring of the ER to a specific subset of non-centrosomal microtubules, which must be disrupted for ER expansion. ER-MT anchoring in turn modulates the stability of non-centrosomal microtubules, and cell protrusiveness and polarity. Thus, PERK activity coordinates ER homeostasis and remodeling with cell morphogenesis and behavior (*Sánchez-Álvarez et al. Cell Reports 2025*).

We are also studying a specific ER remodeling event that is engaged in cells subjected to different forms of stress, including innate immunity activation. Our observations support this ER remodeling contributes to minimize self-damage in the cell. The molecular mechanisms of this protective remodeling, which are partly regulated by the UPR, are being investigated through collaborative research.

A novel regulator of ER-Golgi trafficking

We are studying the cell and organismal function of NFXL1, a novel, very poorly characterized E3 ligase localized to ER domains. Our in vitro and in vivo observations strongly support a role for this factor in the regulation of ER-

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to-Golgi trafficking to enable secretion, with a relevant contribution to the development of bone structures in zebrafish and mice. Apart from detailed mechanistic studies underlying this activity, we are interested in exploring the specific potential role of NFXL1 in neural homeostasis and function, as human pathogenic variants described to date link this factor to syndromes affecting learning and memory. This is notable as multiple neurological syndromes are predominant phenotypes associated with mutations affecting ER shaping and ER-Golgi trafficking regulators.

Emerging roles of lipid droplets

Research conducted during the last 15-20 years has refuted our previous conception of lipid droplets (LDs) as inert lipid storage structures, passively subjected to growth/consumption cycles. On the contrary, lipid droplets are extremely dynamic organelles with a complex proteome, engaging in communication with other cell structures, and non-intuitively serving as regulators of functions beyond triacylglyceride and cholesterol ester accumulation and supply, including proteostasis, stress adaption and immunity. We contributed to studies demonstrating a role for LDs in defence responses against intracellular pathogens: LDs are safe platforms on which toxic antipathogen proteins can be accumulated to engage with intracellular invasors, while contributing to the metabolic rewiring that takes place in the infected cell. We continue to explore the dynamics of the LD proteome in this response, and different aspects of its communication with other cell organelles such as mitochondria and the ER.

Sponsored by the AECC, we have launched a research line to understand how lipid metabolism and the dynamics of specific organelles are linked to genome integrity and telomere maintenance. Aberrant immortalization mechanisms engaged in specific cancer types are associated with a particular rewiring of lipid metabolism and altered pathways for its compartmentalization, including the formation of nuclear LDs. We intend to characterize these novel links.

We are also studying how the expanding LD receives information from mechanosensing structures located in the plasma membrane (PM), called caveolae *(Aboy et al. Nat Commun 2024)*. These studies could shed light on a very poorly understood event: how the accommodation of volume expansion by the PM of the cell feeds into metabolic control through the regulation of the LD proteome, and the molecular mechanisms underlying certain hereditary muscle dystrophy disorders. We also participate in studies focused on systems-level caveolae biology and their contribution to cell function and physiopathology *(Jiménez-Jiménez et al. IJMS 2025).*

PUBLICATIONS:

Jiménez-Jiménez V, Sánchez-Cabo F, Schwartz MA, Sánchez-Álvarez M§, del Pozo M§ (§ co-correspoding author) A survey for human tissue-level determinants of CAV1 regulation and function. *IJMS*. **2025**, *26(8)*, 3789 DOI: 10.3390/ijms26083789

Sánchez-Álvarez M§, Lolo FN, Fulgoni G, Sailem H, Pascual-Vargas P, del Pozo MA, Vázquez J, López JA, Bakal C§ (§ co-correspoding author). PERK-dependent reciprocal crosstalk between ER and non-centrosomal microtubules coordinates ER architecture and cell shape. *Cell Rep.* **2025**, *44(5)*, 115590. DOI: 10.1016/j.celrep.2025.115590

DOCTORAL THESES AND OTHER WORKS:

Sofía Rodríguez Álvarez

Final degree's project. *Optimización de Casilio, un sistema basado en CRISPR-Cas9 para el estudio de la elongación alternativa de los telómeros (ALT).* Universidad Francisco de Vitoria. 2025. Supervisor/s: Miguel Sánchez Álvarez. Grade: 10/10

FUNDING:

Lipid metabolism and organelle homeostasis: novel opportunities for understanding, detecting and intervening genomic instability and telomeric alterations in tumor cells (LIPALT).". «LAB AECC 2024» LABAE246690SANC Fundación Científica AECC. Miguel Sánchez-Álvarez. Jan 2025-Dec 2028

"Architectural remodelling of the endoplasmic reticulum: an essential component in cell defence responses and homeostasis protection across tissues (DefendER)". «Consolidación Investigadora 2023» CNS2023-144831 MICINN. PI: Miguel Sánchez-Álvarez. Apr 2024-Mar 2026

“Novel mechanisms coupling cell secretion with inflammation control: physiopathological relevance (SECRETMMUNE)”. «Proyectos de Generación de Conocimiento» PID2021-128106NA-I00 MICINN. PI: Miguel Sánchez-Álvarez Jan 2023-Dec 2025

“Cell organelles as signaling hubs in disease: novel roles in mechanoadaption and innate immunity”. Ramón y Cajal contract RYC2020-029690-I MICINN. PI: Miguel Sánchez-Álvarez. Feb 2022-Jan 2027.

Transcriptional Control of Metabolic Homeostasis

TENURED SCIENTIST

Vallejo Fernández de la Reguera, Mario
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Group Coordinator

CONTRACT RESEARCHER

Mirasierra Cuevas, Mercedes

TECHNICAL SUPPORT PERSONNEL

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Velo Alcalde, Marina
Pozos Gil, Laia Welhua

KEYWORDS

Diabetes, Energy expenditure, Hypothalamus, Metabolic homeostasis, Pancreatic islets.

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RESEARCH LINES:

Overview

The interest of our group is the study of the mechanisms that maintain systemic metabolic homeostasis. These mechanisms require the coordinated involvement of peripheral organs such as pancreatic islets, muscle and adipose tissue, whose function is orchestrated centrally by brain nuclei located mostly in the hypothalamus. Although a great deal of our past work has been focused on pancreatic islets, during 2025 we finalized our studies on the regulatory role of the transcription factor Alx3 in the hypothalamic regulation of metabolism and reinitiated earlier studies on the role of Alx3 in the regulation of glucose-stimulated insulin secretion (GSIS) in pancreatic islets.

Hypothalamic regulation of energy metabolism by Alx3

We found that Alx3 regulaties feeding and metabolic partitioning in peripheral organs including muscle and adipocytes. Mice lacking Alx3 display decreased food intake without changes in body weight, along with reduced energy expenditure and altered respiratory exchange ratio. Using magnetic resonance imaging and spectroscopy we found that these mice exhibit increased adiposity and decreased muscle mass, which was associated with markers of motor and sympathetic denervation. When fed with a high-fat diet, Alx3-deficient mice gained weight at a lower rate than wild type animals despite their initial relatively higher adiposity. In addition, by performing glucose and insulin tolerance tests, we found that feeding with high-fat food improves insulin sensitivity in these animals. At the molecular level, gene expression analysis demonstrated altered lipogenic and lipolytic gene profiles, thus indicating the importance of Alx3 in lipid metabolism. We characterized the expression of Alx3 in the arcuate nucleus of the hypothalamus, where we found it present in Agrp and Pomc neurons regulating food intake. In consonance with this finding, using positron emission tomography and functional diffusion-weighted magnetic resonance imaging, we observed that Alx3-deficient mice exhibit selective hypothalamic responses to fasting in the arcuate nucleus, altered expression of Pomc and melanocortin-3 receptor mRNA in the hypothalamus, and impaired regulation of feeding behavior. Thus, this line of research has provided solid evidence on the crucial role for Alx3 in governing food intake, energy homeostasis, and metabolic nutrient partitioning, thereby influencing body mass composition.

Regulation of glucose-stimulated insulin secretion by Alx3

Alx3 plays an important role in the regulation of metabolic homeostasis at the systemic level, not only by its activity in the hypothalamus levels but also by modulating the responses of pancreatic islets to changes in glucose concentrations. Our previous work demonstrates that Alx3-deficient mice retain the ability to respond to GSIS, albeit with a significantly reduced magnitude relative to wild-type animals. We found that GSIS from pancreatic islets ex vivo is significantly reduced in Alx3-deficient mice. Decreased GSIS in Alx3-deficient mice is not directly related with decreased insulin content, because our data indicate that insulin secretion induced by membrane depolarization with 30 mM KCl is similar in wild type and mutant islets. In addition, we found that the levels of glucokinase, a key protein for glucose concentration sensing by β cells, are reduced in these mice. Based on these observations, we have initiated experiments to investigate the mechanisms by which Alx3 deficiency impairs signaling pathways regulating insulin secretion. Gene expression profiling using RNAseq and Gene Set Enrichment Analyses from isolated islets indicated that free fatty acid receptors (FFAR) are likely candidates for altered insulin secretion associated with Alx3 deficiency. Consistently, we have observed that in isolated mouse pancreatic islets, activation of some of these receptors by selective agonists enhances GSIS in wild-type but not in Alx3-deficient mice. Furthermore, glucose tolerance tests indicate that administration of these agonists decrease blood glucose levels in wild type but not in Alx3-mutant animals. We have further characterized the levels of key downstream effectors of the FFAR signaling pathways—PLCβ1, PLCβ3, and Itpr1— and found evidence of disruption of the PLC-IP3-Ca^{2+} signaling axis. We have also studied the possible involvement of components of the cAMP-dependent pathway (Adcy2 and Epac2), which suggests the existence of broader signaling defects. Gene ontology analyses following RNA sequencing revealed altered expression of genes related to endoplasmic reticulum stress, insulin processing and calcium dependent exocytosis. These studies are advanced and a publication is predicted in the following year.

FUNDING:

Multilevel dysregulation of insulin secretion as a pathogenic mechanism of pancreatic islet dysfunction in diabetes. PID2023-150719OB-I00. Ministerio de Ciencia, Innovación y Universidades. 1 de septiembre de 2024 a 31 de agosto de 2027. PI: Mario Vallejo Fernández de la Reguera.

OUTREACH ACTIVITIES:

1er Simposio de islote pancreático (Organizadores: Alberto Bartolomé, Irma García-Martínez y Mercedes Mirasierra). 19 de febrero de 2025.

Event: Día Internacional de la Mujer y la Niña en la Ciencia-Todas Hacemos Ciencia, CSIC. Title: Taller sobre Técnicas Biomédicas en Biomedicina (organizadora, Mercedes Mirasierra). 26 de febrero de 2025.

Mitochondrial Function in Health and Disease

TENURED SCIENTIST

Monsalve Pérez, María
(Investigador Científico, CSIC).
Group Coordinator

Martínez-Costa Pérez, Oscar Hernán
(Profesor Titular, UAM).

Samhan Arias, Alejandro
(Profesor Titular, UAM).

Aragón Reyes, Juan José
(Catedrático Emérito, UAM).

CONTRACT RESEARCHER

Selinger Galant, Leticia
Bernet García-Santesmases

PREDOCTORAL SCIENTIST

Doblado Bueno, Laura
Hidalgo López, Manuela

MASTER THESIS STUDENT

Sastre Arcones, Marta
Porta Roca, Alba
Solla Márquez, Paula
Moreno Maleno, Jesús Miguel

UNDERGRADUATE STUDENT

López Gutiérrez, Carla
Moreno Venegas, Olivia
Sánchez Cabeza, Carlos
Nieto Entrena, Isabel
Menéndez Orejas, Irene
Quintanilla Capawana, Ángela

VISITING SCIENTIST

Casaus Pielago, Ana
Llaque Puertas, Andrea Belén
Peleman, Carlijn
Pilatasig Hurtado, Daritza

KEYWORDS

CRC, CVD, HCC, Intestinal Dysbiosis, Intestinal Inflammatory Disease, IOs, Mitochondria, oxidative metabolism, oxidative stress, MASLD, PGC-1α, T2D.

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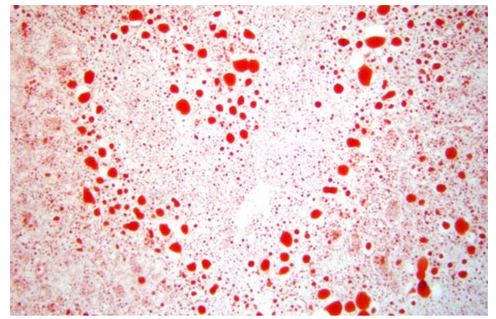
RESEARCH LINES:

Overview
Our laboratory is interested in the study of the regulation of mitochondrial function and the role played by mitochondrial oxidative stress in human pathology, with a special interest in cardiovascular diseases (CVD), metabolism associated steatotic liver disease (MASLD), type 2 diabetes (T2D), cancer (hepatocellular carcinoma, colorectal cancer, thyroid cancer) and intestinal inflammatory disease.

Cardiovascular diseases (CVD)
Researchers involved: Doblado Bueno, Laura; Sastre Arcones, Marta; Monsalve Pérez, María
The first line of investigation is currently focused on understanding how the impact of atypical antipsychotics (AAPs) on mitochondria drives CVD development.

Hepatocellular Carcinoma (HCC)
Researchers involved: Hidalgo López, Manuela; López Gutiérrez, Carla; Monsalve Pérez, María.
The second line of research aims to elucidate the impact of the loss in mitochondrial plasticity on HCC development and the mechanisms involved.



Colorectal Cancer (CRC)
Researchers involved:Moreno Venegas, Olivia; Hidalgo López, Manuela; Belén Llaque Puertas, Andrea; Monsalve Pérez, María.
The third line of research focuses on understanding how the systemic metabolism impacts on CRC development.

Type 2 Diabetes (T2D)
Researchers involved: Solla Márquez, Paula; Casaus Pielago, Ana; Porta Roca, Alba; Doblado Bueno, Laura; Monsalve Pérez, María.
The fourth line of research investigates how mitochondrial dysfunction drives the development of CVD in T2D subjects.

Intestinal Inflammatory Disease (IID)
Researchers involved: Selinger Galant, Leticia; Bernet García-Santesmases, Clara; Peleman, Carlijn; Monsalve Pérez, María.
The fifth line of research studies how nutrition modifies both systemic and intestinal microbiome oxidative metabolism impacting on intestinal immune-metabolic health.

PUBLICATIONS:

Selinger Galant, L.; Doblado, L.; Radi, R.; Teixeira da Rocha, J.B.; Fabro de Bem, A.; Monsalve, M. Differential effects of diphenyl diselenide (PhSe)2 on mitochondria-related pathways depending on the cellular energy status in endothelial cells. *bioRxiv* (preprint). **2025**, https://doi.org/10.1101/2024.06.14.599060.

Doblado, L.; Estebaranz C.; Carrillo, E.; Samhan-Arias, A.K.; Nova, E.; García-Perea, E.; Marcos, A.; Díaz, L.E.; Monsalve, M. Alkalinized Filtered Water Induces Changes in the Gut Microbiome in Inflammatory Bowel Disease. *Submitted* (AMS-19844-2025-01). bioRxiv. **2025**. https://doi.org/10.1101/2025.09.19.677298.

DOCTORAL THESES AND OTHER WORKS:

Alba Porta Roca
Master´s thesis. *Targeting mitochondria: the impact of hypoglycemic drugs in type 2 diabetes and its connection to neurocognitive disorders.* Universidad Autónoma de Madrid. 2025. Supervisor: María Monsalve Pérez. Grade: Sobresaliente.

Marta Sastre Arcones
Master´s thesis. *Impacto de la mitotoxicidad derivada de la ingesta crónica de fármacos en el riesgo cardiovascular.* Universidad Complutense de Madrid. 2025. Supervisor: María Monsalve Pérez.

Paula Solla Márquez
Master´s thesis. *Evaluación de la plasticidad mitocondrial como predictor del riesgo cardiovascular en diabetes tipo II.* Universidad Complutense de Madrid. 2025. Supervisor: María Monsalve Pérez. Grade: Sobresaliente.

Olivia Moreno Venegas
Final degree's project. I*dentificación y evaluación de biomarcadores metabólicos para el seguimiento de la respuesta al tratamiento en cáncer de colon.* Universidad Autónoma de Madrid. 2025. Supervisor: María Monsalve Pérez.

Carla López Gutiérrez
Final degree's project. *Estudio del papel de la proteína PGC-1α en el desarrollo de MASLD a carcinoma hepatocellular.* Universidad de Alcalá de Henares. 2025. Supervisor: María Monsalve Pérez. Grade: Sobresaliente.

FUNDING:

Caracterización del papel jugado por el estres oxidativo mitocondrial en patología humana. PID2021-122765OB-I00. Ministerio de Ciencia e Innovación. 01/09/2022-31/08/2025. María Monsalve Pérez.

Linking Intestinal Bacteria and Host Metabolism to Tackle Type 2 Diabetes with Novel Food (DiBaN). GAP 101162517. European Innovation Council.01/10/2024-30/09/2028. María Monsalve Pérez & Ángela Martínez Valverde.

Enfermedades Metabólicas, COMETA. Conexión CSIC. 2025-2026. Lisardo Bosca &Marta Casado.

Mechanisms connecting mitochondrial function with cell transformation (MinT). PID2024-159721OB-I00. Ministerio de Ciencia, Innovación y Universidades. 01/09/2025-31/08/2029. María Monsalve.

Evaluación del efecto del consumo de agua alcalina sobre el proceso de envejecimiento (AGEN). ALKANATUR S.L.U. Contrato de Apoyo Tecnológico. 15/12/2025-14/12/2028. María Monsalve.

AWARDS:

Best TGF Award of the Colegio Oficial de Biólogos de Madrid to Carla López Gutierrez. Madrid. 2025.

OUTREACH ACTIVITIES:

X: @MitoxiLabIIBm

Linkedin: linkedin.com/in/maría-monsalve-475b2671.

Conference. Dia de la mujer y la ciencia. IES Senda Galiana, 2025.

Conference. Ciclo Conferencias CSIC dirigidas al sitema educativo de la CAM. ICFP Santa Rosa de Lima, 2025.

Conference. Programa Científicos en Prácticas. Unidad de Cultura Científica del CSIC. IES Santa Eugenia, 2025.

Visit to the IIBM. Programa Científicos en Prácticas de la Unidad de Cultura Científica del CSIC. IES Santa Eugenia, 2025.

Visit to the IIBM. Semana de la Ciencia del CSIC “Ruta Científica: Trabajar en un Instituto de Biología Molecular”. FP Superior UEM, 2025.

One-week long practical training for Secondary School Sudents. IES Santa Eugenia, 2025.

Oxygen Homeostasis in the Cardiovascular System

TENURED SCIENTIST

Martín Puig, Silvia
(Científico titular). Group Coordinator

PREDOCTORAL SCIENTIST

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Urra Balduz, Sonia
Castro Mecinas, Rosana

UNDERGRADUATE STUDENT

Mateo Rueda, Irene

TECHNICAL SUPPORT PERSONNEL

Novillo Pérez, Miriam
Martín Yáñez, Laura
Mejías De Frutos, Eva

KEYWORDS

Cardiovascular Diseases, Heart, HIF, Hypoxia, Oxygen, Pulmonary Diseases, VHL

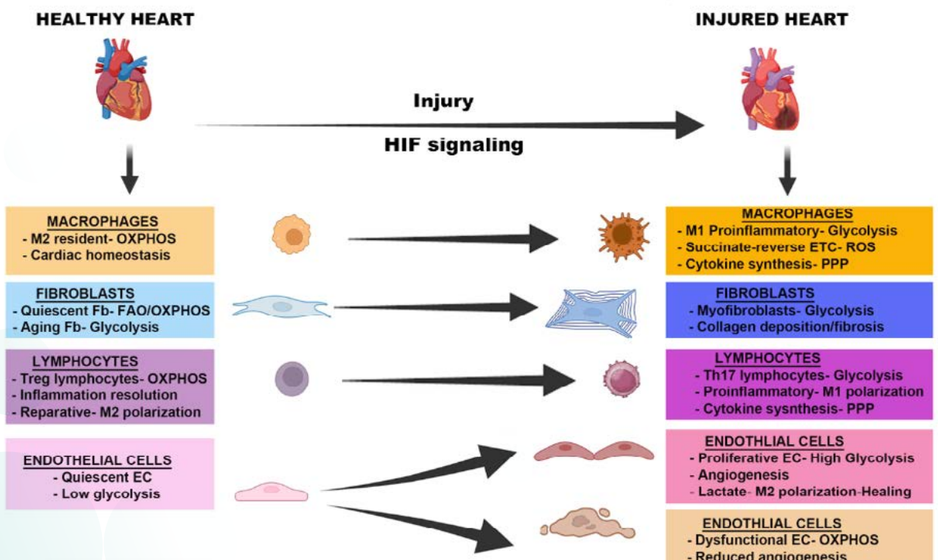

Figure 1. HIF signaling and Cardiac Metabolism

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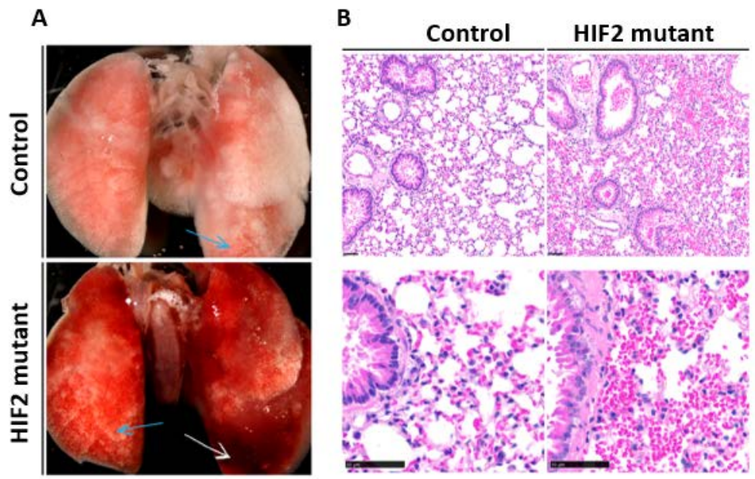

Figure 2. Pulmonary defects in HIF2/Wt1 mutants exposed to chronic hypoxia

Pulmonary defects in HIF2/Wt1 mutants exposed to chronic hypoxia. (A). Whole mount hearts of control (Top, Hif2 flox/flox-Wt1-Cre+/+) and HIF2 KO (Bottom, Hif2 flox/flox-Wt1-Cre+/-) showing significant hemorrhages in the mutant mice. Blue arrows point to well-oxygenated regions, while the white arrow highlights collapsed areas of the lung. (B). Histological cardiac sections stained with hematoxylin and eosin from control (Left panels, Hif2 flox/flox-Wt1-Cre+/+) and HIF2 KO (Right panels, Hif2 flox/flox-Wt1-Cre+/-) mice showing reduction of the alveolar space and substantial erythrocyte accumulation within the lung parenchyma on the HIF2 mutant mice. Bottom panels are magnifications of top panels.

RESEARCH LINES:

Overview

Cardiovascular diseases (CVD) represent the main cause of death and their high prevalence implies a high health cost, in addition to increasing physical dependence and reducing the quality of aging of the population. Therefore, understanding the molecular basis of CVD is a priority to mitigate the high number of deaths and current chronic patients. Likewise, knowledge of the mechanisms involved in correct cardiac formation and function could contribute to developing health prevention strategies. The canonical response to **hypoxia** provides a ubiquitous mechanism of adaptation to low oxygen supply. The varie-

ty of processes regulated by hypoxia include *metabolic reprogramming, vascularization, immune response modulation, pluripotency, differentiation and survival or migration,* among many others. Therefore, the **pathophysiology of hypoxia** is broad and complex, and it is a clinical priority to unravel the molecular mechanisms that link **HIF**-mediated signaling with highly prevalent human diseases, such as metabolic disorders or CVD. **Oxygen** is *an essential modulator* of the **cardiovascular system** and is involved in the appearance and evolution of numerous CVD.

The general objective of our group is focused on **understanding how hypoxia signaling impacts cardiovascular development and homeostasis** and on **defining** the **molecular mechanisms that orchestrate adaptation to changes in oxygen levels in both physiological and pathological conditions.** To determine the function of HIFs and other hypoxia pathway elements during homeostasis and cardiac pathology, we have generated new genetic mouse models of gain or loss of function of the hypoxia signaling pathway in different cardiac populations to mimic CVD and investigate new molecular mechanisms that connect alterations in oxygen homeostasis with cardiovascular pathology. In addition, we have established a clinical collaboration network to investigate the molecular basis of low-prevalence pediatric vasculitis and understand the role of hypoxia in its onset and progression. Our specific scientific interests are depicted bellow.

Decipher the role of the VHL/HIF axis in the development and maturation of the heart

One of our research lines is dedicated to investigating the influence of HIF signaling during heart development and maturation. We have discovered the fundamental role of VHL/HIF1 axis signaling in the establishment of metabolic territories in the embryonic heart that are essential for myocardial maturation and proper formation of the ventricular chambers. Our studies reveal that HIF1 signaling controls a metabolic switch in embryonic cardiomyocytes from glycolysis to fatty acid oxidation (*Developmental Cell, 2016; STAR Protoc, 2021)*. Furthermore, we have determined that in the absence of glycolysis in HIF1 null cardiomyocytes there is an activation of compensatory mechanisms based on amino acids transport and consumption in parallel to HIF2 and ATF4 expression *(iScience 2021, Adv Exp Med Biol, 2024*). Moreover, our research points to new functions of VHL and HIF2 in the proper formation of cardiac valves that are under investigation. In addition, we are exploring the impact of maintaining HIF signaling activation on the postnatal cardiomyocyte maturation and cardiac performance. We are determining the influence of HIF signaling in neonatal cardiomyocyte metabolism, sarcomeric and mitochondrial structure, proliferation ability and additional maturational hallmarks like binucleation and electrical coupling. Moreover, we are interested in exploring the crosstalk between cardiomyocytes and other cardiac cell types in close contact with them and to determine whether changes in hypoxia could modulate these cell-cell interactions.

Characterization of the cellular and molecular events controlled by HIF transcription factors and VHL in coronary homeostasis and vascular pathologies

A second line of interest is dedicated to exploring the influence of HIF signaling in the stability of coronary vessels and capillaries. We have characterized the impact of HIF activation in epicardial progenitors (labelled by Wt1: Wilms tumor 1) contributing to endothelial and vascular smooth muscle cells of the coronary vessels and cardiac fibroblasts, finding that mutant mice develop several vascular and myocardial alterations. We have elucidated the critical role of HIF2 in the development of cardiovascular abnormalities and inflammation by means of RNA sequencing, genetic mouse models, and functional assays (Escobar B et al. under revision). Furthermore, we have established a clinical network of collaborators to explore the influence of HIF signaling in pediatric rare diseases displaying vascular inflammation and cardiac complications.

To further understand the relative impact of HIF signaling from endothelial and vascular smooth muscle cells, we have generated novel mouse models of hypoxia activation in these lineages. We are currently characterizing the phenotype of these mutants by classical histology, molecular analysis, advanced cardiac imaging and functional analysis.

Understand the importance of hypoxia-mediated signaling in cardiac regeneration

In the lab we are also exploring the influence of HIF transcription factors in cardiac regeneration during the neonatal window, when mammalian hearts can still response to a cardiac insult by efficient proliferation of pre-existing cardiomyocytes. To this aim, we are performing left anterior descending coronary artery ligation of neonatal pups deficient for HIF1, HIF2 or both isoforms in the epicardium or cardiomyocytes at day 0 (efficient regeneration) or day 7 (non-efficient regeneration) and investigating whether HIF mutants display any beneficial or detrimental change in terms of cardiac function (measured by echocardiography), scar size or inflammatory resolution after myocardial infarction. In addition, we will explore the impact of transient activation of HIFs during the onset of myocardial infarction in the regenerative capacity of neonatal and adult hearts.

Identifying novel molecular mechanisms connecting hypoxia signaling with CV diseases

Finally, we are interested in the identification of molecular mechanisms that connect HIF signaling with the development and progression of CV diseases like cardiac hypertrophy or pulmonary hypertension. On the one hand, we are investigating how endothelial cells, pericytes and fibroblasts with activation of the hypoxia pathway could signal to cardiomyocytes and favor indirect hypertrophic responses using novel genetic models in these cellular compartments. On the other hand, we are uncovering novel roles of HIF2 in the response to chronic hypoxia beyond its classical relevance in pulmonary endothelial cells. Our results showed that the absence of HIF2 in the Wilms-tumor 1 (Wt1) lineage protects against pulmonary vascular remodeling and elevation of right ventricular systolic pressure. However, HIF2/Wt1 mutants displayed microvascular instability, inflammation and pulmonary hemorrhages, with significant reduction of the alveolar space. Moreover, HIF2 mutant mice present cardiac abnormalities (ventricular hypertrophy and left ventricular systolic dysfunction) together with carotid body dysfunction. These results highlight novel protective roles of HIF2 in the adaptation to chronic hypoxia and warn on the design of therapeutic strategies for pulmonary hypertension based on HIF2 pharmacological inhibition *(ATVB, 2025).*

PUBLICATIONS:

Albendea-Gomez T, Mendoza-Tamajon S, Castro-Mecinas R, Escobar B, Ferreira Rocha S, Urra-Balduz S, Nicolas-Avila JA, Oliver E, Villalba-Orero M, Martin-Puig S. Vascular HIF2 Signaling Prevents Cardiomegaly, Alveolar Congestion, and Capillary Remodeling During Chronic Hypoxia. *Arterioscler Thromb Vasc Biol.* **2025** *Mar;45(3)* :e78-e98. DOI: 10.1161/ATVBAHA.124.321780

DOCTORAL THESES AND OTHER WORKS:

Teresa Albendea Gómez

Ph.D. thesis. *Caracterización de nuevas funciones de HIF2a en fisiopatología cardiovascular en respuesta a hipoxia crónica.* Universidad Francisco de Vitoria. 2025. Supervisor: Silvia Martín Puig. Grade: Sobresaliente Cum Laude (por unanimidad).

Irene Mateo Rueda

Final degree's project. *Descifrando el papel de la señalización por hipoxia en progenitores cardiovasculares Isl1.* Universidad Autónoma de Madrid. 2025. Supervisor: Silvia Martín Puig. Grade: Matrícula de Honor (9.8)

FUNDING:

Disfunción vascular en hipertrofia cardiaca isquémica: identificación de nuevos biomarcadores y tratamientos basados en el uso de hiperoxia. CARDIO.COM. Área de Biomedicina y Salud. Fundación Domingo Martínez. 2023-2025. PI: Silvia Martín Puig

Bioingeniería de células satélite de músculo esquelético como nueva estrategia de diferenciación a cardiomiocitos y regeneración cardiaca. CARDIOBOOST-CM (P2022/BMD-7245). Comunidad de Madrid. 2023-2026. PI: Silvia Martín Puig

Deciphering the role of HIFs in myocardial maturation and cardiac regeneration. OxyHeart. PID2023-149528OB-I00. Ministerio de Ciencia, Innovación y Universidades. 2024-2027. PI: Silvia Martín Puig

AWARDS:

Best Flash Talk IIBM Retreat 2025. Residencia La Cristalera, Miraflores de la Sierra, Madrid. 6-7 November 2025.

OUTREACH ACTIVITIES:

11F Día internacional de la Mujer y la Niña en la Ciencia. Cuidamos nuestro corazón. Colegio Rabindranath Tagore. 11 de Febrero 2025. Susana Mendoza Tamajón y Silvia Martín Puig

11F Día internacional de la Mujer y la Niña en la Ciencia. Ciencia y género: retos para eliminar el sesgo de género en la ciencia. Colegio Legamar. 11 de Febrero de 2025. Irene Mateo Rueda

Semana de la Ciencia. Proteins, the machines of life. Colegio Rabindranath Tagore. 26 de Noviembre de 2025. Silvia Martín Puig

Semana de la Ciencia. Juegos de mesa científicos. Organizada por el club de juegos Mecatol Rex en colaboración con el Consejo Superior de Investigaciones Científicas (IIBM-CSIC y CIB-CSIC). Madrid, 22 de noviembre de 2025. Susana Mendoza Tamajón.

Immunity, Immunopathology and Emergent Therapies

TENURED SCIENTIST

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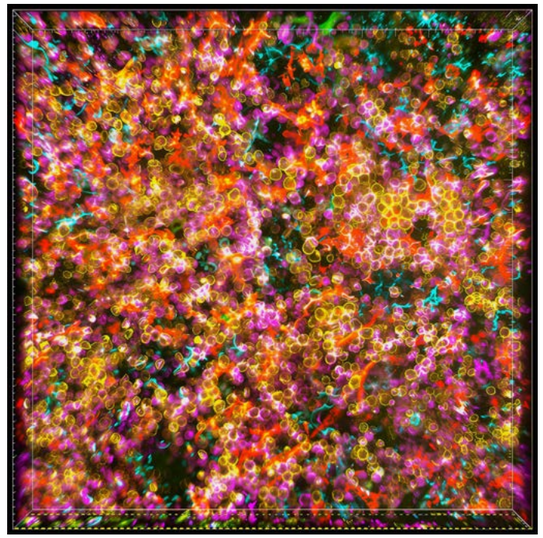
KEYWORDS

Immunotherapy, T cells, CAR, Macrophages, LXR, Immunometabolism



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RESEARCH LINES:

Overview

Our group goal is to understand the molecular mechanisms that control the metabolic homeostasis of the immune system and the pathologies derived from its deregulation, including infectious, inflammatory, neoplastic and autoimmune processes, many of which still lack a cure. We also have a translational vocation with projects focused on the development of new approaches and immunotherapeutic tools against cancer.

Development of a new CAR targeting B cell neoplasias and new therapeutic tools for cancers with bad prognosis

Researchers involved: Zapata, Juan Manuel; Pérez Chacón, Gema; Aldea, Marcos; Gaibar, María, Smith Gallego, Cristina; González Muñoz, Celia, Anubla, Irene.

Overview:

Chimeric antigen receptor (CAR) T-cell therapy has demonstrated remarkable efficacy against some B-cell leukemias and multiple myeloma, but the development of refractory/relapsed disease is common. Furthermore, effective treatments for many types of B-cell lymphoma have not yet been achieved. Follow-up of patients treated with CARs targeting these neoplasms shows that, in some cases, more than 50% of patients relapse. Among the most important factors causing the failure of this therapy are the loss of target antigen expression and the lack of efficacy and persistence of the CAR T cells. In fact, between 30% and 70% of patients with relapsed disease after CAR T-cell treatment have lost expression of CD19 or BCMA, two of the target antigens used in the development of CARs. Therefore, it is necessary to identify new targets in B-cell malignancies for the development of CAR T cells that can offer better personalized options to patients and help prevent or limit antigen escape.

Current Research:

We have developed a CAR based on a single-chain variable region (scFv) derived from an anti-CD45 antibody, developed by us, that recognizes an epitope in the RA region of CD45 (CD45RA*). This epitope is restricted to B cells, both normal and malignant, and is not expressed in T lymphocytes or activated NK cells; therefore, these CAR cells lack fratricidal activity. Furthermore, this target is not expressed in stem B cells nor in B memory and plasma cells, so the patient would maintain intact his/her acquired humoral immunity. This CAR is functional and readily forms immune synapses similar to those by the TCR.

In addition, we have generated lentiviral vectors for 4th generation CAR T/NK (TRUCKs, T cells redirected for universal cytokine-me-

diated killing) that use metabolites and physiological conditions found characteristic of tumors, such as glioblastoma and osteosarcoma, to trigger the production of anti-tumor cytokines. Expression of these cytokines are under the control of specific promoters activated by these metabolites and would restrict their expression to the tumor, minimizing the risk of cytokine release syndrome.

Macrophages, Nuclear Receptors and Inflammation

Researchers involved: Castrillo, Antonio; Alemany, Susana; Guerrero, Erika; Navarro, Eliezer

Overview:

Inflammation is a protective response against infections and injuries, but if it progresses uncontrolled, it can aggravate several diseases, such as diabetes, cancer, or cardiovascular diseases. During the onset and resolution/repair phases of inflammation, macrophages are particularly important cells. Macrophages are a type of immune cell present in all tissues, with a great functional diversity in different anatomical locations. Macrophages are specialized in the protection of the organism against foreign elements, mainly through their excellent phagocytic and inflammatory capacity, and also participate in multitude homeostatic processes in the different sites of residence. Thus, macrophages contribute to the homeostatic and inflammatory balance in all tissues.

Our studies are focused on understanding the functions of macrophages in different tissues and different pathophysiological situations, through the control of their activities exerted at the transcriptional level by members of the nuclear receptor superfamily.

LXR Nuclear Receptors:

Liver X receptors (LXRα and LXRβ, encoded by Nr1h3 and Nr1h2 respectively) play crucial roles in mammalian cholesterol homeostasis, and are also involved in the inflammatory response. LXRα is expressed in liver, adipose tissue, intestine and macrophages, whereas LXRβ is ubiquitously expressed. LXRs function together with Retinoid X Receptors, RXRs, and their endogenous ligands include several intermediates of the cholesterol biosynthetic pathway, termed oxysterols. Mechanistically, LXRs positively regulate gene expression through direct binding to DNA response elements (LXREs) within the regulatory regions of target genes. Endogenous activation of LXR in macrophages occurs, for example, when macrophages accumulate cholesterol derived from lipoprotein uptake or cellular debris, which triggers the induction of a transcriptional program to promote cholesterol utilization. This is believed to be one of the crucial functions of LXR in tissues. However, LXR also plays important roles in inflammatory macrophages in response to injury or infection. Genetic deletion of LXR results in defective innate immunity against various pathogens, suggesting that endogenous LXR activity potentiates innate immune responses. The molecular mechanisms that activate innate immune pathways and their connections with endogenous LXRα or LXRβ activities have not been explored in depth, and is one of the main interests of our group.

Current Research:

In recent years, scientific progress has increased the spectrum of tissue macrophage activities, broadening the range of macrophage identities, their plasticity and heterogeneity, derived from their tissue-specialized properties. In fact, there are certain organs, such as lymphoid, or metabolic tissues such as the liver, or secondary lymphoid organs, that present several distinct macrophage subtypes, and whose individual functions have not been studied in depth. Our group has made an important contribution to unraveling the mechanisms of macrophage action in experimental mouse models. In particular, our work has shown that LXR transcription factors regulate several macrophage functions, including control of the inflammatory response, defense against pathogens, and their involvement in phagocytosis and functional specialization of the different macrophages present in lymphoid tissues such as the spleen. Our current interests are oriented towards the cellular and molecular study of the nuclear receptor LXRα in macrophages, through in vitro and in vivo studies with mouse models of LXRα deficiency, and knockin transgenics of conditional absence or reporter mice. Our recent results suggest that LXRα exhibits distinct activities in macrophages. On one hand, in healthy tissues it exerts homeostatic functions in certain subtypes of tissue-resident macrophages, and on the other hand, in situations of inflammation or infection it promotes antimicrobial and inflammatory polarization actions in recruited, monocyte-derived, macrophages. Specifically, we approached these studies from 2 main angles. First, we study the role of LXRα in the differentiation and transcriptional activity of tissue-resident macrophages. Using genomic strategies we search for the genome-wide localization of LXRα, and we will analyze by proteomic approaches the molecular interactions of LXRα in different situations, using tissue-derived macrophages and with macrophages in culture. The second major direction of our research will aim to analyze the function of LXRα in macrophages in pathological situations through inflammation and infection models. We envision that our contributions may translate into future strategies for therapeutic intervention in diseases by manipulating macrophage activity.

PUBLICATIONS:

Baena-Nuevo M; Vera-Zambrano A; Martinez-Laperche C; Buño I; Muñoz-Calleja C; Valenzuela C; Zapata JM; Perez-Chacon G. Indole-3-carbinol is an inhibitor of KV1.3 potassium voltage-gated channel activity in chronic lymphocytic leukemia cells. *Eur J Pharmacol.* **2025**; *1007*:178281. DOI: 10.1016/j.ejphar.2025.178281.

Sánchez-García, S.; Povo-Retana, A.; Marin, S.; Madurga, S.; Fariñas, M.; Aleixandre, N.; Castrillo, A.; de la Rosa, JV.; Alvarez-Lucena, C.; Landauro-Vera, R.; Prieto, P.; Cascante, M.; Boscá, L.; Immunometabolic Effect of Nitric Oxide on Human Macrophages Challenged With the SARS-CoV2-Induced Cytokine Storm. A Fluxomic Approach. *Adv Healthc. Mater.* **2025** *Jan;14(1):* 2401688-2401688. DOI: 10.1002/adhm.202401688.

Glaría, E.; Martínez, PR.; Font-Díaz, J.; De la Rosa, JV.; Castrillo, A.; Crawshaw, DJ.; Vidal Taboada, JM.; Saura, J.; Matalonga, J.; Chini, E.; Caelles, C.; Valledor, AF.; Liver X Receptors and Inflammatory-Induced C/EBPβ Selectively Cooperate to Control CD38 Transcription. *J. Innate Immun*. **2025**;*17(1)*:56-77. DOI: 10.1159/000543274.

DOCTORAL THESES AND OTHER WORKS:

Celia González Muñoz
Master Thesis. *"Explotando el microambiente tumoral para combatir el cáncer: Caracterización de la expresión y función de un nuevo vector para CAR-T de 4ª generación"* Supervisor/s: Gema Pérez Chacón & Juan Manuel Zapata Hernández. Grade: Sobresaliente (9).

FUNDING:

"Reprogramación Transcripcional Del Receptor Nuclear LXRalfa Y El Control De La Identidad De Los Macrofagos En Homeostasis E Inflamacion. PID2022-137696OB-I00". Agencia Estatal de Investigación (AEI), Ministerio de Ciencia, Investigación y Universidades. 2023-2026.

"Reprogramación De Macrófagos Como Estrategia Terapéutica Frente A Covid-19 Severo". (RETAR-A-COVID-CM). P2022/BMD-7274. Entidad financiadora: Comunidad de Madrid CAM 2022. Años: 2022-2025. Consorcio. Antonio Castrillo Viguera.

"New Functions of TRAF1 in T Lymphocyte immune Responses, Homeostasis and Aging. PID2022-136909OB-I00. 01/09/2023-31/08/26. Entidad financiadora: Agencia Estatal de Investigación (AEI), Ministerio de Ciencia, Investigación y Universidades y Fondo Europeo de Desarrollo Regional (FEDER), 2023-2026. Juan Manuel Zapata Hernández

"Next Generation multitarget STAB and CART Immunotherapies". Consorcios en Biomedicina CAM. P2022/BMD-7225. Miembros del Consorcio: Antonio Pérez-Martínez (UAM, IdIPaz), Juan M Zapata (CSIC), Luis Álvarez Vallina (H12O), Manuel Ramirez Orellana (FIBHNJS), Javier García Castro (ISCIII), Beatriz Martín Antonio (FJD). 01/01/2023-31-12-2026.

"Análisis de las alteraciones en expresión génica en respuesta al tratamiento combinado fludarabina/Indol-3-carbinol en células de leucemia linfática crónica: identificación del mecanismo de acción" Fludaindol. Ayudas a la Investigación Vicerrectorado de Investigación, Ciencia y Doctorado. Universidad Camilo José Cela.". 2025. Marcos Aldea Romero y Gema Pérez Chacón.

OUTREACH ACTIVITIES:

Semana de la Ciencia. Taller científico: Explorando la biología celular: Detección de mutaciones y marcaje celular obre el aislamiento del DNA. November 27, 2026

Laboratory recipient of awardees of the Spanish Biology Olympiad. July 2025.

Laboratory participant in the program “Científicos en Prácticas” (CSIC and Spanish Association for Science Advancement), aimed at young people in the 3rd year of Compulsory Secondary Education (ESO) and 1st year of the Curricular Diversification Program (PDC), from public Secondary Education Institutes. July 2026.

Participant in the program Cienciaterapia, Bringing science to hospitalized children with interactive workshops and safe experiments with everyday materials. November 2025

SCIENTIFIC REPORT 2025

department

of Neurological Diseases & Aging

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Neuroprotective Strategies in Neurodegenerative Diseases

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Fernández Ginés, Raquel
Carnicero Senabre, Daniel
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KEYWORDS

Chronic Diseases, Inflammation, NASH, NRF2, Neurodegeration, Oxidative.

RESEARCH LINES:

Overview

Aging is the main factor contributing to non-communicable diseases such as neurodegenerative Parkinson's (PD) and Alzheimer's (AD) diseases and non-alcoholic steatohepatitis (NASH/MASH). These chronic, incurable diseases can have debilitating effects for years. Many degenerative organ changes stem from local stress networks, such as oxidative stress, closely related to inflammatory and proteotoxic stress. The research team studies protective mechanisms to maintain homeostasis and how these mechanisms could be targeted pharmacologically. The team is focusing on the transcription factor NRF2, which regulates genes involved in stress responses and metabolism. Using rodent models and pharmacological approaches, we are investigating the role of NRF2 in protecting against oxidative damage and neuroinflammation in models of neurodegenerative diseases as well as in protectin the liver for fat accumulation, fibrosis and inflammation in NASH models. Our main objectives are related to: 1) Understand the mechanisms of NRF2 regulation.2) Determine its role in protection against unwanted redox alterations, chronic inflammation and metabolic disturbances, 3) Find putative biomarkers of NRF2 activity 4) Identify novel drugs targeting NRF2 that could be translated to clinical practice.

Targeting NRF2 for Neuroprotection: Investigating Small Molecule Therapeutics for Alzheimer's Disease.

Researchers involved: García-Yagüe, AJ; Rojo, AI; Escoll, M; Carnicero-Senabre, D; Cuadrado, A.

We explored the potential of NRF2 activation as a neuroprotective strategy in Alzheimer's disease (AD) by investigating small molecule therapeutics. In colaboratio nwith Prof. Masayuki Yamamoto (Tohoku University, Sendai, Japan), we analyzed the effects of CDDO-2P-Im, a potent NRF2 inducer, in two AD mouse models: AppNLGF and APP/TAU and found . We found that APP/TAU mice exhibited a milder AD phenotype compared to AppNLGF mice, making them more suitable for assessing early-stage interventions. Our treatment experiments revealed that CDDO-2P-Im significantly reduced Aβ42 accumulation in APP/TAU mice without affecting Aβ40 levels. This reduction led to a lower Aβ42/Aβ40 ratio, a key indicator of amyloid plaque formation. We further examined whether CDDO-2P-Im reached the brain and successfully activated NRF2 target genes. Using LC-MS/MS, we detected CDDO-2P-Im in the brains of treated mice. We also observed increased expression of detoxification and antioxidant genes, including Nqo1, Ugt1a6, and Gclc, confirming NRF2 pathway activation. Additionally, we found that CDDO-2P-Im elevated glutathione (GSH) levels in the brain, which likely contributed to its protective effects against oxidative stress. Our findings suggest that NRF2 activation through small molecule therapeutics can effectively modulate AD-related pathology by reducing amyloid accumulation and enhancing antioxidant defenses. These results support the potential of NRF2 inducers as early-stage therapeutic candidates for AD.

Advancements in Blood-Brain Barrier Integrity and Neurodegenerative Disease Prevention

Researchers involved: Cazalla E; García-Yagüe AJ.; Escoll, M; A; Rojo, AI; Cuadrado, A..

In 2024, our team conducted extensive research on the role of the transcription factor NRF2 in maintaining the integrity of the Blood-Brain Barrier (BBB) and its implications for neurodegenerative disease prevention. We analyzed the mechanisms by which NRF2. Our findings suggest that NRF2 plays a crucial role in protecting the BBB from damage caused by oxidative stress and inflammation, which are common pathological features in neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease (PD), and Huntington's disease (HD). We found that NRF2 activation enhances the expression of tight junction proteins, such as Claudin-5 and

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Occludin, which are essential for maintaining BBB integrity. Additionally, NRF2 reduces the expression of matrix metalloproteinases (MMPs), which are known to degrade BBB components, thereby preventing BBB disruption. Our results also indicate that NRF2 mitigates neuroinflammation by suppressing pro-inflammatory cytokines and promoting antioxidant responses, which are critical for neuronal protection. We also explored the therapeutic potential of various NRF2 inducers, including phytochemicals like sulforaphane, which showed promising results in preclinical studies. By enhancing NRF2 activity, we believe it is possible to mitigate BBB dysfunction and slow the progression of neurodegenerative conditions.

Development and Pharmacokinetics of NRF2-Activating Compounds for Brain Disorders.

Researchers involved: Fernández-Ginés, R; Míguez, R; Olazabal-Chias M; García-Yagüe, AJ.; Rojo, AI Cuadrado, A.

We have investigated the therapeutic potential of NRF2-activating compounds for brain disorders, focusing on their development and pharmacokinetic properties. We started by reviewing the current literature on various NRF2 activators, including synthetic triterpenoids, natural compounds, and electrophilic agents, known for their ability to enhance antioxidant and detoxification pathways in the brain. Then, we analyzed the structure-activity relationships of these compounds, identifying key structural features that contribute to their NRF2-inducing efficacy and blood-brain barrier permeability and neurodegeneration. Our results suggest that specific structural modifications can significantly enhance the pharmacokinetic properties of NRF2 activators, leading to improved brain delivery and target engagement. In particular, we found that CDDO-2P-Im derivatives exhibit prolonged NRF2 activation in the mouse brain.

NRF2 as a Therapeutic Target for Non-Alcoholic Steatohepatitis (NASH): Addressing Oxidative Stress and Lipid Metabolism.

Researchers involved: Fernández-Ginés, R; Escoll, M; Carnicero-Senabre, D; Jiménez-Villegas, J; García Yagüe, AJ.; Rojo, AI; Cuadrado A.

We investigated the role of NRF2 as a therapeutic target in non-alcoholic steatohepatitis (NASH) by addressing oxidative stress and lipid metabolism. We focused on the NRF2/β-TrCP interaction as a novel approach to modulate NRF2 activity in the liver while avoiding the side effects associated with KEAP1 inhibition. We assessed the effects of PHAR, a selective NRF2/β-TrCP protein-protein interaction inhibitor, in liver cells and a STAM mouse model of NASH. Our experiments demonstrated that PHAR effectively activated NRF2 in hepatocytes, Kupffer cells, and hepatic stellate cells, leading to an increase in antioxidant and metabolic gene expression. PHAR also suppressed lipopolysaccharide (LPS)-induced inflammation in Kupffer cells and attenuated TGF-β-induced fibrotic responses in hepatic stellate cells. Using the STAM model, which mimics the full spectrum of human NAFLD, we analyzed PHAR's effects on liver fat accumulation, oxidative stress, inflammation, and fibrosis. MRI imaging revealed that PHAR significantly reduced hepatic steatosis, and molecular analysis showed a reduction in inflammatory markers such as IL-6 and TNF-α, as well as oxidative stress indicators including glutathione (GSH/GSSG ratio), malondialdehyde (MDA), and carbonylated proteins. Histological analysis confirmed that PHAR reduced hepatocellular lipid accumulation and inflammation. Furthermore, PHAR treatment attenuated fibrosis progression, as evidenced by decreased collagen deposition (Sirius red staining) and reduced expression of fibrotic markers such as α-SMA (Acta2), collagen genes (Col1a1, Col3a1), and pro-fibrotic signaling molecules (TGF-β, PDGF). Our transcriptomic analysis indicated that PHAR upregulated anti-fibrotic genes (Plg, Serpina1a, Bmp7) while downregulating pro-fibrotic (Acta2, Col3a1), extracellular matrix remodeling (Mmp3, Mmp9, Timp1), and inflammatory (Nfkb1, Il1b, Ccl3) genes. These findings suggest that NRF2 activation via β-TrCP inhibition provides a milder but effective therapeutic strategy against NASH without the adverse effects observed with strong NRF2 activation. Overall, our results support PHAR as a promising therapeutic candidate for NASH by reducing oxidative stress, inflammation, and fibrosis. Further studies are needed to evaluate its long-term safety and efficacy in clinical settings.

PUBLICATIONS:

Rojo AI, Buttari B, Cadenas S, Carlos AR, Cuadrado A, Falcão AS, López MG, Georgiev MI, Grochot-Przeczek A, Gumeni S, Jimenez-Villegas J, Horbanczuk JO, Konu O, Lastres-Becker I, Levonen AL, Maksimova V, Michaeloudes C, Mihaylova LV, Mickael ME, Milisav I, Miova B, Rada P, Santos M, Seabra MC, Strac DS, Tenreiro S, Trougakos IP, Dinkova-Kostova AT. Model organisms for investigating the functional involvement of NRF2 in non-communicable diseases. *Redox Biol*. **2025**, *79*:103464. DOI: 10.1016/j.redox.2024.103464

Cuadrado A, Cazalla E, Bach A, Bathish B, Naidu SD, DeNicola GM, Dinkova-Kostova AT, Fernández-Ginés R, Grochot-Przeczek A, Hayes JD, Kensler TW, León R, Liby KT, López MG, Manda G, Shivakumar AK, Hakomäki H, Moerland JA, Motohashi H, Rojo AI, Sykiotis GP, Taguchi K, Valverde ÁM, Yamamoto M, Levonen AL. Health position paper and redox perspectives - Bench to bedside transition for pharmacological regulation of NRF2 in noncommunicable diseases. *Redox Biol. 2025*, *81*:103569. DOI: 10.1016/j.redox.2025.103569

García-Yagüe ÁJ, Cañizares-Moscato L, Encinar JA, Cazalla E, Fernández-Ginés R, Escoll M, Rojo AI, Cuadrado A. A novel β-TrCP1/NRF2 interaction inhibitor for effective anti-inflammatory therapy. *J Biomed Sci.* **2025**, *32(1)*:65. DOI: 10.1186/s12929-025-01157-3.

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Carnicero-Senabre D, Barata MA, Jiménez-Villegas J, Guimas Almeida C, Cuadrado A, Rojo AI. NRF2 deficiency is associated with synaptic alterations and ether-linked phospholipid imbalance in the hippocampus. *Redox Biol.* **2025**, *86*:103853. DOI: 10.1016/j.redox.2025.103853

Carnicero-Senabre D, Jiménez-Villegas J, Álvarez-Garrote S, Escoll M, Cuadrado A, Rojo AI. NRF2 activation by 6-MSITC increases the generation of neuroprotective, soluble α amyloid precursor protein by inducing the metalloprotease gene ADAM17. *Free Radic Biol Med*. **2025**, 227:94-102. 10. DOI: 1016/j.freeradbiomed.2024.11.048

Kozlov A, Blazquez-Llorca L, Benavides-Piccione R, Kastanauskaite A, Rojo AI, Muñoz A, Cuadrado A, DeFelipe J, Grillner S. Mouse and human striatal projection neurons compared - somatodendritic arbor, spines and in silico analyses. *PLoS Comput Biol.* **2025**, *21(10)*:e1013569. DOI: 10.1371/journal.pcbi.1013569

Jakubowska M, Costa VM, Krzeptowski W, Dominkuš PP, Santos M, Demirdöğen BC, Genç Ş, Trougakos IP, Kanninen KM, Winklhofer-Roob BM, Copple IM, Cuadrado A, Dolžan V, Morgenstern C. Altered NRF2 signalling in systemic redox imbalance: Insights from non-communicable diseases. *Redox Biol.* **2025**, *87*:103891. DOI: 10.1016/j.redox.2025.103891

Romero-ElKhayat L, Dakterzada F, Huerto R, Carnes-Vendrell A, Mínguez O, Pujol Sabaté M, Targa A, Barbé F, Milanesi E, Dobre M, Manda G, Cuadrado A, Piñol-Ripoll G.
Inflammatory and Redox Blood Gene Expression Fingerprint of Severe Obstructive Sleep Apnoea in Patients With Mild Alzheimer's Disease. *J Inflamm Res*. **2025**, *18:*1609-1621. DOI: 10.2147/JIR.S475776

DOCTORAL THESES AND OTHER WORKS:

José Jiménez Villegas
Ph.D. *NRF2 at the intersection of redox imbalance and stress granule dynamics in amyotrophic lateral sclerosis*. Universidad Autónoma de Madrid. 2025. Supervisor/s: Ana Isabel Rojo Sanchís y Antonio Cuadrado Pastor. Grade: Sobresaliente Cum Laude.

FUNDING:

NRF2 as a novel therapeutic target in early and intermediate age-related macular degeneration. HR21-00703. La Caixa. 2022-2025. PI: Antonio Cuadrado

Optimización y validación in vivo de fármacos innovadores para el tratamiento de taupatías. S2022/BMD-7230. Comunidad Autónoma de Madrid. 2023-2026. PI: Antonio Cuadrado y Ana Isabel Rojo

Papel del factor de transcripción NRF2 en protección sináptica en las Taupatías. PID2022-141786OB-I00. MICINN. 2023-2026. PI: Antonio Cuadrado y Ana Isabel Rojo

Bench to bedside transition for pharmacological regulation of NRF2 in noncommunicable diseases" (BenBedPhar). AGA CA20121. Programa COST. 2021-2025. PI: Antonio Cuadrado

PATENTS:

"Tetrahydro-Spiroindoline-Pyrrolopyrrole-Triones Inhibitors of the NRF2-β-TrCP Interaction for Use in the Treatment of Fatty Liver Disease". Antonio Cuadrado Pastor, Raquel Fernández Ginés, José Antonio Encinar, Rafael León, Juan Felipe Franco-González, Manuela García-López, María Isabel Rodríguez Franco y Ana Isabel Rojo Sanchis. PCT/EP2022/050657 WO 2022/152800 A1". 2024

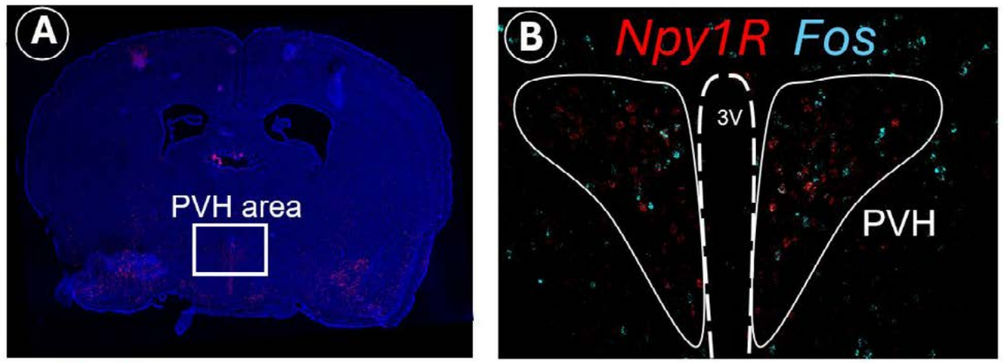
AWARDS

Mejor Publicación en el Área de Neurociencias del IdiPAZ. Madrid. 2025

Metabolic Neurocircuits Laboratory

TENURED-TRACK SCIENTIST
Juan de Solis, Alain
(Investigador Ramón y Cajal, CSIC)

KEYWORDS
Hypothalamus, Metabolism, Neurocircuits, Obesity,



A. ***Npy1R*** expression in a coronal section of the adult mouse brain. **B.** In the paraventricular hypothalamic area (PVH), located bilaterally around the third ventricle (3V), activated ***Npy1R*** **$^+$ neurons** are identified by colocalization with ***Fos***, a marker of neuronal activation, using RNAscope

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RESEARCH LINES:

Overview

Our research focuses on the development and function of neurocircuits involved in the regulation of metabolism. The hypothalamus is the primary region of the central nervous system responsible for controlling metabolic responses and maintaining energy balance. It contains specialized neurocircuits that integrate internal signals (neuronal input, hormones, and nutrients) and external cues (food availability, circadian rhythms) to modulate appetite, energy expenditure, and the metabolic responses of peripheral organs. Dysfunction in these circuits leads to a sustained positive energy balance and contributes to the development of obesity.

Obesity, a chronic disease characterized by excessive lipid accumulation, has tripled in prevalence among adults worldwide and has also increased markedly in children and adolescents. It significantly elevates the risk of developing cardiometabolic diseases (CMDs), including diabetes and cardiovascular disorders, thereby reducing quality of life and creating a substantial burden on healthcare systems.

To investigate the mechanisms underlying metabolic regulation, we use transgenic mouse models combined with advanced neuroscience approaches (chemogenetics, AAV-mediated manipulation, circuit mapping, brain clearing, in situ RNA detection, etc.), sequencing technologies (bulk and single-cell RNA-seq), molecular biology techniques (RT-PCR, Western blotting), and comprehensive metabolic phenotyping (metabolic cages, indirect calorimetry, ITT, GTT, etc.). We also implement developmental programming interventions by assessing the impact of high-fat diets (HFD) during the lactation period. Our research aims to unravel the causes of obesity, diabetes, and other metabolic diseases, identify new druggable targets involved in metabolic control, and contribute to the development of improved strategies to reduce childhood obesity.

Role of hypothalamic neurocircuits in the control of peripheral metabolic responses

Researchers involved: Juan de Solis, Alain

Recent publications using deep sequencing analysis have delineated the main neuronal subpopulations within the hypothalamus, and several key metabolic neurocircuits are located in the paraventricular nucleus (PVH). The PVH receives abundant neuronal input from other hypothalamic nuclei and projects axonal terminals to multiple hypothalamic and extra-hypothalamic regions, including the median eminence, the striatum, and the brainstem. The dorsal vagal complex (DVC) of the brainstem contains several neurons that form part of the autonomic nervous system, which receives direct neural connections

from PVH neurons. Because of its position and connectivity, the PVH acts as an integration center that coordinates hypothalamic neural activity and modulates feeding behaviors, energy expenditure and the metabolic responses of peripheral organs.

A recent innovative study classified PVH neuronal populations proposed that PVH neurons expressing the Npy1R gene—the receptor for neuropeptide Y (NPY)—function as a key integrator population based on their responses to physiological states such as hunger, satiety, thirst, and fear. In line with this, my previous research uncovered the critical role of Npy1R-PVH neurons in the coordinated control of feeding, although other metabolic functions were not characterized. Furthermore, results also suggested that Npy1R-PVH neurons are resilient to the effects of chronic HFD adult exposure.

This research line will therefore investigate the metabolic roles of Npy1R-PVH pre-autonomic neurons and address the following objectives:

- Delineate Npy1R-PVH neurocircuits and determine their role in energy balance.
- Characterize the impact of HFD-induced obesity on the function of Npy1R-PVH neurocircuits.

Impact of obesogenic diets during the postnatal maturation of hypothalamic neurocircuits.

Researchers involved: Juan de Solis, Alain

Epidemiological studies show a strong link between environmental changes during critical developmental periods and the early onset of obesity and CMDs. These developmental disruptions can alter growth trajectories and increase susceptibility to adverse nutritional environments later in life. Obesogenic diets are the main environmental factor driving molecular, cellular, and systemic changes that promote a positive energy balance. In mice, hypothalamic neurocircuit formation occurs postnatally, corresponding to the third trimester of human gestation. Maladaptive maturation of these circuits, triggered by obesogenic diets, can impair the coordinated metabolic responses of peripheral organs and facilitate the onset of childhood obesity.

This research line will characterize the development of PVH neurocircuits and their susceptibility to developmental programming by altered nutritional states. To achieve this, we will pursue the following objectives:

- Map the temporal development of PVH axonal projections across the postnatal period.
- Evaluate the impact of HFD exposure during lactation period on PVH axonal projections.
- Analyze transcriptional changes in PVH neurons induced by HFD exposure during lactation.

OUTREACH ACTIVITIES:

Outreach seminar at IES Joaquin Rodrigo (Vicalvaro), as part of “Cientific@s en Practicas” program (27 November)

Thyroid Hormones and Central Nervous System

TENURED SCIENTIST

Guadaño Ferraz, Ana
(Investigador Científico). Group Coordinator

ASSOCIATED INVESTIGATOR

Grijota Martínez, María del Carmen
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Ausó Monreal, Eva
(Profesora Titular, UA)

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Valcárcel Hernández, Víctor

Fornachiari Reveco, Arantxa Jeannette

PRE-DOCTORAL INVESTIGATOR

Guillén Yunta, Marina

Muñoz Falder, Beatriz

Pedraza Lozada, Diego

MASTER THESIS STUDENT

Navarro Martorell, Noelia

UNDERGRADUATE STUDENT

Yuste Rodríguez, Gonzalo

SENIOR RESEARCH TECHNICIAN

Montero Pedrazuela, Ana

KEYWORDS

Thyroid hormones, Thyroid hormone transport, MCT8 deficiency, Brain, Blood-brain barrier, Thyromimetics

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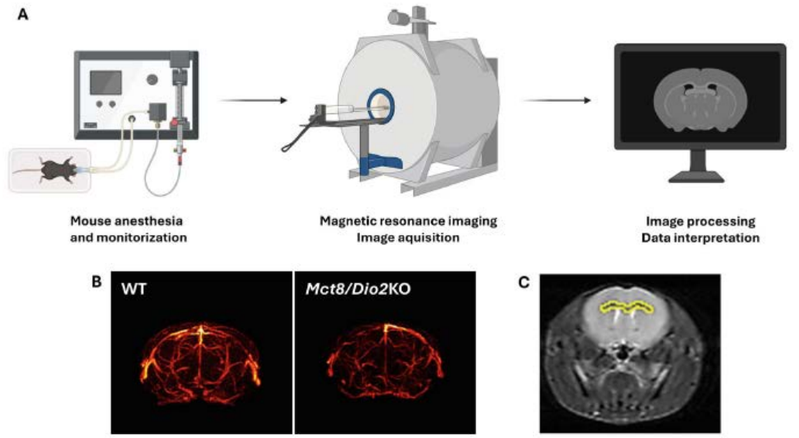

Fig. 1 Experimental procedures to explore MCT8 deficiency pathophysiology in vivo by magnetic resonance imaging techniques. (**a**) Representative protocol for magnetic resonance imaging procedures in mice. (**b**) The panel shows representative Pseudocolor Image Look-Up Table (LUT) maps from maximal intensity projections of 2D Time of Flight-Magnetic Resonance Angiography (TOF-MRA) acquisitions of the cerebral blood-vessel network of WT and *Mct8/Dio2*KO mice, an animal model for MCT8 deficiency. Modified from Guillén-Yunta M et al. Fluids and Barriers of the CNS (2023) 20:79. (**c**) T2 weighted image from a 6-month-old WT animal with embedded ROI for Diffusion Tensor Imaging (DTI) maps analyses

RESEARCH LINES:

Overview

Thyroid hormones (THs) are essential for vertebrate development, regulating growth and metabolism in all tissues. The developing central nervous system (CNS) is particularly sensitive to TH deficiency, which can cause neurological alterations depending on timing and cause. Maintaining normal TH levels during neurodevelopment is therefore critical. However, the regulation of TH availability and action in the CNS remains poorly understood, and this knowledge is key for understanding CNS neurodevelopment and CNS repair and regeneration in pathological conditions.

Our research group is committed to advancing the understanding of the pathophysiology and disease mechanisms in the CNS associated with rare disorders caused by defects in TH signaling and altered TH availability. Our research aims to characterize potential therapeutic targets and innovative approaches that can facilitate the development of therapeutic strategies for these conditions. Furthermore, our investigations will enhance our comprehension of the role THs play in neurodevelopment, brain function and plasticity.

We are interested in investigating the pathophysiology of an X-linked, inherited rare disease caused by mutations in the Monocarboxylate transporter 8 (MCT8), a specific TH transporter. This condition, known as the Allan-Herndon-Dudley syndrome (AHDS) or MCT8 deficiency, leads to peripheral hyperthyroidism and profound neurological impairments, primarily due to brain hypothyroidism. However, the underlying pathophysiological mechanisms remain poorly understood. Additionally, we aim to develop preclinical studies to explore potential therapies for the unmet neurological needs of patients.

We are also interested in understanding the role of MCT8, other TH transporters and deiodinases, the enzymes that metabolize THs in the CNS, during neurodevelopment.

To achieve our goals:

- We analyze the histopathology of human autopsy brain tissue from patients with a genetic diagnosis of AHDS.
- We investigate the phenotype of several disease animal models using different experimental approaches, primarily in vivo studies. The majority of these experimental models have been developed in our laboratory. These include animals with congenital hypothyroidism, mice deficient in MCT8, the main TH transporter at brain barriers and in neural cells, and knockout models for other TH transporters and deiodinases involved in TH metabolism and action. Using these models, we aim to dissect the role of MCT8, additional TH transporters, and deiodinases in regulating TH availability and signaling in the CNS during neurodevelopment, as well as their impact on CNS plasticity processes such as glial remodeling and adult neurogenesis.
- We conduct preclinical studies using AHDS animal models to evaluate the effects of different TH analogs on TH target neural cells under MCT8-deficient conditions. More recently, we have also been evaluating the effects of various gene therapy approaches on MCT8-deficient disease animal models.

Unraveling the Pathophysiological Basis of MCT8 Deficiency

Our current and future general objective is to contribute to the understanding of how MCT8 deficiency leads to neurological deterioration in affected patients. To this end, we are investigating histopathological alterations in the CNS of affected patients with different histological techniques and immunohistochemistry, and magnetic resonance imaging techniques, as well as other non-invasive techniques to evaluate motor alterations. We also investigate histopathological and neurological alterations in AHDS disease animal models.

We have identified important disease mechanisms affecting glial cells and blood–brain barrier permeability and function, and we are now further dissecting these processes to better understand their role in disease progression.

To develop therapeutic strategies aimed to prevent or reverse the neurological alterations associated with MCT8 deficiency.

MCT8 deficiency is a rare disorder characterized by peripheral hyperthyroidism and cerebral hypothyroidism, leading to severe neurological impairments. As there is currently no effective treatment to ameliorate the brain impairments in MCT8-deficient patients, we have devoted significant efforts to developing therapeutic strategies to address these neurological deficits.

The neurological manifestations in the AHDS mainly result from impaired TH transport across the brain barriers, leading to reduced TH availability in the brain. Based on this pathophysiological mechanism, we have conducted preclinical studies using TH analogs capable of crossing cellular membranes independently of MCT8. We have obtained promising results with one of these analogs and are currently expanding and deepening these studies to further evaluate its therapeutic potential.

More recently, we have been investigating the potential of gene therapy to mitigate the neurological alterations associated with MCT8 deficiency, including the development of targeted gene therapy strategies in collaboration with international institutions.

PUBLICATIONS:

Vázquez, P.; Escalona-Garrido, C.; Pescador, N.; Hitos, A.B.; González-Moreno, D.; de Benito-Bueno, Á.; Sierra-Filardi, E.; Boya, P.; Montero-Pedrazuela, A.; Guadaño-Ferraz, A.; Valverde Á.M. Sirtuin 1 overexpression in mice preserves insulin and thermogenic responses in subcutaneous inguinal white adipose tissue under proinflammatory conditions. *J Physiol Biochem* **2025**, *81(4)*:1019-1035. DOI: 10.1007/s13105-025-01109-3.

Borges-Canha, M.; Leite, A.R.; Conceição, G.; Vale, C.; Von-Hafe, M.; Martins, D.; Miranda-Silva, D.; Sousa-Mendes, C.; Chaves, J.; Lourenço, I.M.; Grijota-Martínez, C.; Bárez-López, S.; Miranda, I.M.; Leite-Moreira, A.; Falcão-Pires, I.; Neves, J.S. Evaluation of the hepatic and subcutaneous adipose tissue effects of triiodothyronine treatment in an animal model of metabolic syndrome. *Obes Res Clin Pract*. **2025** *19(2)*:115-121. DOI: 10.1016/j.orcp.2025.04.001.

Montero-Pedrazuela, A.; Grijota-Martínez, C.; Guadaño-Ferraz, A.; Bárez-López, S. A Sensitive Radioimmunoassay for T3 and T4 Determinations in Plasma and Tissues. *Methods Mol Biol.* **2025**; *2876*:37-59. DOI: 10.1007/978-1-0716-4252-8_3.

Guillén-Yunta, M.; Guadaño-Ferraz, A.; Valcárcel-Hernández V. Magnetic Resonance Imaging Techniques for Investigating the MCT8-Deficient Brain in Murine Disease Models. *Methods Mol Biol*. **2025**; *2876*:175-186. DOI: 10.1007/978-1-0716-4252-8_12.

Montero-Pedrazuela, A.; Contreras-Jurado S.C. (2025) Mouse embryonic fibroblasts reprogramming to induced pluripotent stem cells by T3. *Methods Mol Biol.* **2025**; *2876*:117-130. DOI: 10.1007/978-1-0716-4252-8_8.

DOCTORAL THESES AND OTHER WORKS:

Noelia Navarro Martorell
Master´s thesis. *Evaluación de los efectos tiromiméticos de sobetirome en cerebelo y tejidos periféricos del modelo murino Mct8/Dio2 KO del Síndrome de Allan-Herndon-Dudley.* Universidad de Alcalá. 2025. Supervisor/s: Ana Montero Pedrazuela, Soledad Bárez López. Grade: Sobresaliente

FUNDING:

Regulation of thyroid hormone availability during neurodevelopment in health and disease. PID2023-152523OB-I00. MICINN. 01/09/2024-31/08/2027. PI: Ana Guadaño Ferraz.

Exploring a personalized gene replacement therapy approach for the AHDS. OTR10255. Sherman Foundation. 01/05/23-28/02/2026. PIs: Soledad Bárez-López, Ana Guadaño-Ferraz.

Pharmacological stimulation of mitochondrial metabolism as a therapeutic approach in a mouse model of AHDS (GSA24L006) Fondazione Telethon. 1/08/2025-31/01/2027. PI: Dr Emanuela Bottani, Università di Verona, Italia

Allan-Herndon-Dudley Syndrome: pathological studies and development of a novel pharmacological strategy at the preclinical level. PID2020-113139RB-I00. Ministerio de Ciencia e Innovación (MICINN). 01/09/2021-28/02/2025
PI: Ana Guadaño Ferraz

PATENTS:

Registration of the Allan-Herndon-Dudley Syndrome Mouse Model *Mct8/Dio2*-KO as Designated Biological Material. Soledad Bárez López, Ángel García Aldea, Carmen Grijota Martínez, Ana Cristina Guadaño Ferraz, Marina Guillén Yunta, Ana Montero Pedrazuela, Víctor Valcárcel Hernández. CSIC Technology Transfer Department (Registration Number: 129/2025). 2025.

OUTREACH ACTIVITIES:

Cheers to women in neuroscience. Title: “Neurociencia” es femenino: contando el viaje de dos jóvenes neurocientíficas. 07/02/2025. Organization.

Yo investigo, yo soy CSIC. Title: Allan-Herndon-Dudley. 11/04/2025.

Satellite Symposium “La Brecha de Género en Neurociencia: Explorando Soluciones” Within the framework of the XX Congress of the Spanish Society of Neuroscience, activity open to the general public. Co-organization. 5/09/2025.

Noche europea de los y las investigadoras. Title: El CSIC te llama esta noche. 26/09/2025.

Redox Biology and Neurodegeneration

TENURED SCIENTIST

Rojo Sanchís, Ana Isabel
(Profesora titular). Group Coordinator

PREDOCTORAL SCIENTIST

Jiménez Villegas, José
Carnicero Senabre, Daniel
Olazabal Chias, Marta

CONTRACT RESEARCHER

Kratochvil, Marko

KEYWORDS

Amyotrophic lateral sclerosis, Lipidomics, Neurodegeneration, NRF2, Redox, Tauopathies

RESEARCH LINES:

Overview

Neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis (ALS), are characterized by a progressive loss of synapses and neurons in the central nervous system. Although their origin is complex and multifactorial, the disruption of redox homeostasis plays a central role in these processes. Aging is the main risk factor, and the disease often progresses silently for decades before the appearance of the first clinical symptoms.

Currently, there are no curative therapies for these conditions, highlighting the urgent need to identify new therapeutic targets and early biomarkers that enable early diagnosis and the development of personalized treatments. Research in redox biology and the study of molecular mechanisms involved in synaptic loss are essential to advance the fight against neurodegenerative diseases.

Redox imbalance and stress granule dynamics in ALS

Exploring the interaction between oxidative stress, mitochondrial dysfunction, and stress granule behavior as a reflection of RNA metabolic pathway functionality.

Study of the synaptic lipid profile

Integrated evaluation of data obtained through transcriptomic and lipidomic analyses of synaptic dysfunction in Alzheimer's disease.

Peripheral redox and inflammatory signatures in Parkinson's disease

Aiming to identify gene and protein biomarkers in blood.

Development of NRF2-based neuroprotective therapies for tauopathies

Integrating molecular and translational approaches to validate NRF2 as a pharmacological target.

PUBLICATIONS:

Rojo AI, Buttari B, Cadenas S, Carlos AR, Cuadrado A, Falcão AS, López MG, Georgiev MI, Grochot-Przeczek A, Gumeni S, Jimenez-Villegas J, Horbanczuk JO, Konu O, Lastres-Becker I, Levonen AL, Maksimova V, Michaeloudes C, Mihaylova LV, Mickael ME, Milisav I, Miova B, Rada P, Santos M, Seabra MC, Strac DS, Tenreiro S, Trougakos IP, Dinkova-Kostova AT. Model organisms for investigating the functional involvement of NRF2 in non-communicable diseases. *Redox Biol.* **2025**, *79*:103464. DOI: 10.1016/j.redox.2024.103464

Cuadrado A, Cazalla E, Bach A, Bathish B, Naidu SD, DeNicola GM, Dinkova-Kostova AT, Fernández-Ginés R, Grochot-Przeczek A, Hayes JD, Kensler TW, León R, Liby KT, López MG, Manda G, Shivakumar AK, Hakomäki H, Moerland JA, Motohashi H, Rojo AI, Sykiotis GP, Taguchi K, Valverde ÁM, Yamamoto M, Levonen AL. Health position paper and redox perspectives - Bench to bedside transition for pharmacological regulation of NRF2 in noncommunicable diseases. *Redox Biol.* **2025**, *81*:103569. DOI: 10.1016/j.redox.2025.103569

García-Yagüe ÁJ, Cañizares-Moscato L, Encinar JA, Cazalla E, Fernández-Ginés R, Escoll M, Rojo AI, Cuadrado A. A novel β-TrCP1/NRF2 interaction inhibitor for effective anti-inflammatory therapy. *J Biomed Sci*. **2025**, *32(1)*:65. DOI: 10.1186/s12929-025-01157-3.

García-Yagüe ÁJ, Cueto-Díaz EJ, Escoll M, Okunishi I, Hayes JD, Rodríguez-Franco MI, Rojo AI, Cuadrado A. Dual targeting of Keap1 and

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Gsk-3 by hexaraphane in the regulation of transcription factor Nrf2. *Free Radic Biol Med*. **2025**, *239*:579-593. DOI: 10.1016/j.freeradbiomed.2025.07.051

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Carnicero-Senabre D, Jiménez-Villegas J, Álvarez-Garrote S, Escoll M, Cuadrado A, Rojo AI. NRF2 activation by 6-MSITC increases the generation of neuroprotective, soluble α amyloid precursor protein by inducing the metalloprotease gene ADAM17. *Free Radic Biol Med*. **2025**, 227:94-102. DOI: 10.1016/j.freeradbiomed.2024.11.048

Kozlov A, Blazquez-Llorca L, Benavides-Piccione R, Kastanauskaite A, Rojo AI, Muñoz A, Cuadrado A, DeFelipe J, Grillner S. Mouse and human striatal projection neurons compared - somatodendritic arbor, spines and in silico analyses. *PLoS Comput Biol.* **2025**, *21(10)*:e1013569. DOI: 10.1371/journal.pcbi.1013569

Buttari B, Tramutola A, Rojo AI, Chondrogianni N, Saha S, Berry A, Giona L, Miranda JP, Profumo E, Davinelli S, Daiber A, Cuadrado A, Di Domenico F. Proteostasis Decline and Redox Imbalance in Age-Related Diseases: The Therapeutic Potential of NRF2. *Biomolecules*. **2025** Jan *13;15(1)*:113. DOI: 10.3390/biom15010113.

DOCTORAL THESES AND OTHER WORKS:

José Jiménez Villegas
Ph.D. thesis. *NRF2 at the intersection of redox imbalance and stress granule dynamics in amyotrophic lateral sclerosis.* Universidad Autónoma de Madrid. 2025. Supervisor/s: Ana Isabel Rojo Sanchís y Antonio Cuadrado Pastor. Grade: Sobresaliente Cum Laude.

Daniel Carnicero Senabre
Ph.D. thesis. *Definiendo el papel del factor de transcripción NRF2 en el mantenimiento de la homeostasis sináptica*. Universidad Autónoma de Madrid. 2025. Supervisor/s: Ana Isabel Rojo Sanchís. Grade: Sobresaliente Cum Laude.

FUNDING:

Optimización y validación in vivo de fármacos innovadores para el tratamiento de taupatías. S2022/BMD-7230. Comunidad Autónoma de Madrid. 2023-2026. PI: Antonio Cuadrado y Ana Isabel Rojo

Papel del factor de transcripción NRF2 en protección sináptica en las Taupatías. PID2022-141786OB-I00. MICINN. 2023-2026. PI: Antonio Cuadrado y Ana Isabel Rojo

Peripheral Redox and Inflammation Signatures for Biomarker Discovery in Parkinson's Disease. CIUP22A7619. Fundación Ramón Areces. 2025-2028. PI: Ana Isabel Rojo

PATENTS:

"Tetrahydro-Spiroindoline-Pyrrolopyrrole-Triones Inhibitors of the NRF2-β-TrCP Interaction for Use in the Treatment of Fatty Liver Disease". Antonio Cuadrado Pastor, Raquel Fernández Ginés, José Antonio Encinar, Rafael León, Juan Felipe Franco-González, Manuela García López, María Isabel Rodríguez Franco y Ana Isabel Rojo Sanchis. PCT/ EP2022/050657 WO 2022/152800 A1". 2024

AWARDS:

Mejor Publicación en el Área de Neurociencias del IdiPAZ. Madrid. 2025

Biomedical and Biophysics MR lab

CONTRACT RESEARCHER

Lizarbe Serra, Blanca
(Profesor Ayudante Doctor). Group Coordinator

PREDOCTORAL SCIENTIST

Ferreiro de Aguiar, Adriana

SENIOR RESEARCH TECHNICIAN

Holgado Pordomingo, Maya

MASTER THESIS STUDENT

Amellones Iglesias, Celia

UNDERGRADUATE STUDENT

Rodrigo Díaz, Marta

KEYWORDS

Biophysical models, Brain metabolism, Data Analysis, Magnetic Resonance Imaging, Mouse, Obesity

RESEARCH LINES:

Overview

The Biomedical and Biophysics Magnetic Resonance lab focuses its research on the study of brain mechanisms underlying biomedical problems, using advanced imaging techniques (MRI, MRS, PET) combined with biophysical, metabolomic, and machine learning approaches. Our main research lines investigate the neurological changes associated with the development of metabolic diseases (such as obesity) and their treatment, as well as processes related to aging. Our aim is to understand how these conditions or treatments modify brain structure and function, and to provide innovative tools for the standardization, analysis, and interpretation of neuroimaging data.

Brain effects of anti-obesity drugs in animal models

Studies in diet-induced obese mice, exploring modulators such as GLP-1 agonists or dual GLP-1-RA/GIP-RA, with a multidisciplinary approach (MRI, MRS, immunofluorescence, and systemic biomarkers).

Brain effects of pharmacological and surgical treatment in patients with obesity

Longitudinal studies using MRI and MRS to characterize responses to GLP-1-RA/GIP-RA drugs and, in the medium term, compare them with bariatric surgery.

Biophysical models of the MRI signal

Development and implementation of advanced biophysical models, with a strong focus on diffusion imaging (Diffusion Kurtosis Imaging, NEXI, SANDI).

Standardization of MRI pr and post-processing

Optimization of neuroimaging workflows and data analysis through the implementation of state-of-the-art pre-processing pipelines, and advanced statistical methods and machine learning for post-processing.

Machine learning models applied to metabolomics and radiomics

Integration of multiparametric biomarkers to characterize brain processes associated with obesity, metabolism and aging.

New biomarkers of obesity, metabolic dysfunction and aging processes

Exploration of advanced MRI sequences to evaluate neuroinflammation and cerebral metabolism.

PUBLICATIONS:

Tirado-García, P.; Ferreiro, A.; González-Alday, R.; Arias-Ramos, N.; Lizarbe, B.; López-Larrubia, P. Aquaporin-4 inhibition alters cerebral glucose dynamics predominantly in obese animals: an MRI study. *Sci Rep.* **2025**, *15*:15649. DOI: 10.1038/s41598-025-99641-1

DOCTORAL THESES AND OTHER WORKS:

Marta Rodrigo Díaz

Final degree's project. *Resonancia magnética de los cambios cerebrales subyacentes al desarrollo de obesidad.* Universidad Autónoma de Madrid. 2025. Supervisor/s: Blanca Lizarbe Serra Y Adriana Ferreiro de Aguiar. Grade: Sobresaliente.

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FUNDING:

The cerebral changes that underly obesity development and treatment: multimodal imaging and magnetic resonance spectroscopy (PhotOBrains). PID2021-126888OA-I00. Agencia Estatal de Investigación. 2022-2026 PI: Blanca Lizarbe Serra..

Parkinson, ALS and Tauopathies: New Insights

TENURED SCIENTIST
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Rodríguez Cueto, Carmen Aurora

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MASTER THESIS STUDENT
Suárez Fonseca, Javier

UNDERGRADUATE STUDENT
Mihaila, Giulia
León Alonso, Lucía Teresa

VISITING SCIENTIST
Feijen, Dorothea Hendrika Johanna

KEYWORDS
Parkinson's disease, ALS, TAU, neuroinflammation pyroptosis, oxidative stress, drug discovery.

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RESEARCH LINES:

Overview

As our society's population grows older, we face mounting challenges in healthcare and social support systems. The rise in age-related conditions brings increased physical limitations and illnesses, creating significant strain not only on medical resources but also on those affected and their loved ones. Among the most concerning aspects of aging are neurodegenerative conditions, particularly frontotemporal dementia (FTD), Parkinson's disease (PD), and amyotrophic lateral sclerosis (ALS). A critical societal priority is developing effective treatments for these conditions, which requires advances in biomarker identification, pharmaceutical development, and technological innovation. Our laboratory focuses on understanding neurodegeneration at the molecular level. We employ a comprehensive research strategy that bridges fundamental science with clinical applications, utilizing various experimental approaches including cell cultures, mouse models, and analysis of tissue samples from individuals who had FTD, PD, and ALS.

Targeting pyroptosis in TAU-induced neurodegeneration: mechanisms and modulation

Researchers involved: Silva-Llanes, I; Arribas, M; Feijen, D; Lastres-Becker, I.

Frontotemporal dementia (FTD) is an early-onset progressive neurodegenerative disease primarily characterized by neuronal degeneration in the frontal and temporal lobes, followed by hippocampal atrophy. FTD is the second most common cause of dementia in adult patients and the most frequent in patients under 65 years of age. From a molecular perspective, FTD is mainly characterized by aggregates of TAU or TDP-43 proteins. There is also a dysregulation of redox homeostasis and low-grade chronic neuroinflammation. Recent research has revealed a strong connection between neuroinflammation and TAU protein-related neurodegeneration. A key discovery shows that the NLRP3 inflammasome, when activated, can significantly impact TAU pathology and subsequent neuronal death. This process involves pyroptosis - a specific form of cell death that occurs when the NLRP3 inflammasome assembles, leading to GASDERMIN D (GSDMD) cleavage and the subsequent release of inflammatory molecules IL-1β and IL-18. To better understand this relationship, our laboratory investigated pyroptosis's role in TAU-driven neurodegeneration using two distinct experimental approaches.

CB2 Cannabinoid Receptor Modulation as a New Therapeutic Strategy to Protect Against TAU-dependent FTD Neurodegeneration

Researchers involved:Silva-Llanes, I; Suárez, J; Rodríguez, C; Lastres-Becker, I.

TAU protein is the main component of intracellular filamentous deposits that define a series of neurodegenerative diseases called tauopathies. Generally, tauopathies are characterized by alterations in synaptic plasticity, cell death, proteinopathy, and neuroinflammation. Despite enormous efforts to find a cure for these diseases, an effective treatment does not yet exist. In our laboratory, we approach this challenge with two different approaches. We have demonstrated both in vitro and in vivo that neurons with TAU accumulation induce the expression of the CB2 cannabinoid receptor, which enhances neurodegeneration. Therefore, in our first approach, we focus on studying the pharmacological modulation of the CB2 receptor and its effects on TAU-induced neurodegeneration. Currently, there are no specific biomarkers for tauopathies that would allow for prognosis/diagnosis of these diseases.

Differential Function of Mitochondria in Neuron and Astrocyte α-synuclein-dependent Parkinson's Disease

Researchers involved: Solar Fernández, V; Flores, D; León, L; Mihaila, G; Lastres-Becker, I.

Parkinson's Disease (PD) is the most prevalent neurodegenerative disease with motor alterations characterized by the degeneration of dopaminergic (DA) neurons in the substantia nigra and the accumulation of protein aggregates in so-called Lewy bodies, where the most abundant protein is alpha-synuclein (α-SYN). In addition to the neurodegenerative process and protein accumulation, PD is characterized by low-grade chronic inflammation and oxidative stress, which are associated with mitochondrial alterations. PD has been predominantly approached from a neuron-centric point of view, without considering other cell types, such as astrocytes, which are part of the tripartite synapse. However, we cannot rule out that mitochondrial dynamics and function may also be altered in astrocytes and differently from DAergic neurons, contributing to the onset and propagation of the disease. Therefore, in this research project, we aim to address the hypothesis that mitochondrial dynamics and function may be different between neurons and astrocytes in α-SYN-associated PD.

Fighting against Parkinson's Disease with SGK1 inhibitors

Researchers involved: Solar Fernández, V; Flores, D; Mihaila, G; González de Mesa, E; Lastres-Becker, I.

Parkinson's disease (PD) is the second most common neurodegenerative disorder characterized by the degeneration of dopaminergic neurons of the substantia nigra and the accumulation of protein aggregates, called Lewy bodies, where the most abundant is alpha-synuclein (a-SYN). In addition to the neurodegeneration and the accumulation of proteins, PD is characterized by chronic low-grade inflammation and mitochondrial alterations that lead to oxidative stress. Currently, PD patients are only treated with dopamine replacement therapy (levodopa), with serious side effects and which also does not stop the degenerative condition. or this reason, in this innovative research project we want to address this challenge by developing new SGK1 kinase inhibitors to alleviate the 3 main hallmarks: neuroinflammation, autophagy/mitophay and oxidative stress. Preliminary results of our research groups support this hypothesis. For this, we will carry out a multidisciplinary approach from in vitro assays, evaluate new small molecules capable of selectively inhibit SGK1 and finally, we will evaluate the efficacy of these molecules in preclinical trials in a murine model of PD. The results obtained from this project have an immediate projection as a possible clinical tool for the treatment of patients with PD.projection as a possible clinical tool for the treatment of patients with PD.

Exploring the Pathophysiological Links Between Parkinson's Disease, Hypertension, and Acute Lung Inflammation

Researchers involved: Solar Fernández, V; Flores, D; Mihaila, G; González de Mesa, E; Lastres-Becker, I.

This research line examines the interplay between PD, hypertension, and acute lung inflammation (ALI), aiming to elucidate shared pathophysiological mechanisms and their impact on disease progression and patient outcomes. The project focuses on how systemic processes, particularly inflammation, vascular dysfunction, and altered autonomic regulation, may contribute simultaneously to neurodegeneration, cardiovascular dysregulation, and pulmonary injury. By integrating molecular profiling with functional assessments across brain, cardiovascular, and lung systems, the study seeks to clarify whether acute inflammatory events in the lung and chronic blood pressure alterations can exacerbate PD-related pathology or symptom severity. Ultimately, this work aims to identify convergent biological pathways and potential therapeutic strategies capable of addressing neurological, cardiovascular, and respiratory complications in a unified framework.

PUBLICATIONS:

Rojo, AI.; Buttari, B.; Cadenas, S.; Carlos, AR.; Cuadrado, A.; Falcão, AS.;López, MG.; Georgiev, MI.; Grochot-Przeczek, A.; Gumeni, S.; Jimenez-Villegas, J.; Horbanczuk, JO.; Konu, O.; **Lastres-Becker, I.**; Levonen, AL.; Maksimova, V.; Michaeloudes, C.; Mihaylova, LV.; Mickael ME.; Milisav, I.; Miova, B.; Rada, P.; Santos, M.; Seabra, MC.; Strac, DS.; Tenreiro, S.; Trougakos, IP.; Dinkova-Kostova, AT. Model organisms for investigating the functional involvement of NRF2 in non-communicable diseases. *Redox Biol.* **2025** *Feb;79*:103464. DOI: 10.1016/j.redox.2024.103464.

Silva-Llanes, I.; Madruga, E.; Martínez, A.; **Lastres-Becker, I.** RIPK1 expression and inhibition in tauopathies: implications for neuroinflammation and neuroprotection. *Front Neurosci*. **2025** *Jan 27;*18:1530809. DOI: 10.3389/fnins.2024.1530809.

Silva-Llanes, I.; Rodríguez-López, S.; González-Naranjo, P.; Sastre, ED.; López, MG.; Páez, JA.; Campillo, N.; **Lastres-Becker, I**. Targeting CB2 receptor with a novel antagonist reverses cognitive decline, neurodegeneration and pyroptosis in a TAU-dependent frontotemporal dementia mouse model. *Brain Behav Immun.* **2025** *Jul;*127:251-268. DOI: 10.1016/j.bbi.2025.03.008.

DOCTORAL THESES AND OTHER WORKS:

Ignacio Silva Llanes
Ph.D. thesis. *Nuevas estrategias terapéuticas para el tratamiento de la demencia frontotemporal.* Universidad Autónoma de Madrid. 2025. Supervisor/s: Isabel Lastres Becker. Grade: Sobresaliente Cum Laude.

Javier Suárez Fonseca
Master´s thesis. *Analysis of the pyroptosis process in Amyotrophic Lateral Sclerosis: comparative study between a murine model and patient samples.* Universidad Autónoma de Madrid. 2025. Supervisor/s: Isabel Lastres Becker. Grade: Matrícula de Honor (9.8).

Lucía Teresa León Alonso
Final degree's project. *Evaluación de PMI y Omaveloxolona como moduladores de NRF2 en un modelo celular de α-SINUCLEÍNA para la enfermedad de Parkinson.* Universidad Autónoma de Madrid. 2025. Supervisor/s: Isabel Lastres Becker. Grade: Notable (8.9).

Giulia Mihaila
Final degree's project. *Análisis del efecto de la hipertensión arterial sobre el proceso neurodegenerativo en un modelo murino de la enfermedad de Parkinson.* Universidad Autónoma de Madrid. 2025. Supervisor/s: Isabel Lastres Becker. Grade: Notable (8.9).

FUNDING:

"Targeting SGK1: Bridging Therapies for Parkinson and Cardiovascular Diseases (SGK1-4PDCar)". Coordinated with National Research Council, U. Complutense de Madrid (UCM), Jiménez Diaz Foundation, U. Autónoma de Madrid (UAM). PI UCM group: Isabel Lastres-Becker. Funding agency: Joint call Centre for Network Biomedical Research (Ciber) of Cardiovascular Diseases and Ciber Neurodegenerative diseases. Duration: 2024-2026.

Convocatoria 2022 - «Proyectos de I+D+i» Ministerio de Ciencia, Innovación y Universidades: "α-Synuclein-dependent neuron-astrocyte differential function of mitochondria in Parkinson's disease (MitAsNeu4PD)". PI: Isabel Lastres-Becker. (Universidad Autónoma de Madrid). 01/09/2023-31/08/2027.

Beca Dr. Luis Álvarez 2023 de grupos emergentes o clínicos asociados "Receptor cannabinoide CB2: Validación como Biomarcador en Demencias TAU-dependientes". PI: Isabel Lastres-Becker. (IdiPaz). 01/02/2024-31/01/2026.

PATENTS:

"Compuestos inhibidores de SGK1 y su uso para el tratamiento de enfermedades neurodegenerativas y/o cardiovasculares". Ana Martínez; Carmen Gil; Enrique Madruga; Alberto Garcia-Rubia; Eduardo Oliver; Ana Briones; Isabel Lastres-Becker. PCT/ES2025/070297. España. 07/06/2024. CSIC-UAM.

AWARDS:

Final degree's project. Pablo Baceiredo Macho Accésit Plus del Certamen Universitario Arquímedes 2024 (recibido 25 noviembre 2025).

31 reunión anual de farmacólogos de la Comunidad de Madrid (Farmadrid 31): premio a la mejor comunicación oral a Ignacio Silva Llanes.

Isabel Lastres Becker: Lista Forbes 50 over 50 España: https://forbes.es/listas/698651/lista-forbes-50-over-50-2025/

OUTREACH ACTIVITIES:

I Congreso Investigadoras ODS en el Lope de Vega. Title: ¿Tiene cura la enfermedad de Alzheimer?. Date: 12-02-2025.

II jornada sobre enfermedades raras-Instituto de Investigaciones Biomédicas Sols-Morreale. Title: Desentrañando los misterios del cerebro: La lucha contra la Demencia Frontotemporal. Date: 24-02-2025.

Therapy, Brain Response & Imaging

TENURED SCIENTIST

Pacheco Torres, Jesús
(Científico Titular, CSIC). Group Coordinator

PREDOCTORAL SCIENTIST

Carretero Navarro, Paula

TECHNICAL SUPPORT PERSONNEL

Nestares de Kok, Rebeca

MASTER THESIS STUDENT

Cordero Pedrero, Carla

UNDERGRADUATE STUDENT

Bachiller Guerrero, Carolina

KEYWORDS

Glioblastoma, Immunotherapy, Magnetic Resonance Imaging, Metabolism, Tumor Microenvironment

RESEARCH LINES:

Overview
The THERABRAIN research group focuses on understanding the biological mechanisms underlying glioblastoma progression and therapeutic response through the integration of advanced imaging, tumor metabolism, and immunotherapy-related processes. The laboratory combines in vitro, in vivo, and computational approaches to identify functional interactions between tumor microenvironment, immune regulation, and metabolic reprogramming, with the ultimate goal of developing non-invasive biomarkers and precision therapeutic strategies in neuro-oncology.

Immunotherapy and Tumor Immunometabolism in Glioblastoma
This research line investigates the functional relationship between immune checkpoint regulation and tumor metabolic pathways in glioblastoma. Experimental modulation of PD-L1, IDH1, IDO1, and Chk-α in murine and human glioblastoma models has revealed coordinated alterations in metabolite profiles, including lactate, glutamate, and phosphocholine, supporting the existence of an immunometabolic regulatory axis. Transcriptomic analyses from public datasets further reinforce the inverse relationship between PD-L1 and Chk-α expression in human tumors, highlighting translational relevance. These findings provide a mechanistic basis for the design of combined metabolic-immune therapeutic strategies.

Multimodal Imaging and Biomarker Development in Glioblastoma
This line aims to develop and validate imaging-based biomarkers capable of characterizing tumor metabolism, immune microenvironment, and therapeutic response in vivo. Advanced magnetic resonance imaging protocols—including quantitative T1/T2/T2*, diffusion, magnetization transfer, and spectroscopy—have been optimized in orthotopic murine glioblastoma models. Complementary mass spectrometry imaging enables spatial molecular validation of imaging findings, supporting the identification of predictive biomarkers for precision medicine applications. Current work focuses on integrating multimodal datasets and preparing therapeutic intervention studies.

Exercise, Brain Physiology, and Tumor Hypoxia
A complementary research direction evaluates the impact of aerobic physical exercise on glioblastoma growth dynamics and tumor oxygenation using oxygen-enhanced magnetic resonance imaging (OE-MRI). Preclinical evidence indicates slower tumor progression and increased intratumoral oxygenation in exercised animals, together with sex-dependent biological responses. These results support the role of exercise as a modulator of tumor hypoxia and highlight OE-MRI as a non-invasive monitoring tool in experimental glioblastoma.

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DOCTORAL THESES AND OTHER WORKS:

Carla Cordero Pedrero
Master's thesis. E*valuación del impacto del ejercicio físico en el crecimiento del glioblastoma medido mediante imagen de resonancia magnética*. Universidad Complutense de Madrid. 2025. Supervisors: Pilar López Larrubia; Jesús Pacheco Torres; María Begoña Quintana.

FUNDING:

Inmunoterapia personalizada para glioblastoma: interacción entre puntos de control inmunitario, microentorno tumoral y metabolismo tumoral.
Reference: PID2022-137572OA-I00. Funding organization: Agencia Estatal de Investigación (AEI) – Ministerio de Ciencia e Innovación.
Dates: 01/09/2023 – 31/08/2026. PI: Jesús Pacheco Torres.
Total direct funding: 170,000 €.

OUTREACH ACTIVITIES:

The project has contributed to multiple scientific dissemination and training initiatives, including participation in the Semana de la Ciencia at the Instituto de Investigaciones Biomédicas Alberto Sols and outreach activities within the Ciencia en el Barrio program aimed at pre-university students. In addition, the group has supervised undergraduate and master's research projects and submitted scientific communications to national and international conferences, alongside a manuscript currently under peer review.

ImAIgenelab – Dynamic Cancer Insight With AI

TENURED-TRACK SCIENTIST

Alieva Krasheninnikova, María
(Investigadora Talento CAM, CSIC)

CONTRACT RESEARCHER

Hoffman García, Oliver John
Molina Moreno, Miguel
Villegas López, Lupe Ivette
Zamora Berna, Jorge Aurelio
Falempin, Clarissa Anastasie
Rubio Muñoz, Alejandra
Rios Jiménez, Emilio

TECHNICAL SUPPORT PERSONNEL

Fernández Archidona, Sandra

KEYWORDS

Artificial intelligence, Cancer, Computational biology, Immunotherapy, Invasion, Live imaging, Single cell

RESEARCH LINES:

Overview

The group of Dr. Alieva (imAIgene-lab) is a computational research laboratory focused on the development and integration of advanced functional and molecular omics to study cancer biology. Our work combines quantitative live-cell microscopy, single-cell and spatial omics, and data-driven modeling to extract biologically and clinically meaningful insights from complex biological systems. A central concept of the lab is the treatment of quantitative microscopy as a functional omics layer, capturing dynamic cellular behaviors—such as motility, interaction, and invasion—as high-dimensional, single-cell readouts that are directly comparable and integrable with transcriptomic, proteomic, and spatial molecular data. Through close collaborations with leading experimental laboratories specializing in state-of-the-art imaging, organoid models, and sequencing technologies, we develop integrative frameworks that link cellular function, molecular state, and tissue context.

Our research addresses two main biological questions in oncology:

1. Understanding mechanisms of response and resistance to cellular immunotherapies, and

2. Dissecting the cellular and microenvironmental determinants of tumor invasion.

Across both lines, we focus on integrating functional imaging data with multi-omic molecular profiles to uncover how molecular programs translate into cellular behaviors that ultimately determine therapeutic outcome.

Decoding tumor resistance to cellular immunotherapy

Researchers involved: Zamora Berna, Jorge Aurelio; Molina Moreno, Miguel; Rubio Muñoz, Alejandra; Rios Jiménez, Emilio; Fernández Archidona, Sandra; Alieva Krasheninnikova, María

Despite the clinical success of T-cell–based immunotherapies, their efficacy in solid tumors remains limited and highly heterogeneous. These therapies represent dynamic “living drugs”, whose functional behavior within the tumor microenvironment is not adequately captured by static molecular readouts alone.

In this research line, we apply quantitative live-cell imaging as a functional omics approach to characterize T-cell dynamics during interactions with patient-derived tumor organoids. Our previous work has shown that heterogeneous T-cell behaviors are predictive of distinct functional states and therapeutic efficacy. Building on this concept, we integrate imaging-derived functional phenotypes with single-cell and spatial transcriptomic data to uncover the molecular programs underlying effective and dysfunctional T-cell responses.

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The long-term goal is to leverage this integrated functional–molecular framework to identify mechanisms of resistance and to rationally guide combinatorial therapeutic strategies that restore effective anti-tumor T-cell dynamics.

Understanding microenvironmental drivers of tumor cell invasion

Researchers involved: Ríos Jiménez, Emilio; Falempin, Clarissa Anastasie; Muñoz Rubio, Alejandra; Fernández Archidona, Sandra; Alieva Krasheninnikova, María

Tumor invasion is a complex, multiscale process shaped by intrinsic cellular programs and extrinsic cues from the tumor microenvironment. While modern technologies allow detailed characterization of individual aspects of this process—such as live imaging of invasion dynamics, multiplexed tissue imaging, or single-cell transcriptomics—each modality in isolation provides only a partial view.

In this research line, we develop computational integrative approaches that combine dynamic functional imaging with spatial and single-cell molecular omics to achieve a systems-level understanding of tumor invasion, with a particular focus on brain tumors. By linking invasion phenotypes observed by live microscopy to molecular states and spatial niches, we aim to identify predictive pathways and microenvironmental conditions that drive invasive behavior.

This work provides a framework to bridge phenotypic, molecular, and contextual information, enabling the identification of actionable determinants of tumor infiltration.

Software development for imaging-based immune oncology assays

Researchers involved: Hoffman García, Oliver John; Fernández Archidona, Sandra; Villegas López, Lupe Ivette; Alieva Krasheninnikova, María

Inter-patient variability is a major determinant of therapy resistance, particularly in immuno-oncology, highlighting the need for functional screening assays that capture heterogeneity in therapeutic response and mode of action. To address this challenge, we have developed BEHAV3D, a live-cell imaging and analysis platform that extracts functional single-cell phenotypes from complex 3D co-culture systems.

BEHAV3D treats dynamic imaging data as a functional omics readout, enabling the identification of distinct cellular populations and functional states associated with tumor elimination or therapy failure. While the platform has demonstrated strong predictive potential, its current implementation is limited in throughput and scalability.

Our ongoing work focuses on leveraging recent advances in artificial intelligence to adapt BEHAV3D to large-scale, high-throughput screening assays, facilitating its integration with molecular omics data and enabling systematic, patient-relevant functional profiling of immunotherapy products.

Moreover, we are developing livecell imaging–based morphokinetic analyses to quantify chromosomal instability at singlecell resolution and integrate these dynamic phenotypes with molecular profiles, with the aim of understanding how CIN generates functional heterogeneity and promotes tumor progression.

PUBLICATIONS:

Wezenaar AKL, Pandey U, Keramati F, Hernandez-Roca M, Brazda P, Barrera Román M, Cleven A, Karaiskaki F, Aarts-Riemens T, de Blank S, Hernandez-Lopez P, Heijhuurs S, Alemany A, Kuball J, Sebestyen Z, Dekkers JF, Stunnenberg HG, Alieva M, Rios AC. Mapping T cell dynamics to molecular profiles through behavior-guided transcriptomics. *Nat Protoc.* **2025** *Sep;20(9)*:2453-2480. DOI: 10.1038/s41596-024-01126-4.

Rios-Jimenez E, Zomer A, Collot R, Barrera Román M, Archidona SF, Ariese H, van Ineveld R, Kleinnijenhuis M, Bessler N, Johnson H, Dawson CA, Rios A, Alieva M. BEHAV3D Tumor Profiler to map heterogeneous cancer cell behavior in the tumor microenvironment. *Elife*. **2025** *Oct 15;*13:RP102097. DOI: 10.7554/eLife.102097.

DOCTORAL THESES AND OTHER WORKS:

Capitulo de libro: Vogt, Y., Kalweit, M., Alieva, M., Ullrich, E., Boedecker, J., Kalweit, G. (2025). AI in Modular Concepts of Natural Killer Cell Therapy. In: Zimmer, J., Ullrich, E. (eds) Natural Killer Cells. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-662-68816-8_68-1

FUNDING:

“Deep learning-based 3D Virtual Multiplexing to explore microenvironment drivers of brain tumor progression. LEO23-2-10305-BBM-BAS-144”. Leonardo Grant for Researchers and Cultural Creators 2023 from the BBVA Foundation. 2023-2025

“DCODER: Unravelling cell Dynamics to deCODE tumor cell Resistance to inmunotherapy. 2022-T1/BMD-24021” Programa de atracción de talento de la Comunidad de Madrid. 2023-2028

“Developing an organoid Dynamic Screening Platform to Evaluate Inmuno-Oncology Drug activities in a Clinically Relevant Preclinical Model. TKI”. Netherlands-Holland, Consortium grant. 2024-2026

“Ayudas para la realización de contratos para ayudantes de investigación y ayudante de investigación de la comunidad de Madrid 2023. PEJ-2023-TL/SAL-GL-28092”. Comunidad de Madrid. 2024-2026

“Unraveling the microenvironment niches driving pediatric glioma infiltration in the brain. ASEICA-FERO Vth award”. ASEICA-FERO. 2024-2026

“Exploring tumor heterogeneity with morphocynetic analysis: tools for live imaging. Momentum grant MMT24-IIBM-01”. CSIC The funding for these actions/grants and contracts comes from the European Union’s Recovery and Resilience Facility-Next Generation, in the framework of the General Invitation of the Spanish Government’s public business entity Red.es to participate in talent attraction and retention programmes within Investment 4 of Component 19 of Recovery, Transformation and Resilience Plan. 2024-2028

AWARDS:

Emilio Ríos Jiménez: galardonado con la Beca Predoctoral AECC 2025, que finalmente no pudo aceptarse debido a condiciones salariales precarias y a la imposibilidad por parte del CSIC de complementar el salario con otros proyectos.

OUTREACH ACTIVITIES

Alieva, M. Ponencia divulgativa *“Cohetes y microscopios: el legado de las mujeres computadoras”*, dirigida a estudiantes de 5.º de Educación Primaria. C.E.I.P. Ángel de León, Colmenar Viejo.

Alieva, M. Charla divulgativa dirigida a alumnado del ciclo de Formación Profesional en Anatomía Patológica. IES Rosa Chacel, Madrid. Actividad realizada en el IIBM.

Villegas, L. *EPIC 5 + Dorothy Coding Challenge: Bridging Empirical Data and Simulations – From Astrophysics to Computational Biophysics.* ICTP Physics Without Frontiers, Universidad Internacional del Ecuador, 4–8 de agosto de 2025 (40 horas). Participación como organizadora en un evento internacional e interdisciplinar centrado en simulación computacional y análisis de datos.

Villegas, L. Charla *“Historias ocultas de las proteínas, células y tejidos”.* IES Villa de Vallecas, Comunidad de Madrid, 18 de febrero de 2025. Participación como ponente en una actividad divulgativa con motivo del Día Internacional de la Mujer y la Niña en la Ciencia.

Protective Peptides Against Acute and Chronic Injury of the Nervous System

TENURED SCIENTIST

Díaz-Guerra González, Margarita
(Investigador Científico, CSIC).
Group Coordinator

PRE-DOCTORAL SCIENTIST

Ugalde Triviño, Lola

CONTRACT RESEARCHER

Torres Campos, Elena

MASTER THESIS STUDENT

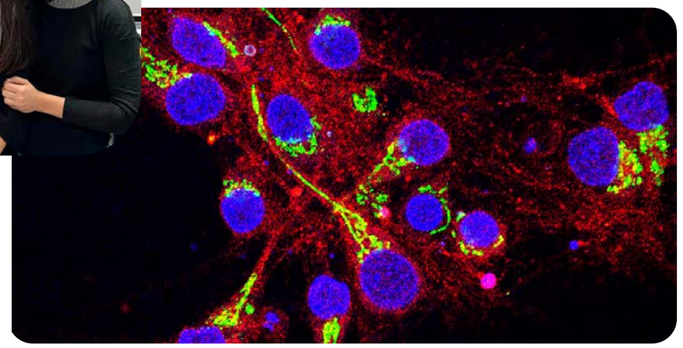
Ruiz Cabrera, Sara

VISITING SCIENTIST

Esteban Ortega, Gema
Hourdillè Paredes, Juana
Prizio, Erica

KEYWORDS

Cell-penetrating Peptides, Excitotoxicity, Neuroprotection, SCI, Stroke, TrkB



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RESEARCH LINES:

Overview

The impact of acute CNS injuries, including stroke, spinal cord injury (SCI) and traumatic brain injury (TBI), is devastating. Their combined global prevalence is extremely high, yet effective treatments are lacking. To meet this clinical need, we must prioritize development of novel neuroprotective therapies that restrict the secondary neuronal death taking place in the nervous tissue, subsequent to an accidental and irreversible primary injury. The mechanisms of this delayed damage are shared by different acute CNS disorders, and mostly rely on the contribution of excitotoxicity, glial reactivity and neuroinflammation.

Our work has focused in the exploration of two alternative therapeutic strategies to prevent secondary neurotoxicity and neuroinflammation after damage: (1) interference of death signaling downstream the overactivation of the N-methyl-D-aspartate type of glutamate receptors (NMDARs), main cause of excitotoxicity, or (2) protection of pro-survival pathways negatively regulated by ischemia. Some proteins, such as postsynaptic density protein-95 (PSD-95) or tropomyosin-related kinase B receptor (TrkB) have dual roles in survival-death choices and, therefore, are promising targets for both types of strategies. We have characterized in depth the mechanisms of excitotoxicity, identified molecules of therapeutic and diagnostic interest, and developed relevant neuroprotective peptides to treat stroke but also other acute CNS pathologies associated with excitotoxicity. We have relied on cell-penetrating peptides (CPPs) formed by two elements: (1) a short sequence of the HIV-1 Tat protein (11 aa), which confers permeability to attached cargoes to cross the plasma membrane and different biological barriers, and (2) a similarly short sequence from the target protein. The use of CPPs is very promising for treatment of neurological diseases since they can cross the blood-brain barrier (BBB), the blood-spinal cord barrier (BSCB) or the blood-labyrinth barrier (BLB) while presenting low toxicity.

PSD-95 stabilization as a relevant target for stroke therapy

PSD-95 is critical to assembly of PSD signaling complexes at excitatory synapses, required for neuronal survival and function. However, calpain processing challenges function of this protein in stroke due to induction of excitotoxicity and protease activation. We have shown before that interference of this PSD-95 processing is a therapeutic target for stroke. For that, we first analyzed the nature and stability of the PSD-95 fragments produced by calpain using a combination of *in vitro* assays, primary neuronal cultures subjected to *in vitro* excitotoxicity

or a preclinical model of permanent stroke. We could establish the location of the PSD-95 cleavage-sites in three specific interdomain linker regions and, based on these results, rationally designed three Tat-derived CPPs containing the cleavage sequences. One of the CPPs, $MTP95_{414}$, was able to interfere PSD-95 downregulation and reduce neuronal death by *in vitro* excitotoxicity. Moreover, *in vivo*, $MTP95_{414}$ was efficiently delivered to mice cortex and significantly improved the neurological outcome after stroke damage, suggesting that this peptide might have potential for ischemia therapy. More recently, we have tested a possible improvement of $MTP95_{414}$ neuroprotection by combined use with a different PSD-95-targeted CPP, nerinetide, a peptide that prevents the establishment of GluN2B-PSD-95-nNOS complexes and formation of nitric oxide (NO) in excitotoxic conditions. Nerinetide has already shown promising results for acute stroke treatment in Phase 3 clinical trials. In contrast to the anticipated results, we found that nerinetide and $MTP95_{414}$ had very similar mechanisms of action, affected the same signaling pathways and, therefore, exerted similar functions in neurons. This results in a lack of potentiation of neuroprotective effects after peptide combination.

Retrograde transport of neurotrophin receptor TrkB-FL regulates Golgi stability in excitotoxicity and is a target for stroke therapy

The full-length isoform of TrkB (TrkB-FL) is the high-affinity receptor for brain-derived neurotrophic factor (BDNF), a binding that induces signaling pathways regulating, among others, neuronal survival. However, BDNF/TrkB-FL signaling becomes aberrant in stroke, mainly due to receptor calpain-processing secondary to TrkB-FL endocytosis induced by excitotoxicity. We previously designed a neuroprotective CPP containing a TrkB-FL sequence, $MTFL_{457}$, which efficiently prevented excitotoxicity-induced receptor processing and neuronal death by a PLC-dependent mechanism. In the stroke model, $MTFL_{457}$ decreased the infarct size and improved the neurological outcome. Our results showed that receptor endocytosis induced by excitotoxicity was followed by TrkB-FL interaction with hepatocyte growth factor-regulated tyrosine kinase substrate (Hrs), retrograde transport to Golgi apparatus (GA) and organelle disruption, considered as a hallmark of neurodegenerative diseases. Interestingly, $MTFL_{457}$ efficiently interfered TrkB-FL/Hrs interaction and receptor trafficking, required for excitotoxic GA fragmentation and TrkB-FL cleavage. Thus, we proposed that TrkB-FL had a central role in GA stability and peptide $MTFL_{457}$ might preserve GA function and promote neuronal survival, not only in stroke but also other diseases affecting the nervous system. In fact, we have shown that $MTFL_{457}$ has similarly a great potential for treatment of noise-induced hearing loss and, probably, other types of sensorineural hearing loss likewise associated with excitotoxicity (collaboration with I. Varela, IIBM). We have employed an *ex vivo* model of excitotoxicity, where this process is induced in cochlear explants by glutamate receptor agonists, and an in vivo model of noise overexposure. Firstly, we demonstrated that $MTFL_{457}$ was able to reach and distribute through the cochlea both *ex vivo* and *in vivo* and, for the latter, using systemic and local routes. Secondly, we observed that excitotoxicity induced calpain activation and reduced levels of TrkB and p-CREB in cochlear explants, decreasing neurotrophic support and resulting in cochlear synaptopathy. However, pretreatment with $MTFL_{457}$ prevented the dysregulation of neurotrophic receptors in cochlear explants, preserving neurotrophin-mediated signaling, and reducing neurodegenerative processes, including cochlear synaptopathy and GA fragmentation. In the model of noise overexposure, pretreatment with $MTFL_{457}$ had a protective effect on hearing function and cochlear synapses in both male and female mice.

TrkB-T1 specific interactome as a target for neuroprotection

In addition to TrkB-FL, neurons express a truncated isoform lacking the tyrosine kinase domain, TrkB-T1, which acts as a TrkB-FL dominant negative mutant and is involved in death pathways. The truncated receptor is also expressed in astrocytes and has TrkB-FL-independent functions, probably mediated by protein interactions established by a highly conserved TrkB-T1 intracellular sequence. Excitotoxicity alters TrkB-T1 levels and activity by mechanisms that include transcriptional upregulation, regulated intramembrane proteolysis (RIP), which produces a receptor ectodomain acting as a BDNF-scavenger and intracellular fragments (ICDs) of unknown function, and changes in TrkB-T1 specific protein interactions. For neuroprotection, we have developed peptides able to prevent TrkB-T1 cleavage by metalloproteinases, first and obligatory step for RIP, or interfere isoform-specific protein interactions. Treatment with such an interfering CPP, $TT1_{Ct}$, results in prevention of reactive gliosis and strongly decreases excitotoxicity-induced damage in cellular and mouse models of stroke. A biotinylated form of $TT1_{Ct}$ has also been very useful to identify the profile of TrkB-T1-interacting proteins in basal conditions or after excitotoxicity, an information critical to establish the role of this truncated receptor in neural cells function and viability. In collaboration with M. Concepción Serrano López-Terradas (ICMM, CSIC), we are currently formulating peptide $TT1_{Ct}$ into natural hydrogels for local administration in SCI models and stroke. Finally, to establish the function of TrkB-T1-ICD, we have designed a mock peptide (Bio-$LTT1_{Ct}$)

containing this short intracellular TrkB-T1 region (23 amino acids) and tested it *in vitro* and *in vivo*. Our results demonstrate that TrkB-T1 RIP is a central mechanism of ischemic damage and that the isoform intracellular region is sufficient to recapitulate stroke-like effects on neurotoxicity, glial reactivity and neuroinflammation.

PUBLICATIONS:

Esteban-Ortega, G.; Torres-Campos, E.; Díaz-Guerra, M. Retrograde transport of neurotrophin receptor TrkB-FL induced by excitotoxicity regulates Golgi stability and is a target for stroke neuroprotection. Cell *Death & Dis*. **2025**,16, 659. DOI 10.1038/s41419-025-07990-6.

Ugalde-Triviño, L.; Tejeda, G.S.; Esteban-Ortega, G.; Díaz-Guerra, M. A brain-accessible peptide modulates stroke inflammatory response and neurotoxicity by targeting BDNF-receptor TrkB-T1 specific interactome. *Theranostics* **2025**,15, 4654-4672. DOI 10.7150/thno.111272

DOCTORAL THESES AND OTHER WORKS:

Elena Torres Campos
Ph.D. tesis. *Nuevas estrategias terapéuticas para la sinaptopatía coclear y la pérdida auditiva neurosensorial: MTFL$_{457}$, un péptido neuroprotector derivado del receptor neurotrófico TrkB-FL*. Universidad Autónoma de Madrid. 2025. Supervisor/s: Isabel Varela Nieto & Margarita Díaz-Guerra González. Grade: Sobresaliente Cum Laude.

Sara Ruiz Cabrera
Master´s thesis. *Estudio comparativo de los mecanismos de acción de los péptidos neuroprotectores nerinetida y MTP95$_{414}$ en excitotoxicidad*. Universidad Complutense de Madrid. 2025. Supervisor: Margarita Díaz-Guerra González. Grade: Matrícula de Honor.

FUNDING:

Mejora de la protección neuronal y cerebral en el ictus mediante técnicas avanzadas de administración de péptidos penetrantes derivados de TrkB y PSD-95. PID2022-137710OB-100. Agencia Estatal de Investigación. 2023-2026. PI: Margarita Díaz-Guerra González.

Preclinical NeuroImaging (PILab)

TENURED SCIENTIST

López Larrubia, Pilar
(Investigadora Científica, CSIC).
Group Coordinator

ASSOCIATED INVESTIGATOR

Gandía González, María Luisa

PRE-DOCTORAL INVESTIGATOR

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Córdova Ascurra, Darwin A.

UNDERGRADUATE STUDENT

Castellar García, María
Felipe Maestre, María de la Jara
Pablo Pasos, Ruth
Ruiz Batista, Lidia
Rodríguez San Pedro, Andrea
Almellones Iglesias, Celia

TECHNICAL SUPPORT PERSONNEL

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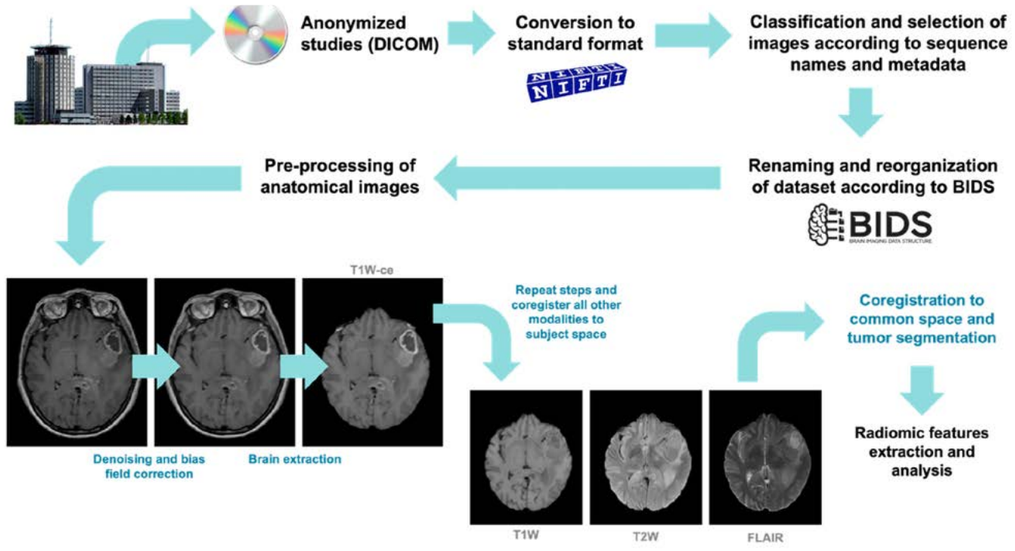
Guillén Gómez, María J.
Domingo Ratia, Andrea C.
López Berenjeno, Carlos
Román Moreno, María

KEYWORDS

Glioblastoma, Multimodal Imaging, Nanomedicine, Neuroinflammation, Biomedical Magnetic Resonance



Schematic representation of the MRI pre- and post-processing workflow for quantitative brain image analysis, including denoising, bias field correction, skull stripping, co-registration, and subsequent radiomics-based feature extraction and imaging biomarker identification. Representative example: clinical MRI of a patient with glioblastoma.

RESEARCH LINES:

Overview

Our laboratory focuses on the application of advanced preclinical magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS) to support precision medicine in neurological disorders. We develop and implement multiparametric MRI and MRS approaches to identify non-invasive imaging biomarkers that enhance disease characterization, stratification, therapy validation, and longitudinal monitoring.

A major research focus of the group is the role of neuroinflammation (NI) in complex disorders such as glioblastoma and obesity. We integrate imaging with metabolomic, molecular, and histological analyses to achieve a comprehensive understanding of disease mechanisms. Our work is specifically aimed at capturing disease heterogeneity and its interaction with key biological modifiers, including sex and metabolic status.

In parallel, we pursue translational strategies designed to bridge preclinical models and clinical imaging data, facilitating bidirectional knowledge transfer. We also explore theranostic approaches based on functionalized nano-

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materials that combine targeted therapeutic interventions with imaging-guided monitoring.

An additional strategic objective of the group is the integration of radiomics and artificial intelligence (AI) -machine learning- into both preclinical and clinical MRI studies. These computational approaches are applied to enhance imaging biomarker extraction, improve quantitative neuroimaging analyses, and advance disease characterization.

Development of a dedicated quantitative MRI and MRS imaging environment for the diagnosis and prognosis of Neuroinflammation

Researchers involved: López Larrubia, Pilar; Arias Ramos, Nuria; González Alday, Raquel.

This research line aims to establish a robust and integrated experimental framework for the quantitative assessment of NI using advanced MRI and MRS methodologies. To support all subsequent research lines, colonies of WT and KO mice for TNF-α and the IL-1R were used.

A comprehensive set of standardized MRI acquisition protocols was developed and validated, including high-resolution anatomical imaging, as well as multiparametric approaches enabling the quantitative assessment of relaxation times, diffusion, magnetization transfer, perfusion, and functional and metabolic parameters. To ensure reproducibility and objective data analysis, an automatic image processing and analysis pipeline has been developed in Python for the correction, normalization and generation of parametric maps from MRI datasets. This software, named RESOMAPER, provides a dedicated environment for quantitative image analysis.

Histological and immunohistochemical workflows were also implemented to support multimodal validation of imaging findings. These protocols included the analysis of key markers associated with microglial activation, astrocytosis, and inflammation (Iba-1, GFAP and CD68).

In addition, an animal model of systemic inflammation was established through intraperitoneal administration of lipopolysaccharide. This model enabled the evaluation of NI in the absence of other coexisting pathologies, providing a controlled framework to isolate NI-related effects. MRI studies performed in this model identified diffusion-derived parameters capable of detecting and quantifying cytotoxic effects associated with NI, which were further supported by HRMAS-based metabolic analyses.

Evaluation and characterization of the contribution and role of neuroinflammation in glioblastoma and obesity

Researchers involved: López Larrubia, Pilar; Arias Ramos, Nuria; González Alday, Raquel; Córdova Ascurra, Darwin A..

This research line investigates the role of NI in glioblastoma (GBM) and obesity development through a translational approach that integrates preclinical models with clinical data. GBM models were generated by stereotactic intracranial injection of GL261 murine glioma cells into WT and KO mice. Experimental models of moderate and severe diet-induced obesity were established by exposing WT and KO animals to a high-fat diet for 10 or 20 weeks, respectively. To evaluate the interaction between metabolic status and tumor progression, equivalent GBM models were also generated in animals with moderate and severe obesity induced by HFD feeding.

Multiparametric MRI studies were conducted in WT animals to characterize the NI component associated with tumor growth and obesity development under control conditions, as well as to assess the impact of obesity-associated NI on GBM progression. Advanced multivariate analytical approaches, including principal component analysis, were applied to identify imaging-derived parameters most strongly associated with neuroinflammatory processes during disease progression. Magnetization transfer and diffusion-derived MRI parameters were identified as particularly sensitive biomarkers of NI-related tissue alterations. In parallel, the development of nosological imaging maps is ongoing to integrate these findings into a comprehensive pathophysiological framework.

Equivalent imaging and analytical protocols are being applied to KO animal cohorts under both control and pathological conditions in male and female animals. This broad experimental design enables systematic dissection of the inflammatory pathways involved in GBM and obesity progression. At the conclusion of each experimental protocol, animals were processed for post-mortem analyses using complementary approaches optimized for NMR-based metabolomics and immunohistological evaluation.

In addition, the functional role of aquaporin-4 channels was assessed in obese versus control animals. These studies revealed a dysregulated response to glucose administration in obesity, which may contribute to the maintenance and progression of NI.

Computation, radiomics and IA approaches in the assessment of preclinical imaging.

Researchers involved: López Larrubia, Pilar; Arias Ramos, Nuria; González Alday, Raquel

Our group has initiated a new research line focused on computational and AI approaches applied to preclinical and clinical imaging, integrating both animal models and human data. Clinical and radiological MRI datasets have been collected from GBM patients treated at Hospital Universitario La Paz, enabling the adaptation and extension of our image-processing pipeline to the clinical setting. A dedicated workflow was developed to curate and prepare the clinical MRI dataset for downstream analyses. As the images were acquired in the context of routine clinical practice, substantial effort was devoted to

data organization and standardization. This process included conversion from DICOM to NIfTI format, classification of sequences based on metadata, and separation of primary acquisitions from derived images. All files were subsequently renamed and structured according to the BIDS standard.

In parallel, a preprocessing pipeline was implemented and optimized, incorporating denoising, bias-field correction, skull stripping, and co-registration to a common reference space. Finally, radiomics analyses are currently being conducted as an initial strategy to quantitatively characterize tumor-related regions and to identify clinically relevant imaging biomarkers.

PUBLICATIONS:

Marcos, A.; Rodríguez del Cerro, M. C.; Fernández, R. M.; Pásaro, E.; Arias-Ramos, N.; López-Larrubia, P.; González-Peramato, P.; Guillamon, A.; De Miguel, M. P. The GnRH Agonist Triptorelin Causes Reversible, Focal, and Partial Testicular Atrophy in Rats, Maintaining Sperm Production. *Int. J. Mol. Sci.* **2025**, *26(14),* 6566. DOI: 10.3390/ijms26146566.

Caro, C.; Arias-Ramos, N.; Urbano-Gámez, J. D.; González-Alday, R.; López-Larrubia, P*.; García-Martín, M. L*. A multiparametric perspective on C6 and F98 cell lines in orthotopic rat models for glioblastoma research. Sci. Rep. 2025, 15, 22547. DOI: 10.1038/s41598-025-06684-5.
Tirado-García, P.; Ferreiro, A.; González-Alday, R.; Arias-Ramos, N.; Lizarbe, B*.; López-Larrubia, P*. Aquaporin-4 inhibition alters cerebral glucose dynamics predominantly in obese animals: an MRI study. *Sci. Rep.* **2025**, *15(1)*, 15649. DOI: 10.1038/s41598-025-99641-1.

Gómez-González, E.; Núñez, N. O.; Caro, C.; García-Martín, M. L.; Monje Moreno, J. M.; Hamdi, A.; López-Larrubia, P.; Becerro, A. I.; Ocaña, M. Sodium lanthanide tungstate-based nanoparticles as bimodal probes for T_1–T_2 magnetic resonance imaging and X-ray computed tomography. *Dalton Trans.* **2025**, *54*, 16562-16572. DOI: 10.1039/D5DT01925G.

FUNDING:

Selective Imaging of Neuroinflammation by Multiparametric MRI/PET Technologies. PID2021-122528OB-I00. Ministerio de Ciencia e Innovación. 2022-2026. PI: Pilar López Larrubia.

Red de Enfermedades Raras CSIC (RER-CSIC). 202420E019. Consejo Superior de Investigaciones Científicas. 2024-2026. PI: Pilar López Larrubia.

Preclinical Optical Imaging System: Fluorescence, bioluminescence and 3D X-rays. EQC2024-008496-P. Ministerio de Ciencia e Innovación 2024-2025. PI: Pilar López Larrubia.

Diagnóstico por imagen molecular: investigación básica y desarrollo traslacional. RED2022-134299-T. Ministerio de Ciencia e Innovación 2023-2025. PI: Pilar López Larrubia. (Coordinador: Fernando Herranz)

RICORS Advanced Therapies. RICORS Code: RD24/0014/0001. Instituto de Salud Carlos III. PI: Joana María Ramis Morey.

Uso de Vesículas Extracelulares que expresan NIS para la teragnosis de tumores infantiles. PI24CIII/00046. Acción Estratégica en Salud Intramural, Instituto de Salud Carlos III. 2024-2026. PI: Pilar Martín Duque.

Nuevas estrategias terapéuticas para el tratamiento de enfermedades raras neurosensoriales (SensoRare). Acciones Cooperativas y Complementarias Intramurales 2023 (ACCI), Instituto de Salud Carlos III. 2024-2025. PI: Silvia Murillo

AWARDS:

Honorable mention among the three best oral presentations in Session 3.1 – Central Nervous System III in FARMADRID 31 Congress. Title: Neuroinflammatory profiling of high-fat diet effects in il-1r1ko mice: Insights from multiparametric mri and indirect calorimetry (Darwin A. Cordova). Universidad CEU San Pablo (Madrid). 17/06/2025.

Cum Laude Poster 5th Annual Meeting of the ISMRM Iberian Chapter "Resomapper: a user friendly and versatile pipeline for multiparametric MRI data processing and mapping". Raquel González-Alday, Adriana Ferreiro, Nuria Arias-Ramos, Blanca Lizarbe, Pilar López-Larrubia. Barcelona. 3-4/07/2025.

Cum Laude Poster 5th Annual Meeting of the ISMRM Iberian Chapter "Evaluating neuroinflammation in-vivo in a mouse model using multiparametric MRI, with ex-vivo insights from immunofluorescence and HRMAS spectroscopy". Raquel González-Alday, Carla Dávila-Yagüe, Nuria Arias-Ramos, Blanca Lizarbe, Pilar López-Larrubia. Barcelona. 3-4/07/2025.

MRI Together mention: "Resomapper: a user friendly and versatile pipeline for multiparametric MRI data processing and mapping". Raquel González-Alday, Adriana Ferreiro, Nuria Arias-Ramos, Blanca Lizarbe, Pilar López-Larrubi. ESMRMB, 41th Annual Scientific Meeting, Marseille (France). 08-11/10/2025.

OUTREACH ACTIVITIES

Semana de la Ciencia 2025. Title: Educational tour of the IIBM-MRI facility for high school students. IIBM, 3 – 16/11/2025.

Noche Europea de los Investigadores e Investigadoras: el CSIC te llama esta noche. Title: Escape Road volunteering. Espacio Fundación Telefónica (Madrid), 26/09/2025

Teaching activity. Aplicaciones de la Resonancia Magnética Biomédica. Programa de Formación del CSIC. IIBM, 22 – 26/09/2025.

Teaching activity. Neurotech EU Summer School - Preclinical Magnetic Resonance Imaging and Spectroscopy: from the bench to the bedside. Instituto de Neurociencias (CSIC-UMH), Alicante. 09-11/07/2025.

Neuroendocrine Pathology

TENURED-TRACK SCIENTIST

Bárez López, Soledad
(Investigadora Talento CAM, CSIC).
Group Coordinator

CONTRACT RESEARCHER

Guillén Yunta, Marina

VISITING SCIENTIST

Dumont, Isabel
Lara Cerezo, Teresa

MASTER THESIS STUDENT

Valero Herrero, Patricia

UNDERGRADUATE STUDENT

Lara Cerezo, Teresa

KEYWORDS

Brain function, Emotional behaviour, Murine models, Neuroendocrinology, Oxytocin, Thyroid hormone

RESEARCH LINES:

Overview

Our laboratory is dedicated to understanding how hormonal signals shape brain function, emotional behaviour, and vulnerability to mental health disorders. We focus on the neuroendocrine mechanisms that regulate mood, stress resilience, and social behaviour, with a particular emphasis on the interaction between thyroid hormones (TH) and oxytocin (OXT).

Thyroid hormones are essential for brain development and adult neural function, while OXT is a key modulator of social behaviour, stress regulation, and maternal bonding. Although these two systems have been studied extensively in isolation, their functional interaction in the brain remains poorly understood. We propose that the TH–OXT axis represents a critical but largely unexplored neuroendocrine pathway linking hormonal homeostasis to emotional health.

Our goal is to elucidate how disruptions in TH signalling affect the oxytocinergic system, neural circuits, and behaviour, and how this interaction contributes to neuropsychiatric vulnerability, particularly in postpartum depression, a highly prevalent mood disorder affecting 10–30% of new mothers worldwide.

To address these questions, we combine murine models, molecular and cellular neuroscience, neural circuit mapping, and transcriptomics with advanced tools such as Cre-dependent genetic models, viral tracing, chemogenetics, and large-scale 3D imaging. We also integrate human genetic data to bridge experimental findings with clinical relevance.

Line 1 - Molecular regulation of oxytocin by thyroid hormones

This line focuses on how thyroid hormones regulate oxytocin synthesis and release in the hypothalamus. We investigate how hypothyroidism alters gene networks, neuron–glia interactions, and structural plasticity within the supraoptic (SON) and paraventricular (PVN) nuclei — the principal sites of OXT production.

Using cell-type-specific genetic tools, we selectively perturb TH signalling in oxytocinergic neurons to distinguish direct hormonal effects from systemic changes. Transcriptomic and spatial analyses allow us to identify TH-dependent molecular pathways that control OXT synthesis, secretion, and neuronal remodelling.

This work aims to define the cellular mechanisms through which thyroid deficiency compromises the oxytocinergic system and predisposes to mood dysregulation.

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Line 2 - Genetic vulnerability in the TH–OXT axis and risk of postpartum depression

In collaboration with Dr.Genevieve Leyden (University of Bristol) we investigate whether genetic variation in key regulators of brain TH availability contributes to susceptibility to postpartum depression. We focus on genes involved in TH transport, metabolism, and signalling in the brain, including DIO2, DIO3, MCT8, OATP1C1, THRA, and THRB.

Using large-scale human datasets (MetaBrain, GTEx, and GWAS for TSH and postpartum depression), we apply genetic colocalization and Mendelian randomization to identify shared biological pathways linking thyroid regulation to mood disorders.

This translational approach complements our animal studies by providing human genetic evidence that the TH–OXT axis may be a risk factor for postpartum depression and a potential therapeutic target.

PUBLICATIONS:

Borges-Canha M, Leite AR, Conceição G, Vale C, Von-Hafe M, Martins D, Miranda-Silva D, Sousa-Mendes C, Chaves J, Lourenço IM, Grijota-Martínez C, Bárez-López S, Miranda IM, Leite-Moreira A, Falcão-Pires I, Neves JS. Evaluation of the hepatic and subcutaneous adipose tissue effects of triiodothyronine treatment in an animal model of metabolic syndrome. *Obes Res Clin Pract.* **2025** *19(2):*115-121. DOI: 10.1016/j.orcp.2025.04.001.

Montero-Pedrazuela A, Grijota-Martínez C, Guadaño-Ferraz A, Bárez-López S. A Sensitive Radioimmunoassay for T3 and T4 Determinations in Plasma and Tissues *Methods Mol Biol.* **2025**, *2876*:37-59. DOI: 10.1007/978-1-0716-4252-8_3.

DOCTORAL THESES AND OTHER WORKS:

Noelia Navarro

Master´s thesis. *Evaluación de los efectos tiromiméticos de sobetirome en cerebelo y tejidos periféricos del modelo murino Mct8/Dio2 KO del Síndrome de Allan-Herndon-Dudley*. Universidad de Alcalá. 2025. Supervisor/s: Soledad Bárez López and Ana Montero Pedrazuela. Grade: Sobresaliente (9,2)

Teresa Lara Cerezo

Final degree's project. *Explorando el papel de la hormona tiroidea en la regulación de oxitocina en ratón común (Mus musculus).* Universidad Politécnica de Madrid. 2025. Supervisor/s: Soledad Bárez López. Grade: Matrícula de Honor

FUNDING:

Explorando la regulación de las hormonas tiroideas sobre la oxitocina: implicaciones en la depresión postparto. PID2024-156039OA-I00. Ministerio de Ciencia, Innovación y Universidades (MCIU) y la Agencia Estatal de Investigación (AEI). 01/09/25 – 31/08/28. PI: Soledad Bárez López.

Ayudas de atracción de talento investigador César Nombela. 2024-T1/ SAL-GL-31247. Comunidad de Madrid. 01/12/24 – 30/09/29. PI: Soledad Bárez López

Exploring a personalized gene replacement therapy approach for the Allan-Herndon-Dudley Syndrome. OTR10255. Sherman Foundation. 01/05/23 – 31/04/2025. PIs: Soledad Bárez López and Ana Guadaño Ferraz

OUTREACH ACTIVITIES

Talleres Científicos para niños hospitalizados. Hospital La Paz. Title: "Llévate a casa tu ADN" and "Construye tu proteína". 01/12/ 2025

Noche Europea de los Investigadores. Title: Taller "Construye tu proteína". 26/09/2025..

Novel Targets in Neurodegeneration and Cancer

TENURED SCIENTIST

Iglesias Vacas, Teresa
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Lasa Benito, Marina
(Profesora Titular)

López Menéndez, Celia
(Científica Titular)

PRE-DOCTORAL SCIENTIST

Cilleros Rodríguez, Darío
Domínguez Salvador, Mª Inmaculada
Moreno Rupérez, Álvaro
Sánchez-Miranda Pajuelo, Luis
Simón García, Ana

MASTER THESIS STUDENT

Ruiz Izaguirre, Carlota

SENIOR TECHNICAL SPECIALIST

López Menéndez, Celia

TECHNICAL SUPPORT PERSONNEL

Prudencio Sánchez-Carralero, Marina
Sanz San-Cristóbal, Marina

KEYWORDS

SINO Syndrome, Stroke, Hydrocephalus, Prostate Cancer, Kidins220, Protein Kinase D

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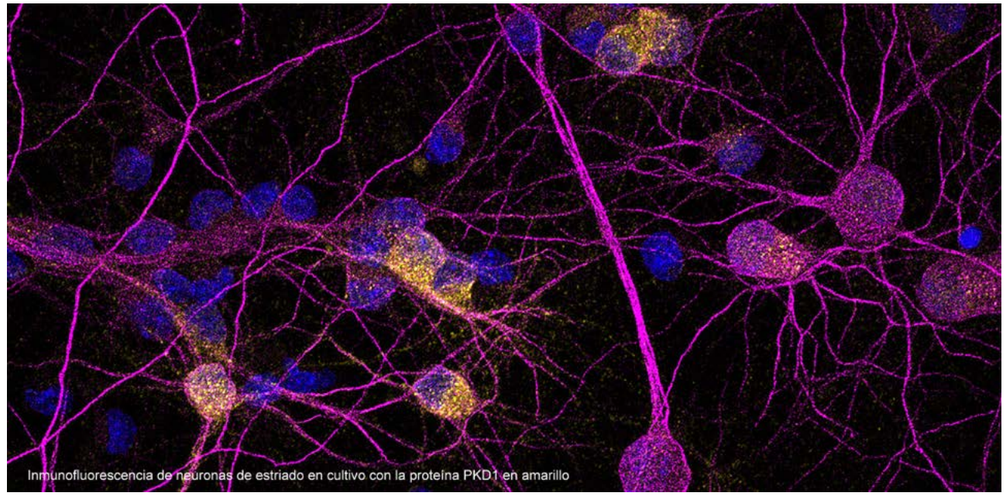

Inmunofluorescencia de neuronas de estriado en cultivo con la proteína PKD1 en amarillo

RESEARCH LINES:

Overview

Our group is dedicated to investigating the mechanisms that underly cell survival in two critical systems where this process plays a pivotal role.

Our primary focus is understanding the cellular and molecular mechanisms behind **neurodegeneration**, with the aim of developing neuroprotective strategies. We concentrate on two key molecules, **PKD1** (Protein Kinase D1) and **Kidins220** (Kinase D interacting substrate of 220kDa), which are crucial for neuronal survival whose enhancement provides neuroprotection, as we have demonstrated. Our goal is to uncover the roles of these molecules in neurological diseases marked by neuronal loss, such as after acute brain injury (e.g. **stroke**), or due to chronic neurodegeneration, including **Alzheimer's disease** (AD) and **Huntington's disease** (HD). AD and ischemic stroke (IS) are the leading causes of **dementia**, a progressive syndrome of memory loss affecting approximately 50 million people worldwide.

To complement this focus, we have developed a parallel line of research aimed at understanding the molecular mechanisms by which PKD regulates the development and progression of prostate cancer, another context where cell survival is critical. P**rostate cancer** is the second most common type of cancer and the fifth leading cause of cancer-related death in men worldwide.

Molecular Mechanisms of Excitotoxicity

Researchers involved: López C; Simón, A; Moreno, Á; Sánchez-Miranda, L; Sanz, M; Prudencio, M

Excitotoxicity is a type of neuronal death associated with several neuropathologies, such as IS and AD, and preventing it could offer neuroprotection across a wide range of neurological diseases. We have shown that a constitutively active mutant form of PKD1 provides neuroprotection in highly excitotoxic environments (Nat Comm, 2017). Our research explores how PKD1 regulates neurodegenerative processes, and we are testing the therapeutic potential of PKD1 in preclinical studies using murine models of both acute and chronic neurodegeneration. In a recent report we have shown PKD1 mediated neuroprotection in Huntington´s Disease experimental models (Cell Death & Dis, 2025) **(Figure 1).** Our approach includes using mice with conditional kinase deletion in different brain cell types and performing celomic, transcriptomic, metabolomic, and proteomic analyses.

Pathophysiological Mechanisms of KIDINS220 Deficiency

Researchers involved: López C; Simón, A; Domínguez, MI; Sanz, M; Prudencio, M

We were the first to clone Kidins220 as the first PKD1 substrate and are now studying its role in two rare diseases characterized by KIDINS220 deficits.

1. Idiopathic Normal Pressure Hydrocephalus (iNPH)

iNPH is the major form of chronic hydrocephalus in adults. It is a neurodegenerative disease associated with AD, presenting with dementia and characterized by the accumulation of cerebrospinal fluid, which enlarges the brain ventricles. Due to limited knowledge of its molecular mechanism, there are no pharmacological treatments for iNPH. We recently discovered that Kidins220-deficient mice develop chronic hydrocephalus, demonstrating that this protein regulates the brain's main water channel, aquaporin-4 (AQP4) (*Mol Psychiatry, 2021*). We also observed a reduction in KIDINS220 and AQP4 levels in the ependymal barrier of brain ventricles in iNPH patients. More recently we have found alterations in the visual system of Kidins220 deficient mice *(Fluids Barriers CNS, 2025).* Our goal is to study neurodegeneration markers in hypomorphic Kidins220 hydrocephalic mice and develop pharmacological and genetic therapeutic strategies to correct or prevent hydrocephalus in preclinical studies. Additionally, we aim to analyse iNPH patient samples to deepen our understanding of this disease.

2. SINO Syndrome.

Pathogenic variants of the KIDINS220 gene are associated with a newly identified rare paediatric syndrome called SINO (spastic paraplegia, intellectual disability, nystagmus and obesity). SINO patients exhibit ventriculomegaly similar to that seen in Kidins220-deficient mice *(Mol Psychiatry, 2021; Genet Med, 2024)*. Through an international collaborative effort, we plan to study the mechanisms underlying hydrocephalus and other SINO syndrome traits using human iPSCs and mouse models carrying these pathogenic variants.

PKD in Prostate Cancer

Researchers involved: Lasa, M; Cilleros-Rodríguez, D; Ruiz Izaguirre, Carlota

Our goal here is to explore the molecular mechanisms that regulate Protein Kinase D (PKD) in the development and progression of prostate cancer. This disease arises from a series of complex events that ultimately lead to an androgen-resistant phenotype, significantly complicating treatment. As a result, advanced prostate cancer is typically treated with chemotherapeutic agents. However, many tumors develop resistance, leading to poor prognosis. At the molecular level, we have demonstrated that prostate cancer progression is driven by the regulation of several key signalling pathways, including MAPKs and DUSP1 *(Mol Oncol, 2014; Food Chem Toxicol, 2019; Cancers, 2021)*. More recently, we discovered that PKD2 activity promotes the migration and invasion of prostate cancer cells (**Figure 2**), via its interaction with ERK and Snail, a key transcription factor in epithelial-mesenchymal transition *(Biochim Biophys Acta Mol Basis Dis, 2024).* Our research also revealed that PKD2 activity increases with the malignancy grade in human tumors, showing a positive correlation with both Snail expression and ERK activity. We are now further investigating PKD's involvement in other critical processes that contribute to prostate cancer formation and progression.progression.

PUBLICATIONS:

Cilleros-Rodríguez, D.; Lasa, M. Analysis of Apoptosis by Thyroid Hormone Induction. *Methods Mol. Biol.* **2025** (*2876* : 77-91). DOI.10.1007/978-1-0716-4252-8_5

Sebastián-Serrano, Á.*‡; Simón-García, A.*; Santos-Galindo, M.; Sánchez-Carralero, M.P.; H-Alcántara, A.; Clemente, C.; Pose-Utrilla, J.; Campanero, M.R.; Porlan, E.; Lucas, J.J.; Iglesias, T.‡ (*Co-first;‡Co-corresponding) Down-regulation of neuroprotective protein kinase D in Huntington´s disease. *Cell Death Dis.* **2025** (*16 (1)* : 418). DOI.10.1038/s41419-025-07688-9

Fernández-Albarral, J.A.*; Simón-García, A.*; Salobrar-García, E.; Salazar, J.J.; López-Menéndez, C.; Pajuelo, L.S.M.; Matamoros, J.A.; de Hoz, R.; López-Cuenca, I.; Elvira-Hurtado, L.; Sanchez-Puebla, L.; Sánchez-Carralero, M.P.; Sanz, M.; Ramírez, J.M.; Iglesias, T.‡; Ramírez, A.I.‡ (*Co-first;‡-Co-corresponding and Co-senior). Kidins220-deficient hydrocephalus mice exhibit altered glial phenotypes and AQP4 differential regulation in the retina and optic nerve, with preserved retinal ganglion cell survival. *Fluids Barriers CNS.* **2025** (*22 (1)* : 16). DOI.10.1186/s12987-025-00626-z

DOCTORAL THESES AND OTHER WORKS:

Luis Sánchez-Miranda Pajuelo
PhD tesis. *Protein Kinase D1 function in astrocytes: Regulation of maturation processes and its association with epilepsy.* Universidad Autónoma de Madrid. 2025. Supervisor/s: Teresa Iglesias Vacas and Celia López Menéndez. Grade: Sobresaliente Cum Laude.

Ana Simón García
PhD tesis. *Effect of excitotoxicity on SNX27 retromer function: design of neuroprotective strategies.* Universidad Autónoma de Madrid. 2025. Supervisor/s: Teresa Iglesias Vacas. Grade: Sobresaliente Cum Laude.

Carlota Ruiz Izaguirre
Master´s thesis. *Molecular mechanisms involved in the regulation of cell survival by protein kinase D in prostate cancer.* Universidad Autónoma de Madrid. 2025. Supervisor/s: Marina Lasa Benito. Grade: Sobresaliente.

FUNDING:

PRKD1- and KIDINS220-related Neurological Disorders: Novel Disease Models to Identify Pathological Mechanisms and Therapeutic Targets and Strategies. PID2023-153284OB-I00. MICIU. 2024-2028. PI: Teresa Iglesias Vacas

Nuevas estrategias terapéuticas basadas en la modulación del metabolismo oxidativo para el tratamiento del cáncer de tiroides V600E-BRAF-positivo. PID2023-147040NB-I00. MICIU. 2024-2027. PI: Pablo Baquero Valls Co.PI: Antonio Chiloeches Galvez

PATENTS:

Methods and Compositions for the Treatment of Disorders Characterized by a Kidins220 Dysfunction in a Subject. Iglesias T, Campanero MR, del Puerto A, Pose J, Simón A, López C, Sánchez-Miranda L. International Patent PCT/EP2022/061794. 2021

OUTREACH ACTIVITIES

Noche Europea de los Investigadores e Investigadoras. Title: Escape Road: a la búsqueda de mujeres Nobel y no Nobel. Espacio Fundación Telefónica (Madrid) – 26/09/2025.

II Jornada sobre Enfermedades Raras IIBM. Speaker: Teresa Iglesias Vacas. Title: Conociendo el Síndrome de Paraparesia Espástica, Discapacidad Intelectual, Nistagmo y Obesidad (SINO).
Instituto de Investigaciones Biomédicas Sols-Morreale (Madrid) – 24/02/2025.

department of **Rare Diseases**

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Rare Diseases Associated to Defects in Autophagy

TENURED SCIENTIST

Escalante Hernández, Ricardo
(Investigador Científico). Group Coordinator

Vincent, Olivier.
(Científico Titular)

ASSOCIATED INVESTIGATOR

Navas Hernández, María de los Ángeles
(Profesor Titular, UCM).

PRE-DOCTORAL SCIENTIST

Antón Esteban, Laura
Monforte Martínez, Beatriz
De León Oliva, Diego

MASTER THESIS STUDENT

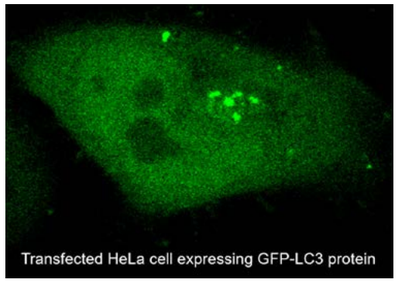
Concha Tello, Lisseth Carolina
Antón Sansano, Miguel
Treviño Domínguez, Alejandro

TECHNICAL SUPPORT PERSONNEL

Valiente Novillo, Mónica

KEYWORDS

Autophagy, BPAN, ChAc, Rare diseases.



Transfected HeLa cell expressing GFP-LC3 protein

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RESEARCH LINES:

Overview

Our research focuses on studying the pathological mechanisms of rare diseases related to defects in autophagy and endo-lysosomal trafficking. Mutations in genes encoding the WIPI and VPS13 protein families give rise to various rare diseases such as BPAN (due to mutations in WDR45 encoding WIPI4) and CHAC (due to mutations in VPS13A). We utilize the model organisms Saccharomyces cerevisiae and Dictyostelium discoideum, as well as human cell lines, to recreate mutations, study the function of the involved proteins, and explore potential therapeutic strategies.

The autophagic machinery and its regulation

Autophagy is an evolutionarily conserved process of cellular degradation in eukaryotes. In response to stressors like starvation or cellular stress, portions of the cytoplasm are sequestered within double-membrane vesicles known as autophagosomes. These structures subsequently fuse with lysosomes, where their contents are degraded. Beyond its role in cellular homeostasis, autophagy is pivotal in clearing protein aggregates, damaged organelles, and invading pathogens. Given its broad implications in various pathologies and the aging process, we are actively exploring the molecular mechanisms underlying autophagosome biogenesis, including the identification of novel proteins through genetic and protein-protein interaction studies.

Characterization of WIPI4 and its role in BPAN disease

Autophagy plays a crucial role in maintaining cellular homeostasis, and its dysregulation is directly linked to numerous human diseases. Some of these diseases are very prevalent, such as neurodegenerative diseases and cancer, while others are rare diseases, including BPAN (beta-propeller-associated neurodegeneration). BPAN arises from mutations in the WDR45 gene, which encodes the WIPI4 protein. We have been investigating the molecular function of WIPI4 and its homologous proteins in the model organisms Saccharomyces cerevisiae and Dictyostelium discoideum. A key objective was to understand how pathogenic mutations impact the molecular function of WIPI4 in terms of its localization and interactions with other proteins.

Study of proteins with a chorein motif: the role of VPS13A

This line of research focuses on the study of proteins with a chorein domain: ATG2 and the VPS13 family. These proteins share a similar tubular structure with a hydrophobic cavity, responsible for transporting lipids between membranes of different organelles at mem-

brane contact sites. Our main goal is to understand how these proteins are recruited to their target membranes and their role in autophagy and endo-lysosomal trafficking. The human genome encodes four VPS13 proteins (A-D), and it was shown that the VAB domain is responsible for the association of Vps13 with various adaptors in yeast. However, the adaptors for the other VPS13 proteins in mammals are still poorly characterized.

PUBLICATIONS:

Bueno-Arribas, M.; Vincent, O. Identification of a novel mechanism for regulation of the early autophagy machinery assembly by PKA. *Autophagy Rep*. **2025**, *4*, 2503226. DOI: 10.1080/27694127.2025.2503226.

Bueno-Arribas, M.; Cruz-Cuevas, C.; Monforte-Martinez, B.; Navas, MA.; Escalante, R.; Vincent, O. The PKA signaling pathway regulates the association of the autophagy initiation complex with the lipidation machinery. *J. Mol. Biol.* **2025**, *437*, 168954. DOI: 10.1016/j.jmb.2025.168954

DOCTORAL THESES AND OTHER WORKS:

Lisseth Carolina Concha Tello
Master´s tesis. *Study of genetic variants of VMP1 and their influence on the autophagy mechanism*. Universidad Complutense de Madrid. 2024. Supervisor/s: Ricardo Escalante. Grade: Sobresaliente

Miguel Antón Esteban
Master´s thesis. *Identification and characterization of interactors of the VPS13/ATG2 protein family.* Universidad Complutense de Madrid. 2025. Supervisor/s: Olivier Vincent. Grade: Sobresaliente FUNDING:

FUNDING:

Molecular and cellular bases of autophagy-related rare diseases. PID2021-127355OB-100. MICINN. 2022-2025. PI: Ricardo Escalante Hernández and Olivier Vincent

Molecular mechanisms and therapeutic strategies in rare diseases related to alterations in autophagy and endo-lysosomal trafficking. PID2024-155990OB-I00. MICIU. 2025-2028. PI: Ricardo Escalante Hernández and Olivier Vincent

AWARDS:

FEBS OPEN BIO award to best poster in SEFAGIA 2025 meeting. Miraflores. 29-31 October 2025.

OUTREACH ACTIVITIES:

Semana de la Ciencia. Title: La ameba Dicty nos ayuda a estudiar las enfermedades raras. 20-11-2025.

Itinerario Cicerón acerca los desafíos científicos en enfermedades raras a la sociedad. https://rer-biomed.csic.es/itinerario-ciceron-acerca-los-desafios-cientificos-en-enfermedades-raras-a-la-sociedad/

Cilia and Ciliopathies

TENURED SCIENTIST

García Gonzalo, Francesc
(Profesor Titular, UAM). Group Coordinator

CONTRACT RESEARCHER

Martín Morales, Raquel (postdoc)

MASTER THESIS STUDENT

Martínez Ceña, Miguel Ángel (UAM)
Portilla Merino, Tatiana del Cisne (UCM)

UNDERGRADUATE STUDENT

Garrido García, Ariadna (TFG, UAM)
Jiménez Eusebio, Blanca (TFG, UAH)
Paradas Cuesta, Claudia (TFG, UEM)
García Murillo, Daniel (UFV)
Harto Garrido, Javier (EPSUM)
Salas Cedeño, Melany Alejandra
(IES Rosa Chacel)

KEYWORDS

Cilia, Ciliopathies, Ellis van Creveld syndrome, Hedgehog signaling, Joubert syndrome, Phosphoinositides

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RESEARCH LINES:

Overview
Primary cilia are microtubule-based plasma membrane protrusions that function as cell type-specific cellular “antennae”. These antennae are essential for multiple aspects of human development and adult physiology. Accordingly, cilia malfunction causes ciliopathies, a diverse group of diseases whose most common manifestations include retinal degeneration, kidney cysts, obesity, and congenital malformations of brain, heart and skeleton. In our lab, we study the molecular mechanisms of primary cilia function during health and disease.

Primera línea de investigación
Researchers involved: Martin-Morales, R; Garrido-García, A; Martínez Ceña, MA; Portilla Merino, T; Garcia-Gonzalo, FR
Ciliary targeting and functions of INPP5E, a phosphoinositide phosphatase implicated in Joubert syndrome.

Segunda línea de investigación
Researchers involved: Martín Morales, R; Jiménez Eusebio, B; Salas Cedeño, MA; Harto Garrido, J; García Gonzalo, FR
Identification of ciliary localization signals (CLSs) in ciliary G protein-coupled receptors (GPCRs), and characterization of their ciliary targeting mechanisms.

Tercera línea de investigación
Researchers involved: MMartín Morales, R; Paradas Cuesta, C; García Murillo, D; Garcia-Gonzalo, FR Role in ciliary Hedgehog signaling of EVC-EVC2, a protein complex involved in Ellis van Creveld syndrome.

DOCTORAL THESES AND OTHER WORKS:

Miguel Ángel Martínez Ceña
Master´s thesis. *Unsuccessful restoration of wildtype phenotype in hTERT-RPE-PS1-INPP5E KO cells via INPP5E Knock-In using PITCh CRISPR-Cas9*. Universidad Autónoma de Madrid. 2025. Supervisors: Francesc García Gonzalo y Raquel Martín Morales. Grade: Notable

Tatiana del Cisne Portilla Merino
Master´s thesis. *Papel de F-actina en el fenotipo ciliar de células mutantes para INPP5E, una fosfoinosítido fosfatasa implicada en ciliopatías*. Universidad Complutense de Madrid. 2025. Supervisors: Francesc García Gonzalo y Raquel Martín Morales. Grade: Notable

Ariadna Garrido García
Final degree's project. *Action mechanisms of ciliary phosphoinositide 5-phosphatase INPP5E in Joubert syndrome*. Universidad Autónoma de Madrid. 2025. Supervisors: Francesc García Gonzalo y Raquel Martín Morales. Grade: Sobresaliente

Blanca Jiménez Eusebio
Final degree's project. *La proteína GPR75 y los residuos aminoacídicos clave que determinan su localización en la membrana ciliar*. Universidad de Alcalá de Henares. 2025. Supervisors: Francesc García Gonzalo y Raquel Martín Morales. Grade: Matrícula de Honor

Claudia Paradas Cuesta
Final degree's project. *Diseño y expresión de un biosensor de la actividad de PKA ciliar en modelos celulares del síndrome de Ellis van Creveld.* Universidad Europea de Madrid. 2025. Supervisors: Francesc García Gonzalo y Raquel Martín Morales. Grade: Sobresaliente

FUNDING:

Sintonizando la Antena Celular: Mecanismos Moleculares de Control de la Composición de Cilios Primarios. PID2023-149472NB-I00. Ministerio de Ciencia e Innovación (MICINN). 2024-2028. PI: Francesc García Gonzalo.

OUTREACH ACTIVITIES

II Simposio Español de Biología Ciliar (online). Instituto de Investigaciones Biomédicas Sols-Morreale UAM-CSIC. Organización, introducción y moderación del simposio a cargo de Francesc García Gonzalo, quien también dio una de las seis charlas (Title: *Joubert syndrome protein INPP5E regulates ciliary microtubule modifications*). Comité organizador: Francesc García Gonzalo, Gemma Marfany, Diana Valverde, Rosa Barrio y José María Millán. 20/02/2025.

Telomeric Diseases and Experimental Therapies

TENURED SCIENTIST

Sastre Garzón, Leandro
(Investigador Científico hasta 20-01-2025; Investigador vinculado Ad Honorem desde esta fecha). Group Coordinator

CONTRACT RESEARCHER

Guerrero López, Rosa
(Investigador contratado)

Fernández Varas, Beatriz
(Investigador contratado)

García Castro, Laura
(Investigador contratado desde 01/03/25)

López García, Iván
(Investigador contratado desde 01/03/25)

PRE-DOCTORAL SCIENTIST

Acero Riaguas, Lucia María

Guillén Morales, Paula

UNDERGRADUATE STUDENT

Calvo Roldán, Sara
(Estudiante de posgrado 13/01/25 a 05/05/25)

Calero Gómez, Martín
(Estudiante de posgrado desde 20/10/25)

Guerrero Martín, Irene
(Estudiante de posgrado desde 01/10/25)

TECHNICAL SUPPORT PERSONEL

Manguán García, Cristina

KEYWORDS

Telomeres, Telomere Biology Disorders, Telomeropathies, Cutaneous squamous cell carcinoma, DUSP1.

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RESEARCH LINES:

Telomere Biology Disorders

Researchers involved: B. Fernández Varas, P. Gillén Morales, C. Manguán-García, I. López García, Laura García Castro, R. Guerrero López, R. Perona, L. Sastre

Our group has been working for several years on rare diseases characterized by the excessive shortening of chromosome's telomeres. These diseases have been name as Telomere Biology Disorders (TBDs), telomere-short syndromes or telomeropathies. Among them are Dyskeratosis congenita, aplastic anemia and idiopathic pulmonary fibrosis. Telomeres are nucleo-protein structures placed at both ends of the chromosomes that protect them from degradation and also from telomere-telomere fusions. Telomere DNA is composed by repetitions of the TTAGGG sequence and is bound to proteins of the shelterin complex for protection. Maintenance of telomeres depends on the activity of the telomerase complex composed by a protein with reverse transcriptase activity (TERT), a template RNA (TR, encoded by the Terc gene) and structural proteins like dyskerin (DKC1). Mutations in genes coding for proteins of the shelterin or telomerase complexes or auxiliary proteins are the genetic cause of TBDs. Our group works in several aspects of these life-threatening diseases that do not have any curative treatment at the present time. Our contribution to the prognosis of these diseases is the determination of telomere length in patient's samples sent by many hospitals. This analysis is accomplished at the Telomeropathies service of the IIBM. In addition, we contribute to the search of causative mutations by whole exome sequencing of selected patients. Pathological mechanisms are also studied using patient-derived cells and mice models of the diseases. In particular, we have developed a model based on a mice strain with telomeres of the same size as the human ones (CAST/EiJ) that carries a pathogenic *Terc* mutation. These mice present phenotypes at the lung and the erythropoietic systems that resemble those of TBD patients. Finally, we are working in the development of a possible therapy based on dyskerin-derived peptides. We have presented several patents to protect these results and in 2023 we have presented a new one that protect the results recently obtained for one of these peptides.

Cutaneous Squamous Cell Carcinoma

Researchers involved: B. Fernández Varas, C. Manguán-García, L. García Castro, L. Acero Riaguas, R. Guerrero López, Perona, R; L. Sastre Perona, A; Sastre, L.

Cutaneous squamous cell carcinoma (cSCC) is one of the most frequent tumors. Fortunately, most of them have a very good prognosis but a small percentage develop resistance to

the therapy and represent a significant challenge for the patients and the health system. Our research is focussed on the possible role played the dual-specificity protein phosphates DUSP1 (MKP1) in these tumors. Dusp1 is expressed at low level in cSCC tumors and expression levels correlate with advanced tumors and worst prognostic. Dusp1 mutant mice developed a larger number of cSCC than wild type animals upon DMBA/TPA chemical mutagenesis treatment. We are presently characterizing these tumors to determine the reasons behind their more aggressive behaviour. We are also generating conditional mutant mice that lack DUSP1 expression specifically at keratinocytes. In addition, cSCC cell lines where DUSP1 has been mutated using the Cas9/CRISPR technique have been generated. We hope that the analysis of these model systems using cell biology, genomic and transcriptomic techniques would give some insight on the role of DUSP1 in cSCC, the possible use as biomarker and/or therapy target molecule.

PUBLICATIONS:

Flores, C., Alonso-González, A., Veliz-Flores, I., Tosco-Herrera, E., González-Barbuzano, S., Mendoza-Alvarez, A., Galván-Fernández, H., Sastre, L., Fernández-Vara, B., Corrales, A., Rubio Rodríguez, L., Jáspez, D., Lorenzo-Salazar, J.M., Molina-Molina, M., Rodríguez-De-Castro, F. and González-Montelongo, R. A tiered strategy to identify relevant genetic variants in familial pulmonary fibrosis: a proof of concept for the clinical practice. *Eur. J. Human Genet*. **2025**. 33(11),1509-1519. (DOI:10.1038/s41431-024-01722-y)

Guerrero-López, R., Manguán-García, C., Carrascoso-Rubio, C., Lozano, M.L., Toldos-Torres, M., García-Castro, L., Sánchez-Dominguez, R., Alberquilla, O., Sánchez-Pérez, I., Molina-Molina, M., Bueren, J.A., Guenechea, G., Perona, R., Sastre, L. Premature ageing of lung alveoli and bone marrow cells from Terc deficient mice with different telomere lengths. *Sci. Reports.* **2025**. 15(1), 6102. (DOI:org/10.1038/s41598-025-90246-2)

DOCTORAL THESES AND OTHER WORKS:

Sara Calvo Roldán
Final degree's project. *Función de DUSP1 en el cáncer escamoso de piel.* Universidad Autónoma de Madrid. Facultad de Ciencias. 2025. Supervisor/s: Beatriz Fernández Varas, Leandro Sastre Garzón. Grade: 8,1.

FUNDING:

Biomarcadores moleculares en la encefalítis de Rasmussen: integración de Proteómica, Metiloma y Modelos mecanísticos para la caracterización patogénica. I convocatoria de ayudas a la invesigación, 2025, Asociación "Guerreros Purpura" PI: Rosa Guerrero López y Verónica Cantarín Extremera.

Molecular Mechanisms of Mitochondrial Pathophysiology

TENURED SCIENTIST

Fernández Moreno, Miguel Ángel
(Profesor Titular, UAM)

Garesse Alarcón, Rafael
(Catedrático Emérito, UAM)

TENURED-TRACK SCIENTIST

Clemente Pérez, Paula
(Investigadora Ramón y Cajal, UAM)

PRE-DOCTORAL SCIENTIST

Antolínez Fernández, Álvaro
Esteban Ramos, Paula

MASTER THESIS STUDENT

Sánchez Calera, Daniel
Castellví Martínez, Elena

KEYWORDS

Mitochondria, OXPHOS, mtDNA, Mitochondrial Diseases, Animal Models.

RESEARCH LINES:

Overview

The main function of mitochondria is the production of vast majority of the cellular energy in the form of ATP. However, they are also involved in lipid metabolism, calcium buffering, apoptosis, the assembly of iron-sulphur clusters or the biosynthesis of nucleotides, cholesterol and amino acids. As central regulators in cell signaling and physiology, mitochondria are essential at the cellular, tissue and organismal level.

Mitochondria contain their own genome (mtDNA), a circular DNA molecule that encodes 13 structural subunits of the OXPHOS system, as well as 2 rRNAs and 22 tRNAs necessary for their translation. However, most mitochondrial proteins (approximately 1100) are encoded by nuclear DNA and imported into the organelle. Mitochondria have reached such a high degree of specialization that their proteome, cristae structure and even their own distribution within the cell, can vary considerably from one cell type to another within the same individual. Due to the dual origin of the mitochondrial proteome, mitochondrial biogenesis requires a precise coordination of the expression of both genomes. An important aspect of this process is mtDNA maintenance and decoding, which involves mtDNA replication to reach the precise copy number of molecules per cell, the transcription of both mtDNA strands into two polycistronic RNAs and their processing, maturation and translation by the mitochondrial ribosome.

Given the central role of mitochondria in cell physiology, mutations in nuclear or mitochondrial genes affecting OXPHOS biogenesis cause the so-called mitochondrial diseases (MDs). Although individually considered MDs are rare, collectively they represent the largest group of inborn errors of metabolism. MDs are genetically and clinically heterogeneous and can present phenotypes varying from a mild single symptom, such as deafness or exercise intolerance, to devastating syndromes incompatible with life.

In our group, we are interested in studying the mitochondrial biogenesis in both physiological and pathological conditions. Specifically, we are carrying out several lines of investigation:

Line 1. Identifying non described genes genes involved in OXPHOS function through genomic data mining.

P.I. Miguel A. Fernández Moreno. Colaborators: Modesto Redrejo Rodríguez (IIBM); Miguel Ángel Martín Casanueva (i+12).

The *Drosophila* genome has proven to be a surprising and highly valuable resource for identifying previously undescribed human genes involved in OXPHOS function. Once the association of a newly identified gene with OXPHOS function is established, in collaboration with Dr. Miguel Ángel Martín Casanueva at the Instituto de Investigación Hospital 12 de Octubre (i+12), these genes are included in the genetic screening of patients suffering an undiagnosed mitochondrial OXPHOS disease, with the objective of finding the causative genes.

Line 2. To characterize the molecular mechanism of action of a group of proteins involved in the synthesis of singular mitochondrial tRNAs.

P.I. Miguel A. Fernández Moreno. Col.: Alvaro Antolínez (IIBM), Elena Castellví (IIBM).

Translation of mitochondrial mRNAs is full of surprises, you can find overlapping coding sequences, mRNAs without untranslated regions, polyadenylated and non-polyadenylated mRNAs, a non-universal genetic code, lack of some aminoacyl tRNA synthetases forcing to develop alternative ways for tRNA synthesis, etc. We are characterizing the singular

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pathway for the synthesis of the mitochondrial Gln-tRNAGln, whose functional defects provoke devastating phenotypes leading to death in the first weeks of life.

Line 3. To further understand the relationship of mtDNA and tumorigenesis.

P.I. Miguel A. Fernández Moreno. Col.: Alvaro Antolínez, Bruno Sainz Jr. (IIBM), Rafael Prados (Dpto Microbiología, epidemiología y salud Pública UAM).

In the context of cancer, we intend to integrate three major areas of action in the understanding of cancer and its translation to clinic and society: i) Extracellular vesicles (EVs; in collaboration with Dr. Rafael Prados, Dpt. Microbiology-UAM), ii) Cancer Stem Cells (which strongly rely on mitochondrial energy metabolism; in collaboration with Dr. Bruno Sainz, IIBM Sols-Morreale UAM-CSIC) and iii) mtDNA as a conditioning agent of tumorigenicity.

Line 4. To integrate some of our basic and biomedical findings to develop zebrafish as a promising animal model for the study of mitochondrial diseases.

P.I.Miguel A. Fernández Moreno. Col.: Alvaro Antolínez (IIBM), Alvaro Arana (U. Santiago de Compostela), Laura Sánchez Piñón (U. Santiago de Compostela)

The ability to replicate human diseases in animal models provides valuable insights into disease mechanisms and helps in the development of putative treatments. We aim to establish zebrafish (Danio rerio) as a model organism to study mitochondrial disorders. Specifically, in collaboration with Prof. Dr. Laura Sánchez Piñón of the ZebraBioRes Research Group at the Universidade de Santiago de Compostela, we have generated a *Danio rerio* knock-out model of the mitochondrial translation factor c6orf203, which is being characterized.

Line 5. *Drosophila melanogaster* models of mitochondrial gene expression defects (Hasta Septiembre 2025)

P.I. Paula Clemente Pérez.

The fruit fly, *Drosophila melanogaster*, is a powerful model system with short generation times, high fecundity, comparably low maintenance costs and well-established biological and genetic tools. Despite the phylogenetic distance, *Drosophila* presents functional orthologs for a great number of human genes. Notably, it shares the same mtDNA gene content and many key metabolic processes, including those necessary for mitochondrial gene expression and the OXPHOS biogenesis, are conserved between human and fly. This makes the fruit fly a highly suitable model system to study mitochondrial pathophysiology. We are currently characterizing the mechanisms and factors involved in the processing and maturation of mitochondrial RNAs using human cell lines and *Drosophila* models. Additionally, we are developing and characterizing *Drosophila* models for the study of mitochondrial pathologies.

DOCTORAL THESES AND OTHER WORKS:

Elena Castellví Martínez

Master's thesis: *Reversión fenotípica de la línea HEK293T knock-out para la subunidad GATC de la enzima mitocondrial GatCAB.*. Máster Universitario en Investigación, Desarrollo e Innovación de Medicamentos. Facultad de Farmacia y Nutrición. Universidad de Navarra / Universidad Autónoma de Madrid / IIBM. Supervisor: Miguel A. Fernández Moreno. Grade: 9,2. Sobresaliente.

FUNDING:

"OXPHOS activity dictates the tumorigenic and metastatic capacity of cancer stem cells through extracellular vesicles. IDEAS222917FERN". Fundación Científica de la Asociación Española Contra el Cáncer. 01/12/2022-31/072025. **Miguel Ángel Fernández Moreno**

"Desafíos OXPHOS: identificación de genes candidatos, diagnóstico, caracterización molecular y desarrollo de modelos de enfermedades mitocondriales. PID2023-148833NB-I00". Ministerio de Ciencia, Innovación y Universidades. 01/09/2024-31/12/2027. **Miguel Ángel Fernández Moreno**

Mitochondrial Gene Expression and Disease

TENURED-TRACK SCIENTIST

Clemente Pérez, Paula
(Investigadora Ramón y Cajal, UAM).

MASTER THESIS STUDENT

Sánchez Calera, Daniel

UNDERGADUATE STUDENT

Hernández Collado, Zoe

Antolín Romero, Alba

KEYWORDS

Mitochondria, OXPHOS, mtDNA, RNA metabolism, mitochondrial diseases, animal models

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RESEARCH LINES:

Overview

Mitochondria are the powerhouse of the cell, generating most of the cellular ATP through oxidative phosphorylation (OXPHOS). Despite their central role in energy metabolism, the function of many mitochondrial proteins remains unknown, and the genetic mechanisms that regulate OXPHOS biogenesis, including mitochondrial DNA replication, transcription, RNA processing, maturation, stability, and translation, are still under intense investigation.

As a remnant of their prokaryotic origin, mitochondria contain their own genome. Human mtDNA is a small, circular genome that contains only 37 genes encoding 13 OXPHOS subunits, as well as 2 rRNAs and 22 tRNAs essential for mitochondrial translation. Expression of mtDNA begins with transcription, which generates two long primary polycistronic transcripts that span almost the full length of both mtDNA strands. These precursor transcripts need to be subsequently processed to release the individual mRNA, tRNA and rRNA molecules. After excision, the individual transcripts are further modified by polyadenylation of most mRNAs and methylation and pseudouridylation of mitochondrial tRNAs and rRNAs. The rRNAs, then, assemble with >80 mitorribosomal proteins in order to form the mitochondrial ribosome. The adequate processing and maturation of mitochondrial mRNAs, rRNAs and tRNAs is regulated by a repertoire of nuclear encoded RNA binding proteins, that are translated in the cytosol and imported into mitochondria.

A correct mitochondrial gene expression is essential for both energy metabolism and mitochondrial function. Remarkably, around 20% of the mitochondrial proteome is devoted to mtDNA expression and maintenance, and over 60 genes involved in mitochondrial RNA metabolism have been found mutated in patients suffering from mitochondrial disorders. These diseases are clinically and genetically heterogeneous disorders, characterized by an OXPHOS dysfunction, which can present at any age with a wide spectrum of different symptoms and clinical manifestations. Individually considered mitochondrial disorders are rare, however, collectively they represent the most usual cause of inborn errors of metabolism.

New factors involved in the regulation of mitochondrial gene expression

Researchers involved: Sánchez Calera, Daniel; Hernández Collado, Zoe; Clemente Pérez, Paula.

In recent years, the known repertoire of proteins that regulate the biogenesis of the OXPHOS complexes has expanded. However, despite their importance, hundreds of nuclear-encoded mitochondrial proteins, including

many of those necessary for OXPHOS biogenesis, remain poorly characterized or entirely uncharacterized. We are currently characterizing the mechanisms and factors involved in the processing and maturation of mitochondrial RNAs using CRISPR/Cas9 knock out human cell lines and Drosophila melanogaster models and an array of molecular biology techniques to study mitochondrial function and gene expression. Through overexpression and immunoprecipitation experiments, we are identifying their interactors and RNA targets.

Drosophila models of mitochondrial disorders

Researchers involved: Antolín Romero, Alba; Clemente Pérez, Paula.

The fruit fly, *Drosophila melanogaster*, is a powerful model system with short generation times, high fecundity, and well-established biological and genetic tools. Despite the phylogenetic distance, Drosophila presents functional orthologs for a great number of human genes. Notably, it shares the same mtDNA gene content and many key metabolic processes, including those necessary for mitochondrial gene expression and OXPHOS biogenesis, are conserved between human and fly. This makes the fruit fly a highly suitable model system to study mitochondrial pathophysiology. We have set up Drosophila as a model to analyze mitochondrial physiology and using the CRISPR/Cas system, we are generating *Drosophila* models representative of mitochondrial disorders. These fly lines will allow us to study the molecular basis of these diseases and the phenotypical consequences of a defective mitochondrial function.

DOCTORAL THESES AND OTHER WORKS:

Daniel Sánchez Calera
Master's thesis. *Investigación del papel de FAMXXX en la expresión génica mitocondrial y la bioenergética en modelos celulares y de Drosophila.* Universidad Complutense de Madrid, Universidad de Alcalá de Henares y Universidad Autónoma de Madrid. 2025. Supervisor: Paula Clemente Pérez. Grade: 9,6. Sobresaliente.

Zoe Hernández Collado
Final degree project. *Caracterización de factores que participan en el procesamiento de los RNA mensajeros mitocondriales*. Universidad Complutense de Madrid. 2025. Supervisor: Paula Clemente Pérez. Grade: 9,6. Sobresaliente.

FUNDING:

Expresión del genoma mitocondrial: nuevos factores e implicación en patologías mitocondriales. PID2024-160512NA-I00. Ministerio de Ciencia, Innovación y Universidades, Agencia Estatal de Investigación. 2025-2028. PI: Paula Clemente Pérez.

Contrato y dotación adicional programa Ramón y Cajal. RYC2022-037640-I. Ministerio de Ciencia, Innovación y Universidades, Agencia Estatal de Investigación. 2024-2028.

New Mechanisms and New Models of DNA Replication and Repair

TENURED SCIENTIST

Arredondo Lamas, Juan José
(Profesor Contratado Doctor).

Redrejo Rodríguez, Modesto
(Profesor Permanente Laboral).
Group Coordinator

TENURED TRACK SCIENTIST

Cobo Simón, Marta
(Profesor Ayudante Doctor).

PRE-DOCTORAL INVESTIGATOR

Mayoral Campos, Carmen
Mateo Cáceres, Víctor

PREDOCTORAL SCIENTIST

Lozano Escobar, Eduardo Diego
Mateo Cáceres, Víctor

MASTER THESIS STUDENT

Marcos Fernández, Jorge
Rueda Bolaño, Kilian

UNDERGRAD STUDENT

Fernández Amestoy, Jorge
Ferrer Vázquez, Laura

TECHNICAL SUPPORT PERSONNEL

Mayoral Campos, Carmen

KEYWORDS

DNA polymerase, DNA amplification, mobile genetic elements, bacterial genomics

RESEARCH LINES:

Overview

Our research centers on uncovering the molecular mechanisms that preserve genomes stability. We aim to deepen the biochemical understanding of the proteins and pathways we investigate, while also exploring their diversity, evolution, and potential biotechnological applications. To do so, we rely on simple biological models—such as bacteriophages and bacterial mobile genetic elements—and employ a multidisciplinary strategy that integrates bioinformatics, biochemistry, molecular biology, and microbiology. We are also committed to implementing high-throughput genomic and functional genetic approaches, often through collaborations with other research groups.

Primer-independent DNA polimerases and their application to novel whole genome amplification methods

Researchers involved: Arredondo Lamas, Juan J.; Ferrer Vázquez, Laura; Mateo Cáceres, Víctor; Mayoral Campos, Carmen; Redrejo-Rodríguez, Modesto; and Rueda Bolaños, Kilian.

Our main efforts are dedicated the biochemical characterization of enzymes involved in alternative mechanisms of DNA replication initiation or priming that operate independently of DNA primases, as well as the replicons that encode them. In recent years, we have significantly advanced the understanding of a novel subfamily of PolB enzymes, the piPolBs (“primer-independent PolBs”), which we first described in 2017. These enzymes likely represent the ancestral forms of family B replicative DNA polymerases, including human polymerases α, δ, and ε. Remarkably, piPolBs can initiate DNA synthesis d*e novo,* without a pre-existing primer. Our recent work has emphasized structure–function analyses to elucidate the molecular basis of their activities and to apply this knowledge to the development of innovative DNA amplification methods. In addition, we have examined the diversity of cargo genes within pipolins, suggesting a role as a versatile reservoir of bacterial defense systems.

Efficient and reliable whole-genome amplification (WGA) is crucial for modern genomics and its applications in biomedicine and environmental studies. We previously developed a primer-independent MDA protocol (piMDA), based on the combined use of piPolB and Φ29 DNA polymerase (Φ29DNAP), which outperformed existing methods for amplifying high-GC genomic and metagenomic sequences (Ordóñez et al., 2023). In collaboration with Andrew Ellington’s laboratory (UT, USA), we have now applied MutCompute, a machine-learning strategy to engineer new piPolB variants with improved amplification performance and increased thermostability. When paired with a commercially available thermostable Φ29DNAP variants, these enzymes enable isothermal WGA at 44–45 °C from very low DNA inputs, including picogram-level amounts of metagenomic or human DNA. Our short-term efforts are focused on integrating long- and short-read sequencing to comprehensively characterize the amplification products, assessing coverage distribution and sequence bias.

High-throughput bacterial functional genomics and transcriptomics

Researchers involved: Lozano Escobar, Eduardo and Redrejo-Rodríguez, Modesto.

We are also strongly interested in the protein-primed DNA replication strategies employed by linear-genome bacteriophages, particularly temperate tectiviruses that infect

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diverse B*acillus cereus sensu lato*, a group of bacteria with notable biomedical and biotechnological relevance. In collaboration with Dr. Annika Gillis (Catholic University of Louvain, Belgium), we are using RNA-seq experiments for analyzing the transcriptional reprogramming of *Bacillus thuringiensis* hosts during lysogeny and following induction of the lytic cycle by both coevolved and heterologous betatectiviruses. Complementary computational efforts, including coexpression clustering and network-based analyses, aim to identify the molecular signatures associated with tectivirus infection.

Furthermore, in collaboration with Dr. Mario Mencía (CBMSO and UAM, Madrid), we have examined *Thermus thermophilus* Tn-seq datasets to identify genes required for growth at high temperatures. Unexpectedly, transposon insertions were found in nearly all genes, complicating the distinction between essential and non-essential loci. Comparisons with core-genome predictions derived from pangenome analyses—from the genus level to the phylum level—revealed no correlation with Tn-seq results. We hypothesize that the organism's known polyploidy, together with potential regulatory mechanisms, may underlie its ability to tolerate insertions in genes typically essential in bacteria, reflecting its remarkable genomic plasticity.

Genomic and Gene-Flow Approaches to Bacterial Species Delimitation

Researchers involved: Cobo Simón, Marta and Marcos Fernández, Jorge..

Our research explores the application of high-throughput comparative genomics and gene-flow–based frameworks to refine bacterial taxonomy and understand the evolutionary forces shaping microbial diversity. We focus on the reclassification of the marine bacterial order *Pelagibacterales* (SAR11), one of the most abundant and ecologically relevant clades in the global ocean. Leveraging complete genomes and metagenome-assembled genomes, we integrate multiple complementary criteria—including Average Nucleotide Identity (ANI), Genome Taxonomy Database (GTDB) assignments, and gene-flow analyses based on the Biological Species Concept (BSC) —to delineate cohesive evolutionary units within SAR11.

Our work combines computational pipelines for large-scale genome retrieval, quality filtering, ANI-based clustering, and phylogenetically informed taxonomic assignment, together with recombination-based approaches such as *ConSpeciFix* and *PopCOGenT*. This integrative strategy allows us to evaluate whether homologous recombination acts as a cohesive force within SAR11 lineages and to identify potential biological species undergoing ecological diversification. These analyses contribute to establishing universal, biologically grounded criteria for species delimitation in prokaryotes and provide insights into the evolutionary dynamics of one of the most diverse and environmentally significant bacterial groups.

PUBLICATIONS:

Gómez-Campos, C.; Gost, M.; Silva de Sousa, B.F.; Álvarez, L.; Berenguer, J.; Redrejo-Rodríguez, M.*; and Mencía, M.* *bioRxiv*. **2025**, https://doi.org/10.1101/2025.04.03.647156

DOCTORAL THESES AND OTHER WORKS:

Kilian Rueda Bolaños
Master's thesis. P*ipolinas y bacteriofagos: explorando la relación entre la replicación del DNA y la defensa bacteriana.* Máster en Virología, Universidad Complutense de Madrid, 2025 Supervisor/s: Modesto Redrejo-Rodríguez and José A. Escudero García-Calderón (UCM).

Laura Vázquez Ferrer
Final degree's project. *Papel biológico de las ADN polimerasas independientes de primer en Escherichia coli en el contexto de daño genotóxico.* Grado en Bioquímica, Universidad Autónoma de Madrid. 2025. Supervisor/s: Juan J. Arredondo Lamas and Modesto Redrejo-Rodríguez

FUNDING:

Functional characterization of primer-independent DNA polymerases in the context of genotoxic stress in bacteria. PID2021-123403NB-I00. Agencia Española de Investigación (MCIN/AEI/10.13039/501100011033) and ERDF A way of making Europe. 2022-2025. PI. Modesto Redrejo Rodríguez.

Molecular characterization of orthogonal replicons and their application as new biotechnology tools. PID2024-156454NB-I00. Agencia Española de Investigación (MCIN/AEI/10.13039/501100011033) and ERDF A way of making Europe. 2025-2028. PI. Modesto Redrejo Rodríguez.

Genetics and Pathophysiological Mechanisms of Congenital Anomalies

TENURED SCIENTIST

Ruiz Pérez, Víctor Luis
(Investigador Científico). Group Coordinator

Flores Mauriz, Carmen Lisset
(Científica Titular)

PREDOCTORAL SCIENTIST

Iturrate Soleto, Asier

García Valentín-Fernández, David
(Funded by Centro de Investigación Biomédica en Red, Enfermedades Raras (CIBERER, ISCIII))

CONTRACT RESEARCHER

Pescador Sánchez, Nuria
(Contract funded by Centro de Investigación Biomédica en Red, Enfermedades Raras (CIBERER, ISCIII))

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VISITING SCIENTIST

Jiménez Estrada, Juan Andrés

KEYWORDS

Ellis-van Creveld syndrome, Hedgehog signaling, Orofaciodigital syndrome, Osteogenesis imperfecta, Primary cilia

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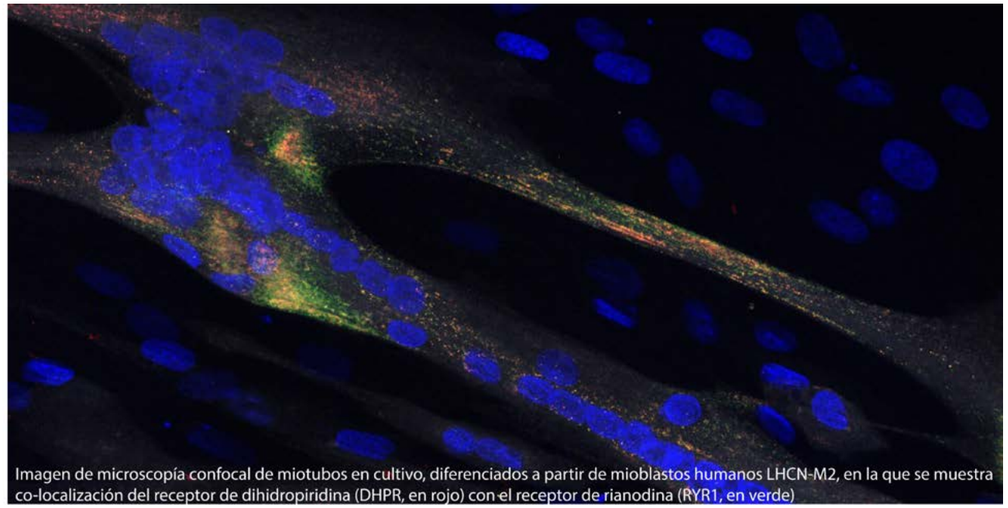

Imagen de microscopía confocal de miotubos en cultivo, diferenciados a partir de mioblastos humanos LHCN-M2, en la que se muestra co-localización del receptor de dihidropiridina (DHPR, en rojo) con el receptor de rianodina (RYR1, en verde)

RESEARCH LINES:

Overview

The scientific activity of our group is centered on the identification of new genes responsible for rare developmental disorders and the study of the underlying molecular pathology through the analysis of cellular and/or animal models.

Ellis van-Creveld syndrome and overlapping ciliopathies.

Ellis-van-Creveld syndrome is a rare autosomal recessive chondro-ectodermal dysplasia primarily caused by mutations in *EVC* or *EVC2*. These genes encode two interacting proteins located at the base of the primary cilium that act as positive mediators of Hedgehog (Hh) signaling, an evolutionarily conserved intercellular communication pathway that is critical for the development of the majority of vertebrate organs. Our laboratory has an interest in improving knowledge on the biology of the primary cilium and on hedgehog signaling through the identification of new genes and genetic variants responsible for ciliopathies.

Osteogenesis imperfecta and bone fragility conditions

Osteogenesis imperfecta (OI) is a bone-related disorder characterized by an increased risk of fractures. Most OI cases are caused by mutations in *COL1A1* or *COL1A2*, which are the genes coding for the two polypeptide chains

of procollagen type I. However, there is also a small fraction of OI cases that have mutations in other genes. Our goal in this line of research is to identify new causes and molecular mechanisms that lead to OI or to other forms of bone fragility

PUBLICATIONS:

Mora-Gómez, M.; Feito, M.; Gallego-Zazo, N.; Maseda-Pedrero, R.; Sobral-Costas, T.G.; Miranda-Alcaraz, L.; Vásquez-Amell, V.; Rodríguez-Canó, M.; Parra, A.; Cazalla, M.; Arias, P.; Silván, C.; Jiménez-Estrada, J.A.; Ruiz-Pérez V.L.; Nevado, J.; Lapunzina, P.; Tenorio-Castano, J. Case Report: Autosomal recessive palmoplantar keratoderma with additional bilateral hearing loss due to a pathogenic frameshift deletion in FAM83G. *Front Med (Lausanne).* **2025**, *12*:1687811. DOI: 10.3389/fmed.2025.1687811.

Iturrate, A.; Tran-Mau Them, F.; Verloes, A.; Pouzet, A.; de Silva, D.; Perrin-Sabourin, L.; Wentzensen, I.M.; Jones, K.; Upadia, J.; Abdalla, E.; Thauvin-Robinet, C.; Ruiz-Perez, V.L.; Bruel, A.L. Expanding the phenotype associated with biallelic SCNM1 variants. *Hum Genomics.* **2025**,*19(1)*:155. DOI: 10.1186/s40246-025-00868-w.

Elhady, G.; Amin, A.K.; Iturrate, A.; El-Dessouky, S.; Nevado, J.; Campos-Xavier, B.; Matsa, L.S.; Giunta, C.; Lapunzina, P.; Ruiz-Perez, V.L.; Abdalla, E. Genetic and clinical spectrum of osteogenesis imperfecta in an Egyptian cohort with a high rate of lethal phenotypes. *Clin Genet*. **2025**, *online ahead of print*. DOI: 10.1111/cge.70091.

Iturrate, A.; Assia Batzir, N.; Jaron, R.; Garcia-Valentin, D.; Nevado, J.; Tenorio-Castano, J.; Lapunzina, P.; Lee, K.; Greenberg, R.; Sassi, D.; Aharoni, S.; Kuzminsky, A.; Basel-Salmon, L.; Orenstein, N.; Fellig, Y.; Ben-Shachar, S.; Marek-Yagel, D.; Ruiz-Perez, V.L. N-terminal truncating variants in CACNB1 cause a new congenital muscular disorder. Eur J *Hum Genet*. **2025**, *online ahead of print*. DOI: 10.1038/s41431-025-01944-4.

Parra, A.; Jimenez-Estrada, J.A.; Vásquez-Amell, V.; Cazalla, M.; Rodríguez-Canó, M.; Gallego-Zazo, N.; Miranda, L.; Mora-Gómez, M.; Vallespín E.; Mena, R.; Fernández L.; Silván, C., Arias , P.; Dominguez-Jiménez, M.; Guillén-Navarro, E.; Nevado, J.; Tenorio-Castano, J.; Ruiz-Pérez, V.L.; Lapunzina P. Identification of a de novo heterozygous frameshift variant in FMR1 in a female with fragile X syndrome. *Clin Genet*. **2025**, *108(2)*:224-226. DOI: 10.1111/cge.

Parra, A.; Cazalla, M.; Jimenez-Estrada, J.A.; Silván, C.; Miranda-Alcaraz, L.; Gallego-Zazo, N.; Mora-Gómez, M.; Rodríguez-Canó, M.; Arias, P.; Rodríguez-Antolín, C.; Nevado, J.; Ruiz Pérez, V.L.; Tenorio-Castano, J.; Lapunzina, P. A Novel Deep Intronic Variant in NSD1 Causing Sotos Syndrome. *Am J Med Genet.* **2025**, *197(8):*e64074. DOI: 10.1002/ajmg.a.64074.

DOCTORAL THESES AND OTHER WORKS:

Alejandro Fuentes Rodríguez
Final degree's Project: *Validación de la patogenicidad de una variante intrónica detectada en EVC en un paciente con el síndrome de Ellis-van-Creveld*. Universidad Europea Madrid. 2025. Supervisor/s: Víctor Luis Ruiz Pérez. Grade: Matrícula de Honor

FUNDING:

“Deciphering pathological mechanisms behind ciliopathies and uncovering new genes responsible for developmental disorders. PID2022-139565OB-I00”. MICINN. 2023-2026

OUTREACH ACTIVITIES

Comité Organizador de la II Jornada sobre Enfermedades Raras en el IIBM. 24 de febrero de 2025.

Neurobiology of Hearing and Myelinopathies

CONTRACT RESEARCHERS

Murillo Cuesta, Silvia
(Investigador senior, CIBER-ISCIII).
Group Coordinator

Jiménez Lara, Ana María
(Investigador contratado Doctor CSIC)

TENURED SCIENTIST

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(Prof. Investigación)

Cosgaya Manrique, José Miguel
(Científico Titular)

ASSOCIATED INVESTIGATORS

Cediel Algovía, Rafael
(Prof. Contratado Doctor, UCM)

Contreras Rodríguez, Julio
(Prof. Contratado Doctor, UCM)

Zubeldia Ortuño, José Manuel
(Profesor Titular, UCM)

PREDOCTORAL SCIENTISTS

Franco Caspueñas, Sandra
García Montoya, Carmen
Méndez Grande, Inés
Ruiz García, Carmen
Tapias Martín, Marina

UNDERGRADUATE STUDENT

Rodríguez Etxebarría, Ariadna

TECHNICAL SUPPORT PERSONNEL

Blanco Herrero, Luz

KEYWORDS

Age-Related Hearing Loss, Cellular Senescence, MKP1-mediated Oxidative stress, NLRP3-mediated Neuroinflammation, Vestibular Schwannomas.

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RESEARCH LINES:

Overview

Our laboratory is interested in understanding the genetic and molecular bases of hearing loss.

Hearing loss, technically referred to as hypoacusis (from the Greek hypo- (ὑπο-), meaning under, decrease, deficiency, and akousis (ἀκούσις), the act of hearing or listening) and more commonly called "deafness," is defined as a decrease in auditory sensitivity in one or both ears, which manifests as an elevation of the hearing threshold. Moderate and severe forms have a direct impact on language development and speech perception, and therefore on oral communication, learning, social relationships, and overall quality of life.

According to reports from the World Health Organization, there are currently more than 1.6 billion people with hypoacusis, and around 480 million (6% of the world's population) have disabling hearing loss—a figure expected to rise to 10% by 2050, mainly due to population aging. This situation carries an extremely high cost for healthcare systems and the global economy, estimated at 1 trillion dollars annually (including direct costs such as devices or rehabilitation, and indirect costs such as loss of productivity). But it also has an intangible impact, which often leads many people with hearing impairment, especially older adults, into isolation, loneliness, and early cognitive decline. In fact, hearing loss is considered the most important preventable risk factor for the development of dementia: for every 10 dB of loss, our risk increases by 4–24%.

Hearing loss has a highly diverse etiology, including genetic factors (mutations in genes relevant to hearing) and a wide array of environmental agents (e.g., noise, toxins, infectious agents) and lifestyle factors (e.g., nutrition) that can damage the ear. Thus, the constant interaction between the genome and the exposome determines the deterioration of auditory function, as demonstrated by studies conducted in monozygotic twins.

Follow us on X and YouTube:
@HearingMadrid
YouTube: https://www.youtube.com/channel/UCYanykLgCm8aGOT9dbTlNWg

Genetic and Molecular Bases of Hereditary Hearing Loss

Researchers involved: Murillo Cuesta, Silvia; Cediel Algovía, Rafael; Contreras Rodríguez, Julio; Varela Nieto, Isabel; García Montoya,Carmen; Méndez Grande, Inés; Tapias Martín, Marina

Our objective is to study hearing loss associated with deficiency of insulin-like growth factor I (IGF-1), its high-affinity receptor, and its intracellular targets—particularly p38/MKP1 and NLRP3—as well as identify the transcriptional networks that regulate inner ear senescence during aging.

Environment–Genome Interaction in Hearing Loss

Researchers involved: Murillo Cuesta, Silvia; Cediel Algovía, Rafael; Contreras Rodríguez, Julio; Varela Nieto, Isabel; Zubeldia Ortuño, José Manuel; García Montoya, Carmen; Méndez Grande, Inés; Tapias Martín, Marina.

Our objective is to analyze animal models of hearing loss to identify genetic predisposition factors and their interaction with environmental factors such as noise exposure, ototoxic agents, and nutritional status, with particular attention to the role of oxidative stress and inflammation in auditory damage.

Study of Human Vestibular Schwannoma

Researchers involved:Murillo Cuesta, Silvia; Varela Nieto, Isabel; Franco Caspueñas, Sandra; Méndez Grande, Inés; Ruiz García, Carmen. Jiménez Lara, Ana María; Rodríguez Etxebarría, Ariadna; Cosgaya Manrique, José.

Our objective is to Investigate this rare tumor of the cochleovestibular nerve from a molecular and translational perspective in collaboration with the ENT service in La Paz University Hospital.

Development of Experimental and Translational Tools

Researchers involved:Murillo Cuesta, Silvia; Cediel Algovía, Rafael; Contreras Rodríguez, Julio; Blanco Herrera, Luz.

Develop and maintain advanced platforms including 2D and 3D primary cultures, animal models, ex vivo cultures, microsurgical techniques and procedures, biomarker panels, and new drug delivery and imaging approaches (MRI) in the inner ear, while ensuring the competitiveness of the IIBM ENNI facility as a unique service within the Community of Madrid.

PUBLICATIONS:

Murillo-Cuesta S, Seoane E, Cervantes B, Zubeldia JM, Varela-Nieto I. NLRP3 inflammasome and hearing loss: from mechanisms to therapies. *J Neuroinflammation*. **2025** *Oct 4;22(1)*:225. DOI: 10.1186/s12974-025-03561-w. PMID: 41046290 Free PMC article. Review.

Murillo-Cuesta S, Contreras J, Chioua M, García-Montoya C, Rodríguez-de la Rosa L, Méndez-Grande I, Piotrowska DG, Głowacka IE, Varela-Nieto I, Marco-Contelles J. Quinolylnitrone 23 Protects from Auditory Cell Oxidative Injury and Noise-Induced Hearing Loss. *ACS Pharmacol Transl Sci.* **2025** *Aug 26;8(9)*:3007-3018. DOI: 10.1021/acsptsci.5c00221. eCollection 2025 Sep 12. PMID: 40969893

Escalera-Balsera A, Robles-Bolivar P, Parra-Perez AM, Murillo-Cuesta S, Chua HC, Rodríguez-de la Rosa L, Contreras J, Domarecka E, Amor-Dorado JC, Soto-Varela A, Varela-Nieto I, Szczepek AJ, Gallego-Martinez A, Lopez-Escamez JA. A rare haplotype of the GJD3 gene segregating in familial Meniere's disease interferes with connexin assembly. *Genome Med*. **2025** *Jan 15;17(1):*4. DOI: 10.1186/s13073-024-01425-1. PMID: 39815343 Free PMC article.

Franco-Caspueñas S, García-Montoya C, Contreras J, Lassaletta L, Varela-Nieto I, Jiménez-Lara AM. Uncovering cellular senescence as a therapeutic target in NF2-related vestibular schwannoma. *Hear Res.* **2025** *Jan;455*:109165. DOI:10.1016/j.heares.2024.109165. Epub 2024 Dec 4. PMID: 39647233 Free article.

AWARDS:

Medalla de Honor de la SEBBM a Isabel Varela Nieto. Cáceres. Septiembre 2025.

Comité Organizador de la II Jornada sobre Enfermedades Raras en el IIBM. 24 de febrero de 2025.

OUTREACH ACTIVITIES:

Día Mundial de la Audición con el World Hearing Forum. Actividad en el MNCN-CSIC. Silvia Murillo Cuesta. Marzo 2025.

Semana de la Ciencia. Visita guiada al IIB. Silvia Murillo Cuesta. Noviembre 2025.

DOCTORAL THESES

Elena Torres Campos
Ph.D. tesis. Nuevas estrategias terapéuticas para la sinaptopatía coclear y la pérdida auditiva neurosensorial: MTFL457, un péptido neuroprotector derivado del receptor neurotrófico TrkB-FL. Universidad Autónoma de Madrid.
2025. Supervisor/s: Isabel Varela Nieto & Margarita Díaz-Guerra González. Grade: Sobresaliente Cum Laude.

FUNDING:

Sensorineural hearing loss and hearing protection: exploring genetic and molecular mechanisms to develop novel therapeutic strategies PROHEAR). MICIN/FEDER PID2023-147347OB-I00 (2025-2028) Nuevas estrategias terapéuticas para el tratamiento de enfermedades raras neurosensoriales (SensoRare). CIBERER ACCI2023-19-761 (2024-2026)

SOLS MORREALE

MaX
MARCO DE AUTOEVALUACIÓN DE LA EXCELENCIA

CSIC
Consejo Superior de Investigaciones Científicas

UAM Universidad Autónoma de Madrid