IBIMA

SCIENTIFIC REPORT 2024

CSIC
Consejo Superior de Investigaciones Científicas

UAM Universidad Autónoma de Madrid

INSTITUTO DE INVESTIGACIONES BIOMÉDICAS SOLS-MORREALE

Index

Welcome from the director

It is my privilege to introduce this report summarizing the activities of the IIBM during the year 2024. Having assumed the role of Director in February, I have worked to maintain the momentum of ongoing initiatives while fostering new opportunities to strengthen our research and institutional development. The foundation laid in previous years has allowed us to consolidate our in biomedical research, ensuring a dynamic and forward-looking approach to scientific challenges.

A significant highlight of 2024 has been our successful completion of the first phase of the CSIC's MaX Excellence Program. This recognition underscores the scientific rigor and impact of our Institute. Throughout the year, we have dedicated substantial efforts to preparing and submitting a comprehensive scientific program and an excellence plan for CSIC's evaluation, aiming to advance to the second phase of the MaX Program. These documents, developed with the invaluable contributions of many committed researchers, outline a strategic vision for our future. Notably, several of the proposed improvements and initiatives from the excellence plan have already begun to be implemented, demonstrating our proactive commitment to institutional growth and scientific advancement.

Beyond this key institutional milestone, 2024 has been a year of consolidation and progress. Our research programs have continued to expand, reinforcing our dedication to investigating the molecular mechanisms of disease through both fundamental and translational approaches. We remain focused on critical biomedical challenges, including cancer, rare diseases, metabolic disorders, immune responses, cardiovascular conditions, and neurodegenerative diseases.

This year has also been marked by remarkable success in securing competitive funding. We have achieved significant financial support from various sources, including prestigious European Union grants, as well as substantial funding for a spin-off company co-founded by one of our researchers. Additionally, our commitment to technology transfer has yielded tangible improvements, with several research groups at the Institute strengthening their collaborations with industry and advancing the application of their discoveries.

In parallel, our ability to attract talented researchers remains strong. The arrival of new scientists through excellence programs, along with permanent appointments by the CSIC and the UAM, has further enriched our scientific community. At the same time, we recognize and appreciate the contributions of those colleagues who have retired this year, acknowledging their long-standing dedication to the progress of our Institute.

Furthermore, we have made significant strides in networking activities. Our Institute has strengthened its participation in international and national research structures, including the European COST actions and the CSIC's Connection and PTI platforms. Notably, we have taken a leadership role in coordinating newly established networks, such as the CSIC Rare Diseases Network (RER-CSIC), initiated from the IIBM. These efforts highlight our commitment to fostering scientific collaboration and expanding our research impact beyond institutional boundaries.

As we look to the future, we remain focused on fostering scientific excellence, strengthening collaborations, and ensuring that the IIBM continues to be a leading institution in biomedical research. I extend my sincere gratitude to all members of our Institute, including administrative staff, core service personnel, and technical teams, whose dedication and efforts are instrumental to our collective success.

With confidence in our continued growth and innovation, I look forward to another year of scientific discovery and institutional development at the IIBM.

Pilar López Larrubia. Director, IIBM

Key **Metrics**

Publications

- **1** Books
- **65** Internationally co-authored publications
- **10** Medium impact
- **1** Low impact
- **103** High impact

Fundraising

- **7.316.122€** Funding
- **418.424€** R&D Contracts

Supervision activities

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

Outreach activities

- **36** Materials
- **93** Events

SCIENTIFIC REPORT 2024

department *of* **Cancer**

Molecular Bases of Chemo and Radioresistance in Tumors

PRINCIPAL INVESTIGATOR
Sanchez Prieto, Ricardo

SENIOR INVESTIGATOR
Belandia Gomez, Borja

KEYWORDS
Chemotherapy, Radiotherapy, Sarcoma, MAPK, Resistance

RESEARCH LINES:

Overview

Our laboratory is interested in understanding the molecular mechanisms underlying cellular transformation and their implications in the response to cancer therapy. To this end, we use a variety of approaches, including cell culture systems, animal models and human tumor samples. We employ advanced techniques in molecular cellular biology, gene perturbation systems (CRISPR/CAS, shRNA, ect...) and omics-based approaches (RNA sequencing, whole-exome sequencing). Our overarching goal is to contribute to the improvement of current diagnostic methods and therapeutic strategies.

Molecular basis of chemo/radioresistance:

Sanchez Prieto Ricardo, Belandia Gomez

Our research group has a large trajectory focused in the study of the molecular bases of chemo- and radioresistance, beginning over 30 years ago with investigations into the chemo- and radiosensitivity associated with the E1a gene. In the last years we have concentrated our efforts on understanding radioresistance and improving the efficacy of Radiotherapy. Radiotherapy is one of the mainstays of cancer treatment, benefiting over 50% of patients, and is in many cases the therapy of choice. However, radioresistance, whether intrinsic or acquired after exposure to ionising radiation, is a limiting step in this type of treatment, contributing to relapses and leading to therapeutic failure on many occasions. We are currently generating radioresistant cell models developed by repeated exposure to radiation, simulating clinical scenarios leading to radioresistance. These models will be analysed using whole-exome sequencing (WES) and transcriptomic studies (RNA-seq). In parallel, we will employ genome-wide CRISPR/Cas9 screens to identify genes essential for the survival of radioresistant cells These approaches aim to uncover novel biomarkers of radioresistance as well as new therapeutic vulnerabilities, with the ultimate goal of achieving more personalized and effective cancer therapy in the context of radioresistance.

In a second line of research, we are evaluating novel targeted therapies as radiosensitizer agents. To this end we evaluate the response to ionizing radiation in different experimental models and study the DNA-damage response as well as molecular targets implicated in the radiosensitizing potential of these antitumor agents. We use in silico, in vitro and in vivo studies combined with radiobiology and “omic” analyses to unveil new radiosensitizing agents that facilitate more personalized radiotherapy.

Role of cell signalling in sarcoma biology and therapy.

Sanchez Prieto Ricardo , Belandia Gomez Borja

Sarcomas are a group of heterogeneous tumors that develop from connective tissue, which provides a supportive matrix throughout the body. More than 150 subtypes of sarcomas have been described. The tissues of origin can be fibrous, muscular, fatty, cartilaginous, osseous, blood vessels, lymphatic, etc., and sarcomas are therefore not limited to a specific location. The classification of sarcomas divides them into soft tissue sarcomas (STS) and bone sarcomas (osteosarcomas). In addition, there is a third group, gastrointestinal stromal tumours (GIST), which are soft tissue sarcomas that are considered a separate group due to their unique diagnostic and therapeutic features. Sarcomas are rare in adults accounting for only 1% of all cancers; however, in children, they account for about 15% of cancer cases.

Our previous studies showed that ERK5 signalling pathway is a key player in sarcomagenesis triggered by the chemical carcinogen 3-methylcholanthrene (3MC) in murine models. Transcriptomic analysis (RNAseq) revealed over 500 differentially expressed genes in the absence of ERK5 that can potentially justify the oncogenic nature of this pathway, as well as its implications in diagnosis and therapy. These genes are related to key biological processes for tumor biology such as angiogenesis, motility, anchoring, genomic stability or transcriptional control, in which ERK5 has been implicated. In fact, we have been able to validate the role of some of them, such as KLF2, in tumorigenesis . Our main objective in this project is to establish human cellular models lacking ERK5 using interference techniques (CRISPRi, shRNA) and gene editing approaches (CRISPR/Cas9). These models will facilitate the identification of and ERK5-related gene signature based on transcriptomic profiling. In addition, this approach will also be carried out using specific chemical inhibitors of ERK5 in order to distinguish between genes regulated by its kinase activity and those regulated independently of this activity. This analysis aims to identify potential therapeutic targets for future pharmacological intervention. The identified gene signature will be validated in the context of tumor biology, diagnosis and therapeutic response including chemo-, radio- and immunotherapy. Subsequently, the differential expression of the different candidate genes will be evaluated in human sarcoma biopsies. Achieving these objectives will enable the identification of novel biomarkers and therapeutic targets, advancing the development of personalized diagnostic and therapeutic strategies for sarcomas—a group of tumors that remain poorly studied and understood.

PUBLICATIONS:

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.* **2024**, *Dec 3.* DOI: 10.1002/1878-0261.13773

Castro-Larefors, S; Marti-Laosa, MM; Lopez-Honrubia,; Rey-Lopez, I; Ruiz-Herrero, B; Murria-Perez, Y; Andres, I; Jimenez-Garcia, IE; Berenguer, R; Aguayo-Martos, M, Sánchez-Prieto, R; Rovirosa, A; Jimenez-Jimenez, ; Arenas M, Sabater S. Longer time to testosterone recovery impacts favorably on outcomes for prostate cancer following androgen deprivation and radiotherapy. *Strahlenther Onkol.* **2024**, *200(8)*:691-697. DOI: 10.1007/s00066-024-02208-8

DOCTORAL THESES AND OTHER WORKS:

Pablo Fernández Aroca
"Master´s thesis: *Estudio de las Bases Moleculares de la Radiosensibilidad Mediada por Dinaciclib*". Universidad de Castilla la Mancha. Medicina. 2024. Supervisor/s: Ricardo Sanchez Prieto, Maria Jose Ruiz Hidalgo. Grade: 9.4/10.

Ignacio Tobarra Gallego
"Final degree's project: *Pronostic value of : CDK7 Y CDK9 mrNA level in clear cell rnal cell carcinoma: in silico study*"
Universidad de Castilla la Mancha. Medicina. 2024. Supervisor/s: Ricardo Sanchez Prieto. Grade 9.4/10.

David Fernández Fernández
"Final degree's project: *Role of CDK12 as cancer biomarker ANÁLISIS IN SILICO*". Universidad de Castilla la Mancha. Medicina. 2024. Supervisor/s: Ricardo Sanchez Prieto. Grade 9.2/10.

Juan Jesús Martínez Gómez
"Final degree's project: *Molecular mechanisms of cytotoxicity of the axl inhibitor dubermatinib in colorectal cancer cell models*". Universidad de Castilla la Mancha. Biotecnología. 2024. Supervisor/s: Ricardo Sanchez Prieto, Maria Jose Ruiz Hidalgo. Grade: Honorable mention.

FUNDING:

"Estudio de nuevos genes dependientes de la ruta erk5 en patología sarcomatoide: implicaciones en biología tumoral y terapia. PID2021-122222OB-I00". Agencia Estatal de Investigación. 2022-2025.

"Campaña de micro mecenazgo "IRRADIANDO Esperanza". 2022-2026.

"Renovación papel de la señalización intracelular mediada por proteínas quinasas en los fenómenos de quimio y radio resistencia". Fundación Leticia Castillejo. 2021-2023.

"Papel de la señalización celular mediada por MAPK en infecciones por patógenos emergentes ". Universidad de Castilla la Mancha. 2023-2025.

"Bases moleculares de la radiorresistencia". Research Contracts, as PI, with the Association ACEPAIN. 2021-2024

"Nuevos determinantes de radiorresistencia" Research Contracts, as PI, with the Association Taller solidario Árbol de la vida las Pedroñeras. 2023 -2025.

"Nuevos determinantes de radiorresistencia" Research Contracts, as PI, with the Association Comarcal contra el cancer de Motilla del Palancar. 2023-2025.

Bioinformatics and Computational Biology of Cancer Evolution

PRINCIPAL INVESTIGATOR
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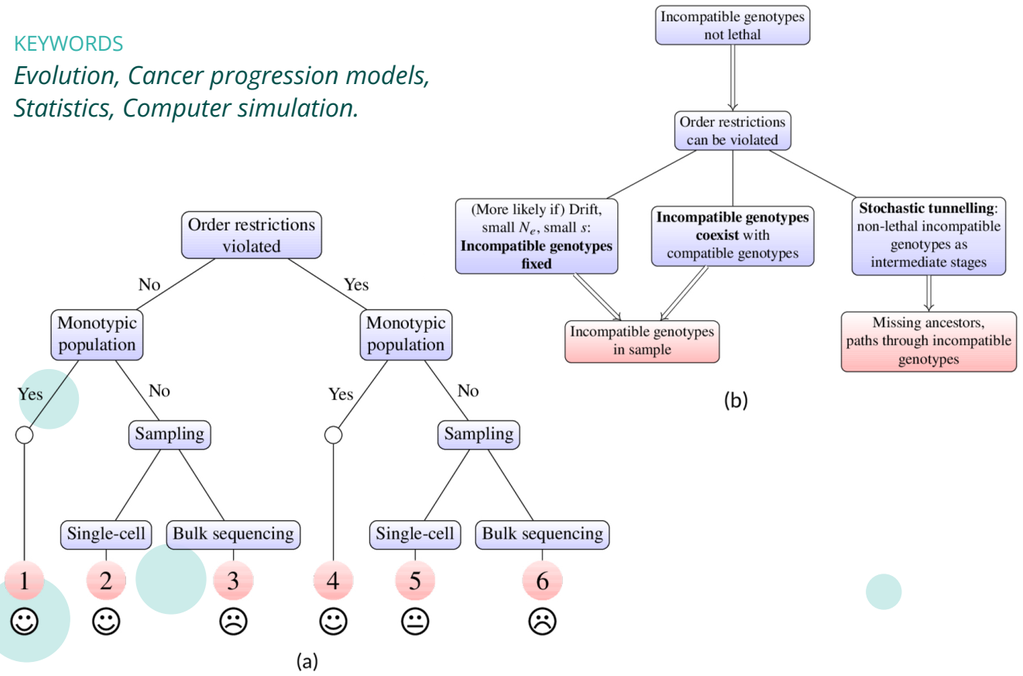
STAFF INVESTIGATOR
Idmbarek, Asmaa

KEYWORDS
Evolution, Cancer progression models, Statistics, Computer simulation.



(a) Deterministic models as within-cell restrictions: six scenarios with respect to order restriction violations, population heterogeneity, and sampling. Order restrictions violated means that genotypes that are incompatible with the models are viable (panel (b)). "Monotypic population": there is a single genotype (defined with respect to the mutations being modelled) present virtually all of the time, except for brief periods of fast clonal sweeps, or during the transient presence of non-fixing mutants. Mixture of tumour types plays no role in this figure, as it as a different problem. Emojis indicate how bad/good each scenario is for inference with cancer progression models.
(b) Order restrictions violated for deterministic models: cause and consequences. A simple scheme of the cause and two main consequences (and mechanisms) of violation of order restrictions. These consequences are relevant for the three scenarios on the right of (a). The presence of small fractions of incompatible genotypes should be handled by the error models. Missing ancestors and paths through incompatible genotypes lead to incorrect estimation of rates, but should not affect structure

RESEARCH LINES:

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 estimate 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. 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 the development of unified interfaces for the analysis of cross-sectional data with cancer progression models (essential both 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:

Johnston, I. G.; Diaz-Uriarte, R. A Hypercubic Mk Model Framework for Capturing Reversibility in Disease, Cancer, and Evolutionary Accumulation Modelling. *Bioinformatics* **2024**, *41*, btae737. https://doi.org/10.1093/bioinformatics/btae737.

Aga, O. N. L.; Brun, M.; Dauda, K. A.; Diaz-Uriarte, R.; Giannakis, K.; Johnston, I. G. HyperTraPS-CT: Inference and Prediction for Accumulation Pathways with Flexible Data and Model Structures. *PLoS Comp. Biol.* **2024**, *20 (9)*, e1012393. https://doi.org/10.1371/journal.pcbi.1012393.

DOCTORAL THESES AND OTHER WORKS:

Laura Yolanda Bermúdez Garrido
"Master´s thesis: *Democratizing personalized oncology treatments: tumor microenvironment sequencing and Artificial Intelligence for cancer diagnosis and real-time treatment monitoring*". Universidad Autónoma de Madrid. Medicina. 2024. Supervisor/s: Ramón Díaz Uriarte.

FUNDING:

"Terapia antitumoral adaptativa usando datos transversales y predicciones de modelos de progression tumoral. PID2019-111256RB-I00". AEI. 2019-2024.

Genomic Biomarkers and Precision Oncology

PRINCIPAL INVESTIGATOR

García Pérez, María J.

KEYWORDS

Ovarian cancer, Susceptibility gene, BRCA1, BRCA2, Cancer genomics, Prognostic biomarker, Targeted therapy, Mismatch repair deficiency, Tumor immunogenicity.

RESEARCH LINES:

Our group focuses on the identification and characterization of prognostic and predictive biomarkers and on the discovery of novel susceptibility genes, primarily—but not exclusively—in ovarian cancer. We take a highly translational approach, with the ultimate goal of improving patient survival and quality of life by applying our findings into clinical practice. We use a multidisciplinary approach that integrates "omics" technologies, molecular and cell biology, bioinformatics, and close collaborations with clinical and computational experts.

Epithelial ovarian cancer is the leading cause of gynecological cancer-related death in adult women in developed countries. Despite advances in treatment, the five-year survival rate has remained unchanged at around 45% over the past decades. Therefore, defining biomarkers that can improve prognosis and predict treatment response is a key priority in the fight against this disease.

Although several risk genes associated with ovarian cancer have been identified (such as BRCA1, BRCA2, RAD51C, RAD51D, and BRIP1), more than 50% of hereditary susceptibility remains unexplained. Determining the specific susceptibility gene within a family is critical, not only for prevention and early detection, but also for therapeutic decisions. For instance, patients with BRCA1 or BRCA2 mutations are particularly responsive to PARP inhibitors, highlighting the clinical relevance of these genetic insights.

With these challenges in mind, our main research lines include:

Definition of novel prognostic and predictive factors based on tumor genomics and immunogenicity

In our group, the genetic landscape of tumors plays a central role in the search for new biomarkers. Beyond individual sequence alterations, we focus on identifying genome-wide patterns with potential clinical impact, such as mutation signatures, microsatellite instability burden, copy number variation or chromosomal instability signatures. These markers help to refine patient stratification based on disease aggressiveness and guide personalized treatments by predicting therapy response. In addition, we are particularly interested in exploring the interaction between genomic biomarkers and tumor immunogenicity, aiming to understand how these factors influence tumor evolution and provide innovative perspectives for the development of more effective therapeutic strategies.

Identification of genes and variants associated with ovarian cancer susceptibility

This second line of research involves a variety of approaches, including the study of highly selected high-risk families and large-scale case-control studies in collaboration with international consortia. It also includes in-

depth characterization of candidate variants to determine their pathogenicity, using in silico tools and functional in vitro studies. As part of an ongoing project, we are using chromosomal instability signatures and Next Generation Sequencing (WES and WGS) to search for novel risk genes in an agnostic and unbiased manner in patients with a family history of ovarian cancer.

Determination of the clinical significance of mismatch repair (MMR) defects in ovarian cancer

While MMR defects are not widespread in ovarian cancer overall, they are present in up to 15-20% of Endometrioid and Clear Cell subtypes. Proper identification of tumors with these repair pathway alterations is essential, as recent studies have shown that they respond to immunotherapy. We are investigating the molecular and clinical characteristics of ovarian tumors with MMR deficiencies and leveraging machine learning techniques to develop a predictor of MMR defects based on tumor genomic features. This predictor aims to overcome the current limitations of standard diagnostic methods, ensuring accurate patient stratification and improved clinical decision making.

PUBLICATIONS:

Nelson, B.H.; Hamilton, P.; Phung, M.T.; Milne, K.; Harris, B.; Thornton, S.; Stevens, D.; Kalaria, S.; Singh, K.; Laumont, C.M.; Moss, E.; Alimujiang, A.; Meagher, N.S.; Bolithon, A.; Fereday, S.; Kennedy, C.J.; Hendley, J; Ariyaratne, D.; Alsop, K.; Traficante, N.; Goode, E.L.; Karnezis, A.; Shen, H.; Richardson, J.; McKinnonDeurloo, C.; Chase, A.; Grout, B.; Doherty, J.A.; Harris, H.R.; Cushing-Haugen, K.L.; Anglesio, M.; Heinze, K.; Huntsman, D.; Talhouk, A.; Hanley, G.E.; Alsop, J.; Jimenez-Linan, M.; Pharoah, P.D.; Boros, J.; Brand, A.H.; Harnett, P.R.; Sharma, R.; Hecht, J.L.; Sasamoto, N.; Terry, K.L.; Karlan, B.; Lester, J.; Carney, M.E.; Goodman, M.T.; Hernández, B.Y.; Wilkens, L.R.; Behrens, S.; Turzanski, Fortner, R.; Fasching, P.A.; Bisinotto, C.; Candido Dos Reis, F.J.; Ghatage, P.; Köbel, M.; Elishaev, E.; Modugno, F.; Cook, L.; Le N.; Gentry-Maharaj, A.; Menon, U.; García, M.J.; Rodriguez-Antona, C.; Farrington, K.; Kelemen, L.E.; Kommoss, S.; Staebler, A.; Garsed, D.W.; Brenton, J.D.; Piskorz, A.M.; Bowtell, D.D.; DeFazio, A.; Ramus, S.J.; Pike, M.C.; Pearce, C.L. Immunological and molecular features of the tumor microenvironment of long-term survivors of ovarian cancer. *J. Clin. Invest.* **2024**, *134(24),* e179501. DOI: 10.1172/JCI179501.

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

"Global Instability and Mutation Burden genetic signatures in Clear Cell and Endometrioid Ovarian Carcinomas: Immunogenicity and prognostic and predictive relevance. PI19/01730". ISCIII. 2020-2024.

Colon Cancer: Organoids, Microenvironment and Vitamin D

PRINCIPAL INVESTIGATOR

Muñoz Terol, Alberto
Larriba Muñoz, María Jesús
González Sancho, José Manuel

SENIOR INVESTIGATOR

Fernández Barral, Asunción

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Barbáchano Becerril, Antonio
Bustamante Madrid, Pilar
González de la Aleja Molina, Arturo
Pintado Berninches, Laura

PRE-DOCTORAL INVESTIGATOR

Albandea Rodríguez, David
Rodríguez Marrero, Silvia

UNDERGRADUATE STUDENT

Rojo García, Alba

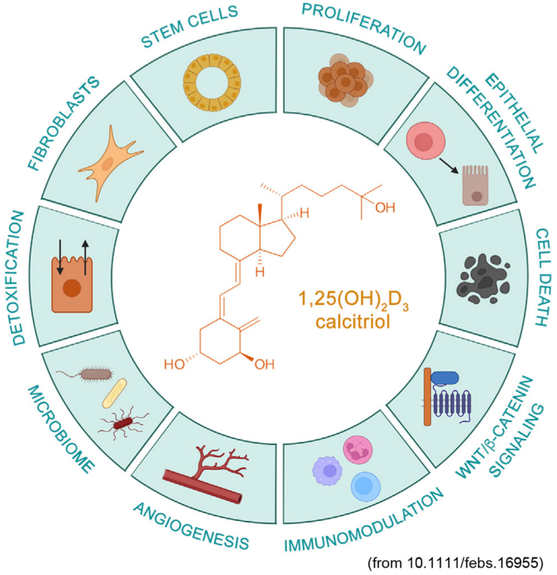
TECHNICAL SUPPORT PERSONNEL

Troya Balseca, Johanna

KEYWORDS

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

Mechanisms for the antitumoral action of vitamin D/calcitriol in colorectal cancer



(from 10.1111/febs.16955)

RESEARCH LINES:

Overview

Our group studies human colon cancer, which is the most frequent malignancy in Spain and a major neoplasia in terms of incidence and mortality worldwide. We use primary cultures of fibroblasts and stem cell-derived organoids established from healthy and tumor tissue of colon cancer patients to characterize the antitumor action of vitamin D and several drugs in this neoplasia. We also study the contribution of cancer-associated fibroblasts and other cell types of the tumor microenvironment to this disease.

Currently, there is an increase in cases of colon cancer in individuals under 50 years of age (early-onset colorectal cancer). The reasons for this rise remain unknown and studies conducted so far are limited, failing to identify the molecular characteristics that differentiate these tumors from those developed in older individuals. We are establishing organoids and fibroblast cultures from these patients to perform comparative analyses (gene expression patterns, pro-tumor properties, vitamin D and drug response) with those obtained from older patients aiming to improve the comprehension of this clinical entity.

The concept of precision/personalized medicine is a hot topic today. Regarding this, 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 the response of their own organoids and fibroblasts to available treatments.

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

Effects of vitamin D and several antitumor drugs on patient-derived colon organoids

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.

Effects of vitamin D on colon cancer-associated fibroblasts and other cell types of the tumor microenvironment

The tumor microenvironment is crucial for cancer initiation and progression and is involved in tumor relapse and therapeutic resistance. Accordingly, the worst prognosis colon 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 colon tumorigenesis.

Our work in this area is focused on the identification and study of calcitriol target genes in primary normal and tumor fibroblasts isolated from colon cancer patients, and in the in-depth characterization of calcitriol phenotypic and metabolic effects in these cells. We are also investigating: (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 other tumor stromal components such as macrophages; and (iii) the hypothetical regulation of these processes by calcitriol.

Collaborations

We actively collaborate with numerous colleagues from different national and international scientific institutions. As experts in vitamin D we have participated in a collaborative effort that led to identify that the epigenetic regulator SIRT1 is involved in the antagonism exerted by vitamin D on the Wnt/beta-catenin pathway in colon cancer.

PUBLICATIONS:

Bustamante-Madrid, P.; Barbáchano, A.; Albandea-Rodríguez, D.; Rodríguez-Cobos, J.; Rodríguez-Salas, N.; Prieto, I.; Burgos, A.; Martínez de Villarreal, J.; Real, F.X.; González-Sancho, J.M.; Larriba, M.J.; Lafarga, M.; Muñoz, A.; Fernández-Barral, A. Vitamin D opposes multilineage cell differentiation induced by Notch inhibition and BMP4 pathway activation in human colon organoids. Cell Death Dis. 2024, 15(4), 301. DOI: 10.1038/s41419-024-06680-z.

Pereira, F.; Fernández-Barral, A.; Larriba, M.J.; Barbáchano, A.; González-Sancho, J.M. From molecular basis to clinical insights: a challenging future for the vitamin D endocrine system in colorectal cancer. FEBS J. 2024, 291(12), 2485-2518. DOI: 10.1111/febs.16955.

García-Martínez, J.M.; Chocarro-Calvo, A.; Martínez-Useros, J.; Regueira-Acebedo, N.; Fernández-Aceñero, M.J.; Muñoz, A.; Larriba, M.J.; García-Jiménez, C. SIRT1 mediates the antagonism of Wnt/beta-catenin pathway by vitamin D in colon carcinoma cells. Int. J. Biol. Sci. 2024, 20(14), 5495-5509. DOI: 10.7150/ijbs.95875.

Van Driel, M.; Muñoz, A.; van Leeuwen, J.P.T.M. Overview of vitamin D actions in cancer. In "Vitamin D, Fifth Edition" 2024, 2(84), 679-718. DOI: 10.1016/B978-0-323-91338-6.00034-3.

Ferrer-Mayorga, G.; Muñoz, A.; González-Sancho J.M. Vitamin D and colorectal cancer. In "Vitamin D, Fifth Edition" 2024, 2(89), 859-898. DOI: 10.1016/B978-0-323-91338-6.00039-2

DOCTORAL THESES AND OTHER WORKS:

Alba Rojo García
"Final degree's project: *El gen supresor tumoral FBXW7 como posible mediador de la acción antiproliferativa del calcitriol en cáncer colorrectal*". Universidad Autónoma de Madrid. Medicina. 2024. Supervisors: José Manuel González and Asunción Fernández. Grade: 9.3

FUNDING:

"Vitamin D effects on colon cancer stem cells and microenvironment: differentiation, metabolism and intercellular communication. PID2022-136729OB-I00". MICINN. 2023-2026

"Cáncer colorrectal en población joven. Estudio farmacogenómico en organoides y efectos del microambiente tumoral. ICI20/00057". ISCIII. 2021-2026

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

"Consorcio CIBER Área Temática de Cáncer (CIBERONC). CB16/12/00273". ISCIII. 2017-2025

"Unidad CIBER de la Plataforma ISCIII de Biomodelos y Biobancos. PT23/00102". ISCIII. 2024-2026

Cancer Stem Cells and Fibroinflammatory Microenvironment

PRINCIPAL INVESTIGATOR
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STAFF INVESTIGATOR
Alcalá Sánchez, Sonia
Yagüe Jiménez, Balbino

ASSOCIATED INVESTIGATOR
Ruiz Cañas, Laura
Palencia Campos, Adrián

PRE-DOCTORAL INVESTIGATOR
López Gil, Juan Carlos

MASTER THESIS STUDENT
García Guzmán, Carol

TECHNICAL SUPPORT PERSONNEL
Navarro Vera, Diego
Batres Ramos, Sandra

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

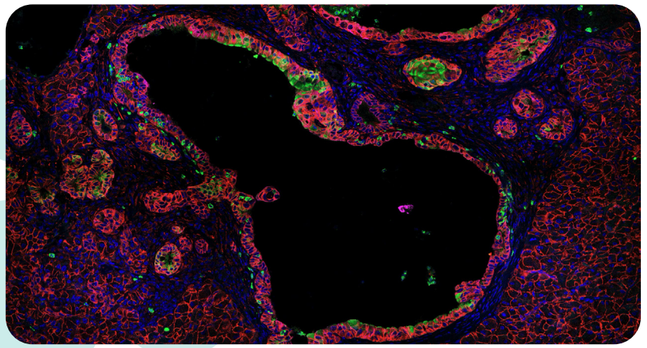

Figure: Pglyrp1 expression (in green) in PDAC lesion in the KPC genetically engineered moused model of pancretic cancer

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 Sainz laboratory, 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: Alcala, S; Navarro, D; López, JC; García, C.

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; Palencia, A; Navarro, D; López, JC; Ruiz, L; Batres, S.

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: Alcala, S; Palencia, A; Navarro, D; López, JC; 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, Alcala, S.

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:

Acalá, S.; Villarino L.; Ruiz-Cañas, L.; Couceiro, J.R.; Martínez-Calvo, M.; Palencia-Campos, A.; Navarro, D.; Cabezas-Sainz, P.; Rodriguez-Arabaolaza, I.; Cordero-Barreal, A.; Trilla-Fuertes, L.; Rubiolo, J.A.; Batres-Ramos, S.; Vallespinos, M.; González-Páramos, C.; Rodríguez, J.; Gámez-Pozo, A.; Fresno Vara, J.A.; Fra Fernández, S.; Benito Berlinches, A.; Moreno Mata, N.; Torres Redondo, A.M.; Carrato, A.; Hermann, P.C.; Sánchez, L.; Torrente, S.; Fernández-Moreno, M.A.; Mascareñas, J.L.; Sainz, Jr., B. Targeting cancer stem cell OXPHOS with tailored ruthenium complexes as a new anti-cancer strategy. *J Exp Clin Can Res.* **2024,** *43(1)*, 33. DOI: 10.1186/s13046-023-02931-7.

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Ibello E.; Saracino F.; Delle Cave D.; Bonaiuto S.; Amoroso F.; Andolfi G.; Corona M.; Guardiola O.; Colonna V.; Sainz, Jr., B.; Altucci L.; De Cesare D.; Cobellis G.; Lonardo E.; Patriarca E.J.; D'Aniello C.; Minchiotti G. Three-Dimensional Environment Sensitizes Pancreatic Cancer Cells to The Anti-Proliferative Effect of Budesonide by Reprogramming Energy Metabolism. *J Exp Clin Can Res.* **2024,** *43(1),* 165. DOI: 10.1186/s13046-024-03072-1.

Alcalá S.; Serralta San Martin G.; Muñoz-Fernández de Legaria M.; Moreno-Rubio J.; Salinas S.; Rojo López J.; Martínez Alegre J.; Cortes Bandy D.; Zambrana F.; Jiménez-Gordo A.M.; Casado E.; López-Gómez M.; Sainz, Jr., B.
Autofluorescent Cancer Stem Cells: Potential Biomarker to Predict Recurrence in Resected Colorectal Tumors. *Cancer Res Commun.* **2024,** 4(10), 2575-2588. DOI: 10.1158/2767-9764.CRC-24-0188.

Palencia-Campos A.; Ruiz-Cañas L.; Abal-Sanisidro M.; López-Gil J.C.; Batres-Ramos S.; Mendes Saraiva S.; Yagüe B.; Navarro D.; Alcalá S.; Rubiolo J.A.; Bidan N.; Sánchez L.; Mura S.; Hermann P.C.; de la Fuente M.; Sainz, Jr., B.
Reprogramming Tumor-Associated Macrophages with Lipid Nanosystems Reduces PDAC Tumor Burden and Liver Metastasis. *J Nanobiotechnology.* **2024,** *22(1),* 795. DOI: 10.1186/s12951-024-03010-5.

Ruz Caracuel I.; Pedraza-Arevalo S.; Alonso-Gordoa T.; Molina-7 Cerrillo J.; Earl J.; Sainz, Jr., B. Everything You Ever Wanted to Know About Cancer Stem Cells in Neuroendocrine Neoplasms but Were Afraid to Ask. *Endocr Oncol.* **2024,** *4(1),* e240006. DOI: 10.1530/EO-24-0006

DOCTORAL THESES AND OTHER WORKS:

Juan Carlos Lopéz-Gil
"PhD: *PGLYRP1 as a Mediator of Pancreatic Cancer Stem Cell Immune Evasion*". Universidad Autónoma de Madrid. Medicine. 2024. Supervisor/s: Bruno Sainz Anding and Susan García Silva. Grade: Sobresaliente Cum Laude

FUNDING:

"Plataforma Biomodelos y Biobancos-IRYCIS. PT23/00098". ISCIII. 2024-2026

"Selectively eliminating cancer stem cells through inhibition of mitochondrial respiration using metal-based small molecules. CC21-20122". LaCaixa Foundation. 2022-2025

"RuCSC - targeting cancer stem cells using ruthenium compounds" GAIN. 2019-2025

"Utility of new pancreatic ductal adenocarcinoma subtype profiles for the discovery of druggable targets or pathways: new tools for personalized medicine. PI21/01110". ISCIII. 2022-2025

"Plataforma Biomodelos y Biobancos-IRYCIS. PT20/00045". ISCIII. 2020-2024

"Preclinical development of apMNKQ2 aptamer targeting MNK1 in gastrointestinal cancer. PMPTA22/00113". ISCIII. 2023-2024

Cell Cycle & Cancer Biomarkers

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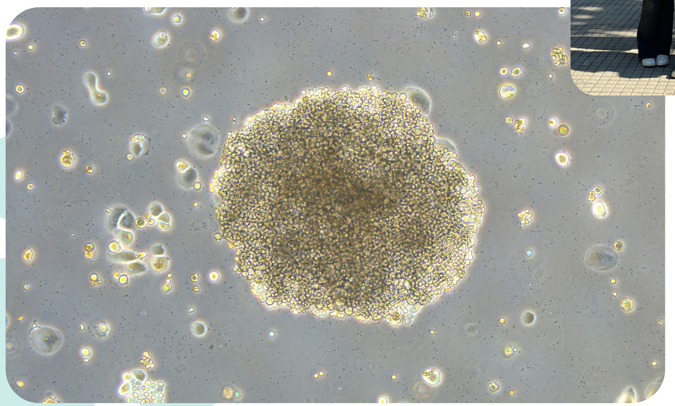
KEYWORDS
Cell Cycle, PLK1, Chromosomal Instability, Aneuploidy, Drug Resistance, CRISPR Screens.



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.

Chromosomal Instability (CIN) genes as biomarkers for cancer therapy.

Researchers Involved: Sanz, Natalia; Monfort, Ana.

Cell division and CIN genes are often overexpressed in tumors, and this commonly confers poor prognosis to the patients. We are testing whether the overexpression of CIN genes can predict sensitivity to different pharmacologic drugs. For this, we collaborated with the pharma company Lilly (SPAIN), where we screened a drug library against breast cancer cells that overexpress CIN-related genes.

We have discovered that overexpression of the gene TPX2, which is well-known to be directly related to CIN, renders sensitivity to the drug Dasatinib in breast cancer cell lines, due to the activation of the YAP/TAZ transcriptional signaling. We further explored the TPX2-YAP/TAZ signaling axis in breast cancer-derived samples, observing that tumors expressing high TPX2 levels, and showing YAP activation, are more aggressive. Our data open a new therapeutic opportunity for aggressive breast cancer CIN tumors, using Dasatinib, which is a drug already in the clinical

setting of leukemia subtypes. This work is a great example of a collaborative network between an academic laboratory (CSIC), a pharma company (Eli Lilly), and the MD Anderson Hospital (Madrid).

Identification of Resistance Mechanisms associated with Cell Cycle Drugs.

Researchers involved: Monfort, Ana; Sanz, Natalia; Montes, Ángela; Cambón, Aitana; Escribano, María; Juan, Carla.

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.

Chromosomal Instability (CIN) is a major hallmark in 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 the cell cycle progression. WGD has two very different outcomes: on the one hand, it regulates tissue homeostasis in the liver, heart, stratified epithelia, etc., leading to a non-proliferative differentiation program. In other words, WGD is detrimental to cell proliferation and may play a tumor suppressor role. On the contrary, WGD has also been described as an early event during tumorigenesis in a variety of tumors, leading to cancer aggressiveness and poor prognosis.

Therefore, tumoral cells that have undergone an early WGD event need to overcome the detrimental effect to be able to proliferate. Despite the efforts done in the last years by different research groups, how cells adapt to WGD in the cancer context is still an open question. Using an elegant genetic trick, to induce WGD in mouse cells, we have identified a genetic signature that might explain this paradox shift, and we are currently exploring the possible molecular mechanism behind it, and trying to find the most suitable cancer type to study this phenomenon.

PUBLICATIONS:

Marugán, C.; Sanz-Gómez, N.; Ortigosa, B.; Monfort-Vengut, A.; Bertinetti, C.; Teijo, A.; González, M.; Alonso de la Vega, A.; Lallena, M. J.; Moreno-Bueno, G.; & de Cárcer, G. TPX2 overexpression promotes sensitivity to dasatinib in breast cancer by activating YAP transcriptional signaling. *Molecular Oncology,* **2024**, *18(6),* 1531–1551. DOI: 10.1002/1878-0261.13602

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Flores, N. G.; Fernández-Aroca, D. M.; Garnés-García, C.; Domínguez-Calvo, A.; Jiménez-Suárez, J.; Sabater, S.; Fernández-Aroca, P.; Andrés, I.; Cimas, F. J.; de Cárcer, G.; Belandia, B.; Palmero, I.; Huertas, P., Ruiz-Hidalgo, M. J.; & Sánchez-Prieto, R. The CDK12-BRCA1 signaling axis mediates dinaciclib-associated radiosensitivity through p53-mediated cellular senescence. *Molecular Oncology,* **2024,** *(advance online publication)* DOI: 10.1002/1878-0261.13773.

Monfort-Vengut, A.; Sanz-Gómez, N.; Ballesteros-Sánchez, S.; Ortigosa, B.; Cambón, A.; Ramos, M.; Montes-SanLorenzo, A.; Rosa-Rosa, J. M.; Martínez-López, J.; Sánchez-Prieto, R.; Sotillo, R.; de Cárcer, G. Osmotic stress influences microtubule drug response via WNK1 kinase signaling. *bioRxiv,* **2024,** DOI: 10.1101/2024.07.08.60203

DOCTORAL THESES AND OTHER WORKS:

Ana Monfort Vengut
"Ph.D. thesis: *Osmotic stress signaling as a modulator of the response to antitumoral cell cycle drugs*". Universidad Autónoma de Madrid. Medicina. 2024. Supervisor/s: Guillermo de Cárcer. Grade: Sobresaliente Cum Laude

Maria Escribano Cebrián
"Master´s thesis: *Identification & characterization of genetic determinants to overcome chromosomal instability in cancer*". Universidad Autónoma de Madrid. Medicina. 2024. Supervisor/s: Guillermo de Cárcer and Natalia Sanz. Grade: Sobresaliente

Aitana Cambón Hernández
"Master´s thesis: *Molecular mechanisms of the stress kinase WNK1 as a modulator of the chemotherapeutic response in cancer*". Universidad Autónoma de Madrid. Medicina. 2024. Supervisor/s: Guillermo de Cárcer. Grade: Sobresaliente

FUNDING:

"Study of novel physiological and tumor biomarkers associated with the therapeutic target PLK1. PID2021". MICINN. 2022-2024

Cytoskeleton And Metastasis

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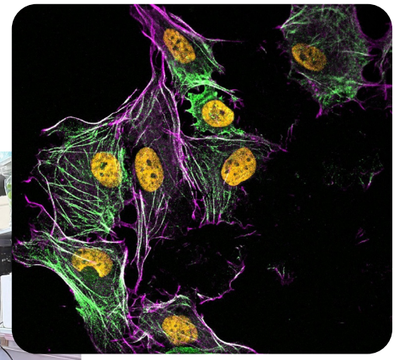
KEYWORDS
Melanoma, Myosin, Cytoskeleton, Therapy Resistance, Metastasis.



Cytoskeleton and Metastasis Group: Jose Orgaz, Lucía Sánchez, Andrea García, Marta Durán

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 (BRAFV600E 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 BRAFV600E 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.; Novo, S.; Cabeza, C.; 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.; Perez de Gracia, N.; Orgaz, J.

This research line is focused on studying the different outcomes and vulneratibilities 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.

PUBLICATIONS:

Orgaz, J.L. Creating a path during melanoma amoeboid migration: When too crowded, start worrying. *Dev Cell.* **2024**, *59(18),* 2395-2397. DOI: 10.1016/j.devcel.2024.07.009.

DOCTORAL THESES AND OTHER WORKS:

Natalia Pérez de Gracia Velázquez

"Master's thesis: *The susceptibility of distinct cutaneous melanoma phenotypes to inhibition of Non-Muscle Myosin II cytoskeleton*". Universidad Autónoma de Madrid. Medicina. 2024. Supervisor: Jose Luis Orgaz Bueno. Grade: 8,8.

Silvia Novo Acedo

"Master´s thesis: *Studying the Regulation of non-muscle Myosin II Cytoskeleton During Adaptation and Resistance to MAPK-targeted Therapy in Cutaneous Melanoma*". Universidad Autónoma de Madrid. Medicina. 2024. Supervisor: Jose Luis Orgaz Bueno. Grade: 9,3.

Lucía Sánchez García

"Final degree's project: *Estudio de la regulación del citoesqueleto de miosina no muscular durante la progresión maligna del melanoma cutáneo*". Universidad Autónoma de Madrid. Ciencias. 2024. Supervisor: Jose Luis Orgaz Bueno. Grade: 8,9.

Carlota Cabeza Arciniega

"Final degree's project: *Estudio de la regulación del citoesqueleto de miosina no muscular durante resistencia a terapias en melanoma cutáneo*". Universidad Autónoma de Madrid. Ciencias. 2024. Supervisor: Jose Luis Orgaz Bueno. Grade: 8,9.

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

"Understanding Myosin II regulation during adaptation to targeted therapies in melanoma and potential therapeutic interventions to delay therapy resistance. 2019-T1/BMD-13642". Comunidad de Madrid. 2020-2024

Pharmacogenomics and Tumor Biomarkers

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

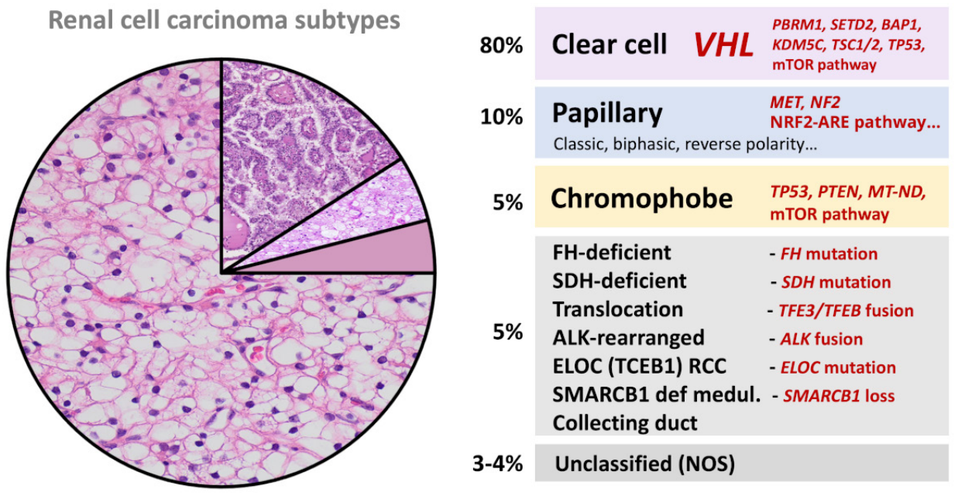

Figure 1. Renal cell carcinoma (RCC) is composed by several histological subtypes characterized by distinct molecular alterations (shown in red).

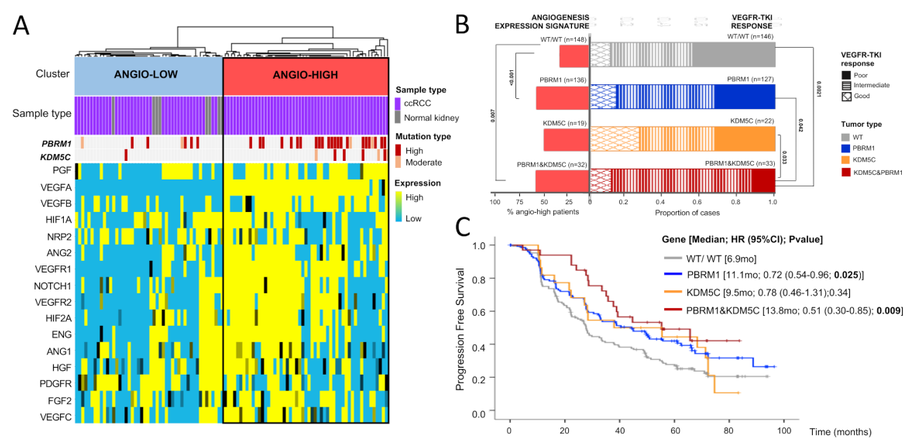

Figure 2. Mutations in *PBRM1* and *KDM5C* are associated with increased clear cell RCC (ccRCC) vasculature and improved patients' response to VEGFR-tyrosine kinase inhibitors (TKI). A) Heatmap showing the expression of 16 angiogenesis-related genes in 93 ccRCC tumors and 8 normal kidney tissue samples. The mutational status of *PBRM1* and *KDM5C* is shown in red. **B)** Response of ccRCC patients to the VEGFR-TKI drug sunitinib (n=343; IMmotion151 clinical trial) according to *PBRM1 and KDM5C* mutational status is shown as a bar chart. **C)** Kaplan-Meier curve showing the progression-free survival of patients. Adaptation from Santos *et al.* 2023 *Am J Cancer Res* PMID: 37293154.

RESEARCH LINES:

Overview

The main interest of our group is **understanding how genomic variation modifies 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 the discovery of new mechanisms of cancer 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.

Molecular-based stratification of metastatic renal cell carcinoma to personalize treatment strategies

Renal cell carcinoma (RCC) is a heterogeneous group of tumors classified into **different histological subtypes (Figure 1).** The most common subtypes are clear cell RCC (ccRCC), papillary RCC, and chromophobe RCC. Less common tumors include FH-deficient and SDH-deficient RCC and those with fusions in *TFE3, TFEB,* and *ALK.* These RCC histologic subtypes present different morphologic features and have **distinct molecular alterations and prognoses.** Worldwide, 431,000 new cases and 179,000 deaths of RCC occur annually. In Spain, 9,200 new diagnoses lead to >2,200 deaths yearly. The survival of patients with **metastatic RCC** has doubled/almost tripled in the last 20 years due to the approval of more than 20 targeted drugs, and the current first-line treatment for metastatic RCC (independently of the histologic subtype) consists of **antiangiogenic drugs** and **immune checkpoint inhibitors.** However, while some RCC patients exhibit long-lasting responses to these drugs, others have intrinsic resistance or develop resistance in a few months. Our group aims to understand this variability in response following the two approaches outlined below.

1. Patients with ccRCC histology (Figure 1)

Antiangiogenics and immune checkpoint inhibitors target key characteristics of ccRCC histology: i) the high tumor angiogenesis derived from the pseudohypoxia caused by VHL inactivation and HIFα accumulation; ii) the high immune cell infiltration typical of this subtype. However, the basis for the variable drug response in patients is unknown. In addition, predictive biomarkers used in other tumor types (e.g., PDL1 and TMB for immunotherapy response) have failed. Thus, at the moment, we cannot predict which patients will be sensitive and which will have a poor response to the treatment, and drug treatment choices cannot be guided. We propose that **secondary mutations that occur after VHL inactivation modulate tumor microenvironment characteristics and modify the response to these drugs.** Specifically, our group is investigating how ccRCC secondary mutations modify the tumor microenvironment and the response to antiangiogenics and immunotherapy. Our main focus is on the chromatin remodeler genes PBRM1 and KDM5C (Santos et al. Am J Cancer Res 2023 and Lanillos et al. Eur Urol 2022; PMIDs 37293154 and 35688666; see **Figure 2**).

This year, we have described that an overexpression of the androgen receptor favors a transcriptomic signature linked to the response to antiangiogenic monotherapy, enhancing precision and personalized therapeutic decisions (Osorio et al. 2024).

2. Patients with histologies different from ccRCC (non-ccRCC) (Figure 2)

We propose that in many of these patients, the drug resistance to current front-line treatments results from a **lack of molecular rationale.** For example, tumors with no hypoxia response or little immune cell infiltration are suggestive of poor response to antiangiogenics and immunotherapy. Unfortunately, clinical trials in non-ccRCC patients pool together multiple RCC histologies with diverse oncogenic drivers and provide unprecise average responses with limited translational potential. Furthermore, as for many rare/ low-frequency diseases, recruitment of the so-called "non-ccRCC" metastatic patients is difficult, and industry-sponsored RCC histology-specific clinical trials are scarce. In this sense, **metastatic non-ccRCC is a neglected disease, treated with unspecific drugs that achieve variable and frequently suboptimal responses in patients.** Our recent work has focused on chromophobe RCC. We have proposed **mTOR pathway** as a promising therapeutic target in metastatic patients (Santos et al. *Mod Pathol* 2020, PMID 32616874); defined how mutations in this pathway modulate the response of patients to mTOR inhibitors (*Int J Cancer* 2020, JNCCN 2017 and 2018, PMIDs 31335987, 29118224, 29632054) and discovered USP9X as a novel therapeutic target with synthetic lethality with mTOR inhibitors (Roldan-Romero et al. *Int J Cancer* 2023, PMID 37260183). Furthermore, **mitochondrial DNA mutations are a hallmark of chromophobe RCC.** In this regard, this year, we have participated in a study that defines mitochondrial mutations in the mtDNA-encoded complex I *gene Mt-Nd5* as functional regulators of cancer metabolism and tumor biology, with potential for therapeutic exploitation and treatment stratification (Mahmood et al. 2004).

Collaborators: To achieve this translational research in RCC, we have to acknowledge our collaboration with national clinical cancer groups, such as the GenitoUrinary Alliance for Research and Development (GUARD), multiple oncology departments in hospitals (key collaborators include Jesus García-Donas at HM Madrid; Guillermo de Velasco at Hospital 12 de Octubre, Ignacio Duran at Hospital Marqués de Valdecilla), uropathologists (key collaborators include Eduardo Caleiras at CNIO and Ainara Azueta at Hospital OSI Basurto-Bilbao) and specialist in computational sciences (key collaborator José Luis Ayala at the Architecture and Technology of Computing Systems Group of UCM).

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. In previous years, we have used targeted NGS panels to determine the impact of germline mutations on metastatic RCC (Santos et al. Genet Med 2021, PMID 37293154). This year, we have collaborated with the CNIO to establish that the prevalence of *MAML3* fusions in **pheochromocytoma and paraganglioma** have a 4% prevalence and that these fusions are associated with increased metastatic risk, increased expression of neuroendocrine-to-mesenchymal transition markers, MYC-targets, and angiogenesis-related genes, leading to a distinct tumor microenvironment with unique vascular and immune profiles (Monteagudo et al. 2024).

Finally, by being part of the **International Consortia OCAC-OTTA** we contribute to **ovarian cancer** research in large multinational collaborative studies. This year, we participated in three publications that defined novel genetic and functional mechanisms underlying ovarian cancer risk regions and identified immunological and molecular features of the tumor microenvironment of long-term survivors of ovarian cancer.

Collaborators: We actively collaborate with the research led by Mercedes Robledo at Hereditary Endocrine Group at CNIO on pheochromocytoma and paraganglioma. We participate in the Internacional Consortia of Ovarian Cancer OCAC-OTTA (https://ottaconsortium.org/).

Germline variation leading to increased risk of adverse drug reactions

An intrinsic part of pharmacogenomics 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.** Our initial results from a Genome Wide Association Study (GWAS) have been presented at the San Antonio Breast Cancer Symposium (Sanchez-Bayona et al. P2-01-16; December 2024).

In addition, to impulse the implementation of personalized medicine in the health systems, we participated in the establishment of the **global repository for allele nomenclature for the polymorphic human *CYP4F2* gene** (Zubiaur et al. 2024). This enzyme impacts the metabolism of vitamin K, which is associated with warfarin dose requirements, and the metabolism of drugs, such as imatinib or fingolimod, and certain endogenous compounds, including vitamin E and eicosanoids.

Collaborators: The PROCURE study is led by the oncologist Rodrigo Sánchez-Bayona at the Hospital 12 de Octubre. We are part of the Expert Panel of International Pharmacogenetic Variation Consortium (PharmVar, https://www.pharmvar.org/), a central repository for pharmacogene variation that focuses on haplotype structure and allelic variation to facilitate basic and clinical research as well as the interpretation of pharmacogenetic test results to guide precision medicine.

PUBLICATIONS:

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Zubiaur, P.; Rodriguez-Antona, C.; Boone, E. C.; Daly, A. K.; Tsermpini, E. E.; Khasawneh, L. Q.; Sangkuhl, K.; Duconge, J.; Botton, M. R.; Savieo, J.; Nofziger, C.; Whirl-Carrillo, M.; Klein, T. E.; Gaedigk, A., PharmVar GeneFocus: CYP4F2. *Clin Pharmacol Ther* **2024**, *116 (4)*, 963-975. DOI: 10.1002/cpt.3405.

Monteagudo, M.; Calsina, B.; Salazar-Hidalgo, M. E.; Martinez-Montes, A. M.; Pineiro-Yanez, E.; Caleiras, E.; Martin, M. C.; Rodriguez-Perales, S.; Leton, R.; Gil, E.; Buffet, A.; Burnichon, N.; Fernandez-Sanroman, A.; Diaz-Talavera, A.; Mellid, S.; Arroba, E.; Reglero, C.; Martinez-Puente, N.; Roncador, G.; Del Olmo, M. I.; Corrales, P. J. P.; Oliveira, C. L.; Alvarez-Escola, C.; Gutierrez, M. C.; Lopez-Fernandez, A.; Garcia, N. P.; Regojo, R. M.; Diaz, L. R.; Laorden, N. R.; Guadarrama, O. S.; Bechmann, N.; Beuschlein, F.; Canu, L.; Eisenhofer, G.; Fassnacht, M.; Nolting, S.; Quinkler, M.; Rapizzi, E.; Remde, H.; Timmers, H. J.; Favier, J.; Gimenez-Roqueplo, A. P.; Rodriguez-Antona, C.; Curras-Freixes, M.; Al-Shahrour, F.; Cascon, A.; Leandro-Garcia, L. J.; Montero-Conde, C.; Robledo, M., MAML3-fusions modulate vascular and immune tumour microenvironment and confer high metastatic risk in pheochromocytoma and paraganglioma. *Best Pract Res Clin Endocrinol Metab* **2024**, *38 (6),* 101931. DOI: 10.1016/j.beem.2024.101931.

Nelson, B. H.; (...); Rodriguez-Antona, C.; Farrington, K.; Kelemen, L. E.; Kommoss, S.; Staebler, A.; Garsed, D. W.; Brenton, J. D.; Piskorz, A. M.; Bowtell, D. D.; DeFazio, A.; Ramus, S. J.; Pike, M. C.; Pearce, C. L., Immunological and molecular features of the tumor microenvironment of long-term survivors of ovarian cancer. *J Clin Invest* **2024**, 134 *(24).* DOI: 10.1172/JCI179501.

Ramachandran, D.; Tyrer, J. P.; Kommoss, S.; DeFazio, A.; Riggan, M. J.; Group, A.; Webb, P. M.; Fasching, P. A.; Lambrechts, D.; Garcia, M. J.; Rodriguez-Antona, C.; (...); Heitz, F., Genome-wide association analyses of ovarian cancer patients undergoing primary debulking surgery identify candidate genes for residual disease. NPJ *Genom Med* **2024**, 9 *(1), 19.* DOI: 10.1038/s41525-024-00395-y.

Dareng, E. O.; (...); Rodriguez-Antona, C.; (...); Gayther, S. A., Integrative multi-omics analyses to identify the genetic and functional mechanisms underlying ovarian cancer risk regions. *Am J Hum Genet* **2024,** *111* (6), 1061-1083. DOI: 10.1016/j.ajhg.2024.04.011

FUNDING:

"Molecular alterations of metastatic renal cell carcinoma of clinical significance for antitumor drug response PID2021-128312OB-I00" PI: Rodríguez-Antona. MICINN. 2022-2025.

"Pharmacogenomic study of the toxicity of trastuzumab-deruxtecan in breast cancer patients". PI: Rodríguez-Antona. Fundación HNA/ Fundación para la Investigación Biomédica Hospital 12 de Octubre. 2022-2024

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

Emerging Genes in Thyroid Cancer

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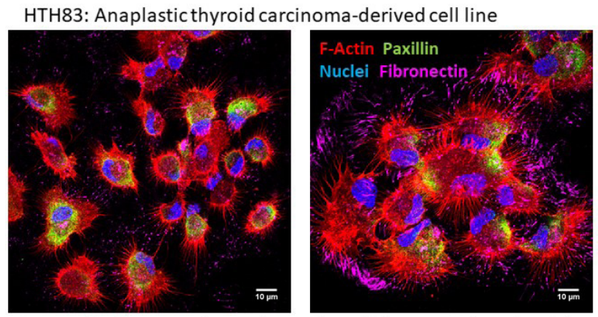
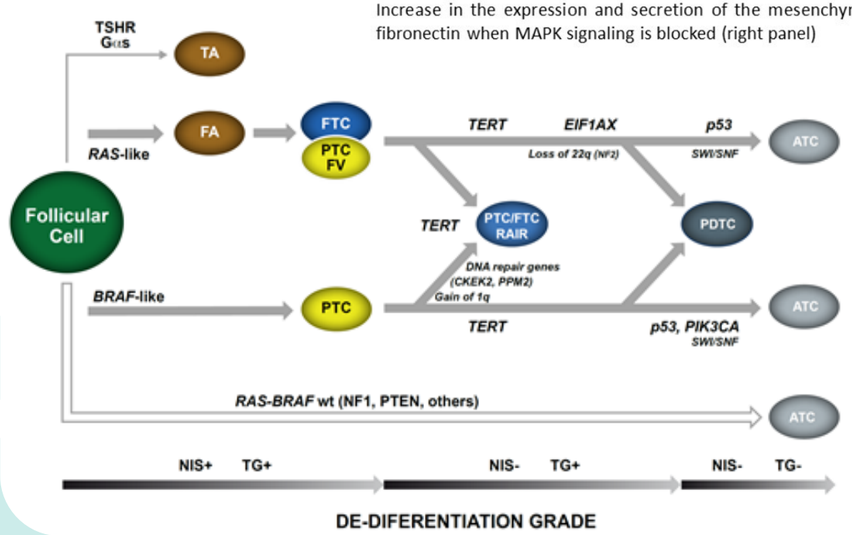
KEYWORDS
Oncogenes, Signaling, RNA editing, MiRNAs, Thyroid cancer



Increase in the expression and secretion of the mesenchymal protein fibronectin when MAPK signaling is blocked (right panel)



The current knowiedge of thyroid carcinogenesis based on the thyroid cancer genome atlas (TCGA).
For more detail see Riesco-Eizaguirre & Santisteban, 1016. doi:10.1530/EJE-16-0202

RESEARCH LINES:

Overview

The main mutations in thyroid cancer are in BRAF, RAS and RET/PTC genes, that lead to aberrant MAPK activation, driving progression invasiveness and metastasis.

The 2024 work is a continuation of the work done in 2023. In this period, we have focused on the transcriptional coactivators YAP1/TAZ1, which are mediators of Hippo pathway. We have focused on studying the function of this pathway in thyroid cells, finding important results of its role in resistance to kinase inhibitors. We have also extrapolated our results to define a crucial role of TAZ in a syndrome associated with congenital hypothyroidism.

1. Role of the Hippo pathway in thyroid cancer and its implication in resistance to cancer therapies.

Researchers involved: Santisteban, Pilar; Zaballos, MA (This research line is the follow-up of Celia Fernández Mendes`s work (see memoir 2021-2022).

Among the emerging genes and signaling pathways identified in our work, in 2024 we focused on better understand the role of YAP/TAZ, which are mediators of the Hippo pathway. In a previous work, we show that TAZ acts a transcriptional corepressor, inhibiting one of the main thyroid differentiation genes the Sodium Iodide Symporter (NIS) through inhibition of the transcription factor PAX8 (Thyroid 2021).

RAF- and MEK-targeted therapies are approved for patients with BRAFV600E Thyroid cancer and in a several other tumor types (melanoma, lung), but resistance remains a major challenge. Since Dr. Trever Bibona's laboratory (University of California San Francisco) had described the role of YAP1 in the mechanism of resistance to RAF-MEK inhibition in lung cancer with BRAF and RAS mutation, Dr. Celia Fernandez joined that laboratory in order to extrapolate this knowledge to thyroid cancer. Due to this collaboration, we demonstrate that the Focal adhesion kinase-YAP signaling axis drives drug-tolerant in lung cancer.

In thyroid cancer, we have demonstrated increased expression of TAZ in the nucleus of thyroid tumor cells due to the over-activation of the MAPK pathway. As previous studies in lung cancer indicated that YAP1 might be mediating kinase inhibitor resistance, we are studying whether the same occurs in thyroid tumor cells. We are using two inhibitors used in the clinic, Dasatinib and Trametinib. In addition, we have initiated a study using the Hippo pathway inhibitor, Verteporfin, in combination with the above kinase inhibitors, with very promising preliminary results.

2. Role of TAZ in congenital hypothyroidism

Researchers involved: Carrasco-López, Carlos; Santisteban, Pilar.

Our previous work had shown that TAZ, plays a transcriptional coactivator role in the thyroid gland, cooperating with the transcription factor NKX2.1 in the regulation of the Thyroglobulin gene in congenital hypothyroidism (Moya et al JCEM 103: 839-852 (2018). Ongoing to study the role of TAZ in this pathology, we have identified a novel pathogenic variant in NKX2-1 causing Brain Lung Thyroid (BLT) syndrome that is inherited through germline mosaicism. The mutation lacks DNA binding capacity, which prevents transactivation of the genes downstream of NKX2-1 in the three organs of the syndrome studied. By functional experiments, we have shown that the mutation studied prevents transcriptional rescue by TAZ.

PUBLICATIONS:

Haderk, F.; Chou, Y.T.; Cech, L.; Fernández-Méndez, C.; Yu, J.; Olivas, V.; Meraz, I.M.; Barbosa-Rabago, D.; Kerr, D.L.; Gómez, C.; Allegakoen, D.V.; Guan, J.; Shah, K.N.; Herrington, K.A.; Gbenedio, O.M; Nanjo, S.; Majidi, M.;Tamaki, W.; Pourmoghadam, Y.K.; Rotow, J.K.; McCoach, C.E.; Riess, J.W.; Gutkind, J.S.; Tang, T.T.; Post, L., Huang, B.; Santisteban, P.; Goodarzi, H.; Bandyopadhyay, S., Kuo, C.J., Roose, J.P.; Wu, W.; Blakely, C.M.; Roth, J.A.; Bivona, T.G. Focal adhesion kinase-YAP signaling axis drives drug-tolerant persister cells and residual disease in lung cancer. *Nature Commun*. **2024** *15,* 3741-3750 DOI: 10.1038/s41467-024-47423-0.

Villafuerte, B*.; Carrasco-López, C*., Herranz, A., Garzón, L.; Simón, R.; Natera-de-Benito, D.; Alikhani. P., Tenorio, J., García-Santiago, F.; Solis, M.; Del-Pozo, Á.; Lapunzina, P.; Ortigoza-Escobar, J.D.; Santisteban, P.#; Moreno JC# (*Co-Firts # Co-corresponding). A novel missense variant at the NKX2-1 homeodomain prevents the transcriptional rescue by TAZ. *Thyroid*. **2024,** *34(7),* 942-948. DOI: 10.1089/thy.2023.0593.

FUNDING:

"Análisis molecular integral para el estudio de la diferenciación en el cáncer de tiroides". MICIN. 2020-2024

"Estudio de la heterogeneidad celular y del entorno inmunológico en las patologías tiroideas: cáncer y enfermedad autoinmune". Programa de Biomedicina Comunidad de Madrid 2023-2022.

"Efectos de la Vitamina D sobre las células troncales y el microambiente tumoral en cáncer de colon: diferenciación, metabolismo y comunicación intercelular". MICIN, 2023-2026.

AWARDS:

Pilar Santisteban: "Medalla de la Ciudad de Villanueva de la Serena (Badajoz)". 2024

Pilar Santisteban: "Premio Imparables Sanitarios 2024, a la trayectoria Científica. Concedido por la Compañía Farmacéutica Italfármaco"

Chromosome Instability & Tumorogenesis

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KEYWORDS
Cell Cycle, Mitosis, Chromosome Instability, Metalodrugs, Preclinical Models.

RESEARCH LINES:

I lead the **Chromosomal Instability and Tumorigenesis** research group at the IIBM Sols-Morreale CSIC-UAM, a multidisciplinary team integrating the Department of Biochemistry and the Department of Inorganic Chemistry at UAM. Additionally, I am a Principal Investigator at IRYCIS within the **Biomarkers and Personalized Approach to Cancer Group (BioPAC).** My involvement in these institutions provides a broad perspective, combining basic science with translational and clinically oriented research.

My research focuses on two fundamental aspects of cancer biology:

1. **Cellular response mechanisms to therapy.**
2. **Identification of biomarkers related to tumorigenesis and therapy response.**

Research Focus: Gastric Cancer (GC)
Gastric cancer, the fifth most diagnosed and third deadliest cancer worldwide, is the primary focus of our research. We pursue two complementary research lines:

1. Molecular mechanisms of mitotic regulation and tumorigenesis
Researchers involved: Melones Herrero J., Delgado Aliseda, P., Calés C., Sanchez-Perez, I.
Gastric cancer exhibits **high chromosomal instability (CIN) and aneuploidy,** often due to **defects in the spindle assembly checkpoint (SAC)**. Our studies analyze key mitotic regulators such as **Mad2 and BubR1,** demonstrating their role in **migration, invasion, and stem cell balance.**

- We identified **MAD2** as a crucial factor for tumor stem cell growth, regulating tumorigenesis through the CXCR4-SNAI2-MMP1 pathway.
- We found that MAD2 overexpression in tumors is driven by post-transcriptional regulation via **miR19a,** *proposing it as a prognostic biomarker* and showing synergistic effects with conventional and novel therapies.

2. Novel metal-based antitumor agents
Researchers involved: Melones Herrero J., Delgado Aliseda, P., Velazquez Gutierrez, J., Figueiras, S. Calés C., Sanchez-Perez, I.
We investigate **cisplatin and novel metallodrugs** to overcome limitations of conventional treatments.

- **Cisplatin Sensitivity Mechanisms:** We explore apoptosis pathways in GC cells with varying cisplatin sensitivity (AGS and MKN45). Higher sensitivity in MKN45 cells correlates with Mcl-1 degradation and DNA repair (NER) alterations, identifying *NER and Bcl-2 proteins as potential therapeutic targets*
- **Platinum(II) trans-complexes:** We studied **cis- and trans-[PtI2(isopropylamine)2]** in gastrointestinal cancer cells, showing they impair *OXPHOS metabolism, induce oxidative stress, and trigger senescence. Notably, I5 reduced tumor growth in pancreatic xenograft models without systemic toxicity, highlighting its potential as an anticancer agent.*
- **Copper(II)-thiosemicarbazone complexes:** We synthesized **$[Cu(L1)_2]$ and $[Cu(L2)_2]$**, demonstrating high cytotoxicity in GC cells via DNA damage, oxidative stress, and autophagy/apoptosis dysregulation. Additionally, **$[Cu(L1)_2]$** targeted tumor stem cells and cisplatin-resistant populations, reducing pluripotency markers TWIST, NANOG, and OCT4.
- **Platinum(II)-phosphine complexes: P2 (trans-$[Pt(amine)Cl_2(PPh_3)]$)** exhibited greater cytotoxicity than cisplatin, with lower toxicity in normal cells. P2 induced ROS generation, DNA damage, and mitochondrial apoptosis via BAX/BAK activation. Moreover, it triggered ER stress and UPR activation, disrupting autophagy markers (p62, LC3). These findings position P2 as a promising prototype for GC therapy.
- **Bio-targeted metallodrug conjugates:** We developed metallodrug-ligand conjugates (Cu(II), Pd(II), Pt(II)) enhancing cell uptake and anticancer activity, synergizing ribonucleotide reductase inhibition and metal center interactions.

Additional Contributions

1. **SARS-CoV-2 Spike Protein and Endothelial Senescence:** Demonstrated that SARS-CoV-2 spike protein induces endothelial cell senescence, impairing vascular function via DNA damage and downregulation of klotho/Nrf2. Pharmacological inhibition of NLRP3 inflammasome prevented these effects, offering new therapeutic strategies for COVID-19 vascular complications.

Our research bridges fundamental cancer biology with **therapeutic innovation,** focusing on **precision medicine and novel drug development** to improve patient outcomes.

PUBLICATIONS:

Fabra, D.; Melones-Herrero, J.; Velazquez-Gutierrez, J.; Matesanz, A. I.; Aliseda, P. D.; Figueiras, S.; Aguilar-Rico, F.; Cales, C.; Sanchez-Perez, I.; Quiroga, A. G., A select thiosemicarbazone copper(II) complex induces apoptosis in gastric cancer and targets cancer stem cells reducing pluripotency markers. *Eur J Med Chem.* **2024**, 280, 116994.DOI: 10.1016/j.ejmech.2024.116994

Melones-Herrero, J.; Delgado-Aliseda, P.; Figueiras, S.; Velazquez-Gutierrez, J.; Quiroga, A. G.; Cales, C.; Sanchez-Perez, I., Trans-[Pt(amine)Cl(2)(PPh(3))] Complexes Target Mitochondria and Endoplasmic Reticulum in Gastric Cancer Cells. *Int J Mol Sci.* **2024**, *25 (14)*. DOI: 10.3390/ijms25147739.

Melones-Herrero, J.; Alcala, S.; Ruiz-Canas, L.; Benitez-Buelga, C.; Batres-Ramos, S.; Cales, C.; Lorenzo, O.; Perona, R.; Quiroga, A. G.; Sainz, B., Jr.; Sanchez-Perez, I., Platinum iodido drugs show potential anti-tumor activity, affecting cancer cell metabolism and inducing ROS and senescence in gastrointestinal cancer cells. *Commun Biol.* **2024**, *7 (1)*, 353. DOI: 10.1038/s42003-024-06052-5

Villacampa, A.; Shamoon, L.; Valencia, I.; Morales, C.; Figueiras, S.; de la Cuesta, F.; Sanchez-Nino, D.; Diaz-Araya, G.; Sanchez-Perez, I.; Lorenzo, O.; Sanchez-Ferrer, C. F.; Peiro, C., SARS-CoV-2 S Protein Reduces Cytoprotective Defenses and Promotes Human Endothelial Cell Senescence. *Aging Dis.* **2024.** DOI: 10.14336/AD.2024.0405

DOCTORAL THESES AND OTHER WORKS:

Sofía Villapuga
"Master´s thesis: *6. Efecto de los metalofármacos I5 e I6 en las líneas célulares de cáncer gástrico MKN45 y AGS*". Universidad Complutense de Madrid. Supervisor/s: Carmela Calés e Isabel Sánchez Pérez. Grade: Sobresaliente

Carmen Viciana
"Master´s thesis: *Synergistic solutions: harnessing metal-organic frameworks for multi-contaminant water treatment and antibacterial action*". Universidad Autónoma de Madrid. 2024. Supervisor/s: Adoración Gomez e Isabel Sanchez Perez. Grade: Sobresaliente

FUNDING:

"New metal complexes as molecular targets and preclinical cancer models. PID2022-137373OB-I00".Ministry of Science and Innovation (MICINN). 2023-2026.

"Advances in Medicinal Chemistry: New approaches and targeted drug delivery". Africa-Europe research collaboration. Funded by: CIVIS-4UA. 2024.

"AptaBreast: Preclinical Development of an Aptamer for Cancer Treatment. RTC2019-007227-1P". Challenges Collaboration. Ministry of Science and Innovation (MICINN). 2020-2024

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. **PCT/ES2006/070152.** España. European EP06849419.4, Japan 5560398, USA US9.074,194B2 Licenciada **ALODIA Farmacéutica S.L.**

Molecular Mechanisms of Aging and Cancer

PRINCIPAL INVESTIGATOR
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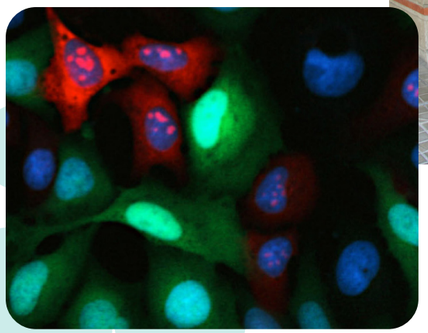
Jiménez Gómez, Lucía

ASSOCIATED INVESTIGATOR
Mayoral Varo, Víctor

UNDERGRADUATE STUDENT
Vaquero Marín, Ignacio
Izquierdo Clemente, Ángel

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



The compound screening revealed a selenazole compound with CRM-1 inhibitory activity. U2redNES and U2foxRELOC cells were treated with CMPD23. After incubation time of one hour, cells were fixed and images were acquired by fluorescent microscopy.

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 anti-cancer and antiviral therapy

Understanding and targeting of FOXO transcription factors in cancer and aging

Researchers involved: Link Wolfgang, Jiménez Gómez Lucía

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 Víctor

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

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:

Vitorino, M.; Pinheiro, G.; Araujo, I. M.; Bibiana, I.; Ferreira, B.I.; Link, W. and Tiscornia, G. Resistance to Carcinogenesis in the African Spiny Mouse (Acomys) correlates with upregulation of tumor suppressor genes. **2024**, DOI: 10.1101/2024.10.24.620065.

Mayoral-Varo, V.; Orea-Soufi, A.; Jimenez, L.; Santos, B.; Serrao, G.; Jociles, M.; Obeso, S.; Ferreira, B.I. and Link, W. The inhibition of three distinct signaling pathways eliminates the oncogenic Tribbles homolog 2 from melanoma cells. *BioRxiv.* **2024**. DOI: 10.1101/2024.07.17.603886.

DOCTORAL THESES AND OTHER WORKS:

Ángel Izquierdo Clemente

"Final degree's project: *Development of a multiplexed system for pharmacological manipulation of FOXO1 and FOXO3*". Universidad Autónoma de Madrid. 2024. Supervisors: Wolfgang Alexander Link and Lucía Jiménez Gómez

Ignacio Vaquero

"Final degree's project: *Estudio de la regulación de TRIB2 por la vía WNT/β-catenina en melanoma*". Universidad Autónoma de Madrid. 2024. Supervisors: Wolfgang Alexander Link and Víctor Mayoral Varo

FUNDING:

"Characterizing and modulating the Tribbles/ AKT/FOXO axis in melanoma (TAFmelanoma). PID2022-136654OB-I00" Ministerio de Ciencia e Innovación. 2023 – 2026

PATENTS:

"*Cellular reporter technology for the use of identifying XPO1 inhibitors that bind in a non-covalent fashion*". Link W, Ferreira B. Patent registration number: 20242005896075. 2024

Drivers and Biomarkers of Metastasis

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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

Biomarkers and Tumor Microenvironment

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KEYWORDS
Colorectal cancer, Tumor Microenvironment, Cancer-Associated Fibroblasts, Biomarkers, 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 both nationally and internationally. Therefore, identifying biomarkers with **clinical applicability** to support **decision-making and patient management** represents a crucial step toward the advancement of **personalized medicine.**

Specific Objectives:

- **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.

We work in c**lose collaboration with clinicians at Ramón y Cajal University Hospital,** ensuring direct insight into **the clinical challenges** faced in managing colon cancer patients. By integrating **molecular and cellular biology** with **translational research,** we maintain a strong **multidisciplinary approach** that prioritizes clinical relevance. Our studies are firmly rooted in **the use of patient-derived clinical samples,** strengthening the translational impact of our findings. Ultimately, our research contributes to **the development of CAF-based biomarkers and targeted therapeutic strategies,** paving the way for novel, CAF-directed treatment approaches in colon

DOCTORAL THESES AND OTHER WORKS:

Macías Gamero, Inés
"Final degree's Project: *Respuesta de los Fibroblastos Asociados a Tumor (CAF) a la radioterapia en el cáncer de colon*". Universidad Alfonso X El Sabio. Biomedicina. 2024. Supervisor/s: Cristina Peña. Grade: Sobresaliente.

FUNDING:
"SBRT radioresistance mediated by Cancer Associated Fibroblasts in oligometastatic Colorectal Cancer Patients. CNS2023-144882". AEI. 2024-2026.

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

"Contratos predoctorales de formación en investigación en salud. FI21/00132". ISCIII. 2022-2024

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

Molecular Mechanisms Involved In Hepatocelular Carcinome Development

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KEYWORDS
Hepatocellular Carcinoma, Fibrosis, Chirrosis, Aurora kinase B, Microbiota, Immunotherapy

RESEARCH LINES:

Overview

Our laboratory is interested in the searching of factors related to the progression and evolution of liver fibrosis and cirrhosis which could be used as predictive hepatocellular carcinoma biomarkers. The project is divided into two main approaches.

AURKB variants as hepatocellular carcinoma biomarkers

Researchers involved: Camblor Murube, Marina.

Hepatocellular carcinoma (HCC) is one of the most common malignancies and the fourth leading cause of cancer-related death worldwide. Hepatitis C virus (HCV) infection has been the major risk factor for liver cirrhosis and alone accounted for 10-25% of HCC cases. Recently, non-alcoholic fatty liver disease (NAFLD) and alcohol misuse have become the foremost reasons for HCC.

Previously, our laboratory has shown that the Hepatitis C virus core protein targets Aurora kinase B (AURKB) in primary hepatocytes and hepatocellular carcinoma cells. This interaction causes a reduction in AURKB activity and the epigenetic and mitotic marker H3Ser10ph affecting cell proliferation and inflammatory response. Significantly, AURKB which regulates segregation and cytokinesis process in mitosis, has been identified as the most significant predictor of aggressive recurrence of HCC in patients after curative hepatectomy. According to these data, microarray analysis revealed the overexpression of several cytokinesis-associated genes in primary HCC. Therefore, AURKB expression has been proposed as an early and late biomarker of HCC.

We are studying the molecular properties of several AURKB variants whose expression has shown alteration in hepatic cell proliferation, DNA damage, chromosome segregation, and cytokinesis. We are performing CRISPR-Cas9 assays to create several cell lines that express those variants. RNA-seq experiments will define the gene expression patterns of those cell lines.

Our group collaborates with clinical groups from several Hospitals in related studies of patients with liver diseases. Two AURKB variants have already been studied in a cohort of patients with chronic hepatitis C. Our results have demonstrated that these AURKB SNPs expression is significantly associated with liver fibrosis progression and HCC outcome. The expression of these two variants, in combination with clinical parameters, could be used to define the risk of HCC development in a specific cohort of patients.

The genetic studies by next-generation sequencing assays of the cell populations expressing AURKB variants might explain

the molecular mechanism inducing fibrosis, cirrhosis, and/or hepatocarcinoma development observed in a cohort of patients infected with the hepatitis C virus.

These studies will be extended to patients with HCC from different etiologies.

Microbiota modifications in HCC patients treated with biological therapy

Researchers involved: Camblor Murube, Marina.

Biological therapy is an effective therapy against cancer that blocks specific targets of tumor cells, such as immune checkpoint blockers such as anti-PD-1 and anti-CTL4 antibodies. Recent research indicates that a significant proportion of patients subjected to this type of therapy undergoes a modification in the composition of the intestinal microbiota, associated with biological therapies. Regarding liver cancer, in 2020, the FDA approved the use of the combination of Nivolumab and Ipilimumab in the treatment of HCC patients previously treated with sorafenib. We are analyzing the effect of biological therapy on the composition of microbiota, and how the variation of populations affects the progression of liver disease. The bacterial translocation suffered by patients with liver cirrhosis related to bacterial overgrowth is of special relevance. A comprehensive study of alterations in the intestinal microbiota and their effect on the host's immune response could contribute to designing innovative treatments for chronic liver disease, including HCC.

DOCTORAL THESES AND OTHER WORKS:

Marina Camblor Murube
"Ph.D. thesis: Variantes de Aurora Quinasa B: nuevos biomarcadores predictivos de desarrollo de carcinoma hepatocelular en pacientes infectados con VHC, tratados con antivirales de acción directa".
Universidad Autónoma de Madrid. *Biociencias Moleculares.* **2024.** Supervisor/s: Aurora Sánchez Pacheco/Antonio Madejón Séiz. Grade: Sobresaliente Cum Laude

Ignacio Fernandez Angel
"Master´s thesis: Análisis de miRNAs en heces como biomarcadores de cáncer de pulmón, ovario y mama". Universidad Autónoma de Madrid. *Biomedicina.* **2024.** Supervisor/s: Aurora Sánchez Pacheco. Grade: 8,5

Alicia Diez Gutierrez
"Final degree's project: Variantes alélicas de AURKB implicadas en el desarrollo de hepatocarcinoma "
Universidad Autónoma de Madrid. *Bioquímica.* **2024.** Supervisor/s: Aurora Sánchez Pacheco.Grade: 9,3

Olalla Bartolome Roselló
"Final degree's project: "El ayuno intermitente desde un punto de vista clínico, nutricional y metabólico". Universidad Autónoma de Madrid. *Nutrición humana y Dietética.* **2024.** Supervisor/s: Aurora Sánchez Pacheco. Grade: 8,5

FUNDING:

"Analysis of music on cell behavior. PID2436". MICINN. 2016-2019TÍTULO DEL PROYECTO: Desarrollo de sistemas de cribado para detección precoz de cáncer y prevención de efectos secundarios asociados a los tratamientos con inmunoterapia. IP: Aurora Sánchez Pacheco. Universidad Autónoma de Madrid. 2024-2025.

"Formulas nutricionales para el control del déficit de ácido docosahexaenoico DHA y ácido araquironico AA en niños extremadamente prematuros". IP: Miguel Saenz de Pipaon. (Hospital Universitario la PAZ). Ramon Areces. XX Concurso Nacional de Investigación en Ciencias de la Vida y la Materia. 09/2021-09/2023. Extended May 2025.

PATENTS:

"A stem-loop primer and a method for short length RNA detection". Sánchez-Pacheco A , López-Lopez A, Camblor Murube M. EP22383065.4 November 7, 2022. Presented in the European patents office (EP0) 24/10/2024.

Melanoma Plasticity in Metastasis and Immunotherapy Resistance

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

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. This unprecedented preclinical platform will allow us to perform functional studies in fully immunocompetent conditions and to overcome the limitations of sample collection in patients. Understanding how melanoma cells orchestrate TME remodeling and brain colonization will provide the rationale for the discovery of new, more effective therapeutic strategies.

Melanoma plasticity role in immunotherapy resistance

Since immune checkpoint blockade (ICB) became the first-line treatment for melanoma, extensive efforts have been invested to understand and additionally target the immune populations involved. However, much less is known about the melanoma cell intrinsic

pathways determining clinical response to ICB. Melanoma arises from the transformation of melanocytes, pigment-producing cells that originate from neural crest progenitors during embryonic development. The ability of melanoma cells to phenotypically switch between melanocytic developmental states, named melanoma plasticity, plays a crucial role in metastatic progression and drug resistance. Although these states are associated with pleiotropic functions essential for melanoma, their contribution to immune modulation is still unclear. Recent work from our group and others have found evidence correlating melanoma plasticity and resistance to ICB, however, the underlying mechanisms remain unknown.

Animal models have demonstrated to be fundamental for understanding the molecular mechanisms underlying therapeutic efficacy. Our research at the National Institutes of Health (NIH) established a panel of melanoma mouse models (M1-M5) etiologically relevant and representative of distinct patient molecular subtypes. These models exhibit a broad range of responses to ICB, associated with specific intratumoral lymphoid and myeloid cell densities that mimic the distribution observed in patients. By the comparative analysis of the gene expression profiles of M1-M4 models and the cross validation with human data sets we identified a "Melanocytic Plasticity Signature" (MPS) that predicts patient outcome upon ICB (Pérez-Guijarro et al., 2020. Nat Med. DOI: 10.1038/s41591 020-0818-3). This predictive signature (MPS) directly linked, for the first time, melanoma multipotency and undifferentiated phenotypes with the clinical resistance to immunotherapy, highlighting the translational value of these models. Expanding on the understanding of embryonic melanocytic development, a study by Marie et al, (Marie et al., 2020. Nat Commun. doi: 10.1038/s41467-019-14085 2) demonstrated the function of these factors in metastasis. These findings provided the premises for a follow up study that defined distinct developmental programs and their dynamics along metastasis progression and immunotherapy treatment by the comparative analysis of the models described in Pérez-Guijarro et al., 2020 and patient data sets (Gopalan et al., 2022. BioRxiv 10.1101/2022.10.14.512297).

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 selected M4 to investigate ITH dynamics in response to immunotherapy due to its mixed ICB responses and high genetic and phenotypic heterogeneity by histological and single-cell RNA sequencing (scRNAseq) analysis. We generated an array of 24 single-cell-derived sublines from M4 cell line exhibiting a wide range of mutational landscapes, developmental states, tumor growth kinetics and ICB responses. Genomic and scRNAseq analyses uncovered the diversity of the sublines and evidenced their plasticity. Further inquiry into melanocytic developmental states and stromal immune cell signatures demonstrated better ICB efficacy in highly inflamed and differentiated melanomas (Gruen et al. 2023. BioRxiv.10.1101/2023.04.03.535074).

In addition, our work generated multi-omics mouse data sets that were used by our collaborators to develop a computational method for the phylogenetic analysis of large scale genomic and transcriptomic single-cell data from tumors (Azer et al., 2020. Bioinformatics. DOI: and Rashidi Mehrabadi et al., 2023. BioRxiv. 2021.03.26.437185). Therefore, our models have emerged as benchmarking tools to develop these and other computational methods (Kizilkale, et al., 2022. Nat Comput Sci. DOI:10.1038/s43588-022 00298-x). Overall, our approach proved to be a powerful platform to study the interactions between melanoma cell intrinsic programs and environmental factors that drive cancer evolution along progression and in response to therapy.

Tumor microenvironment and brain metastasis

As systemic therapies improve, BrM is increasingly a leading cause of cancer patient mortality. While the brain was a paradigm of an immune-privilege microenvironment, accumulating evidence suggests that ICB treatment could significantly benefit BrM patients, achieving response rates close to those with extracranial disease. Recent studies demonstrated that melanoma co-option of developmental pathways enhances the metastatic potential of human and mouse cells. Whether melanoma plasticity plays a role in colonization of the unique brain microenvironment is still unknown. Brain parenchyma is an extremely complex ecosystem of highly specialized cell types whose crosstalk is essential to maintain homeostasis; and understanding how melanoma cells modulate their interaction is fundamental. The overarching goal of this line of research is to understand the molecular mechanisms of immune evasion by melanoma cells determining brain colonization and therapeutic resistance.

Sample collection is especially challenging in BrM patients, highlighting the importance of preclinical models for the discovery of key molecular drivers and predictive biomarkers. Unfortunately, very few animal models of brain metastasis exist and in most the immune system is compromised, precluding evaluation of immunotherapy. To address this deficiency, we have generated a panel of brain-metastatic cells by intracardiac injection that exhibits diverse histopathology and metastatic potential. Importantly, mice implanted with M4-BR1 or M4-BR3 sublines responded differently to anti-CTLA4 and anti-PD-L1 mono- and combination therapies. The results of this study will be soon published, and a pre-print of the manuscript is available in

PUBLICATIONS:

Hirsch, M.G.; Pal, S.; Mehrabadi, F.R.; Malikic, S.; Gruen, C.; Sassano, A.; Pérez-Guijarro, E.; Merlino, G.; Sahinalp, C.; Molloy, E.K.; Day, C.P.; Przytycka, T.M. Stochastic modelling of single-cell gene expression adaptation reveals non-genomic contribution to evolution of tumor subclones. *bioRxiv [Preprint].* **2024,** *Apr 20:2024.04.17*.588869. DOI: 10.1101/2024.04.17.588869.

Daugherty-Lopès, A.; Pérez-Guijarro, E.; Gopalan, V.; Rappaport, J.; Chen, Q.; Huang, A.; Lam, K.C.; Chin, S.; Ebersole, J.; Wu, E.; Needle, G.A.; Church, I.; Kyriakopoulos, G.; Xie, S.; Zhao, Y.; Gruen, C.; Sassano, A.; Araya, R.E.; Thorkelsson, A.; Smith, C.; Lee, M.P.; Hannenhalli, S.; Day, C.P.; Merlino, G.; Goldszmid, R.S. Immune and molecular correlates of response to immunotherapy revealed by brain-metastatic melanoma models. *bioRxiv [Preprint].* **2024**, *Oct 9:2024.08.26*.609785. DOI: 10.1101/2024.08.26.609785.

DOCTORAL THESES AND OTHER WORKS:

María Villoro Agud
"Master´s thesis: *Mechanisms of immune response evasion in brain metastatic melanoma due to interactions with microglia*". Universidad Autónoma de Madrid. Medicina. 2024. Supervisor/s: Eva Pérez Guijarro. Grade: Sobresaliente (9.5)

Silvia Novo Acedo
"Master´s thesis: *Studying the regulation of non-muscle myosin ii cytoskeleton during adaptation and resistance to mapk-targeted therapy in cutaneous melanoma*". Universidad Autónoma de Madrid. Medicina. 2024. Supervisor/s: José Luis Orgaz Bueno y Eva Pérez Guijarro. Grade: Sobresaliente (9.3)

Laura Manso Pérez
"Final degree's project: *Generación de un sistema reportero para el estudio de la plasticidad celular del melanoma*". Universidad Autónoma de Madrid. Medicina. 2024. Supervisor/s: Eva Pérez Guijarro. Grade: Sobresaliente (9.5)

Javier Ramos Gómez
"Final degree's project: *Modulación de los fenotipos de microglía por células de melanoma metastásicas cerebrales*". Universidad Autónoma de Madrid. Medicina. 2024. Supervisor/s: 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

"Dissecting melanoma brain metastasis and response to immunotherapy. MRA-YIA#1037420". Melanoma Research Alliance (MRA). 2023-2026

"Función de la plasticidad celular del melanoma en metástasis y resistencia a inmunoterapia. PID2022-141113OA-I00". AEI. MICINN. 2023-2026

Translational Research in Breast and Gynecological Cancer (Ovarian and Endometrial Cancer)

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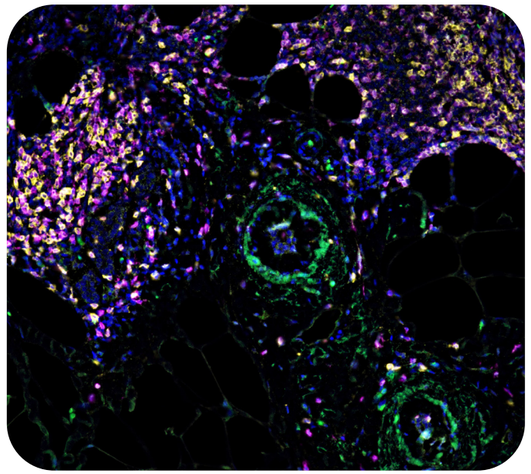
KEYWORDS
Breast Cancer, Gynecological Cancer, Prognosis, Molecular Classification, Immunotherapy, Targeted Therapies.



RESEARCH LINES:

Overview

The group main research interests are to provide deeper knowledge on the mechanisms of drug resistance, tumor progression and metastasis, mainly in breast and gynecological tumors, as well as to develop innovative therapies against the novel molecular targets previously characterized by our group. The interdisciplinary team led by Dr. Gema Moreno-Bueno includes oncologists, pathologists, biochemists, and molecular biologists with a translational experience on the characterization of new prognostic/ predictive cancer biomarkers and molecular targets. As an example, we have decoded a new resistance mechanism to HER2 drugs (antibodies and kinase inhibitors) and subsequently developed novel and effective treatment strategies (including nanomedicines and targeted therapies) for eradicating aggressive tumor cells. In addition, our ambitious translational projects combine state-of the-art molecular approaches (including the characterization of genetic landscape and intratumor heterogeneity) with in vivo approaches (such as novel preclinical models useful to test new treatment options) and comprehensive analyses in clinical tumor series. Thus, our goal is to advance in the personalized oncology and the cancer care management. The group has solid collaborations with several research groups (from CIBERONC, other CIBER areas, as well as from diverse international institutions) that contribute to increase the multidisciplinary and translational potential of our projects. Finally, the group has active participation in diverse CIBERONC sections, such as the Working Module on Experimental Models, the Training and Mobility Program (which is presently coordinated by Dr. Gema Moreno-Bueno), breast and gynecological tumor program (co-coordinated by Dr Gema Moreno Bueno) and the Scientific CIBERONC committee.

Identification and characterization of new resistance mechanisms in breast carcinomas (HER2+).

Researchers involved: Moreno-Bueno, Gema; Sarrió-López, David

The first line of research is based on the analysis and functional characterization of HER2 amplicon genes, which led to the identification of a new marker, Gasdermin B (GSDMB), associated with invasion and metastasis in breast cancer and whose amplification in Her2 tumors is related to non-response to antiHER2 therapies. GSDMB belongs to a family of proteins recently linked to cell death processes. Our current studies are directed to:

- To characterize the involvement of GSDMB in pro-survival autophagy mechanisms in response to anti-HER2 and other drug treatments in breast and gastric carcinoma.

- Characterize the relationship between GSDMB and inflammation in different pathological contexts.
- Development of new anti-GSDMB therapeutic protocols based on the development of biocompatible nanoparticles loaded with an anti-GSDMB antibody.
- Repositioning of new targeted drugs in HER2 breast tumors.

Analysis of intratumoral heterogeneity in cancer

Researchers involved: Moreno Bueno, Gema; Sarrió López, David

The second line of research is centered on the study of clonal evolution in cancer, especially focused on:

- Identify specific patterns of phylogenetic evolution in various tumor types by massive exome and targeted sequencing of regions of the primary tumor and metastatic lesions.
- Validation of phylogenetic patterns and specific clones by sequencing in circulating tumor cells.
- Characterizing complex preclinical models for testing more effective and less toxic therapeutic regimens in gynecological tumors.
- Deciphering new diagnostic tools for improving the risk of recurrence in endometrial cancer using AI.

PUBLICATIONS:

Casas-Arozamena, C.; Vilar, A.; Cueva, J.; Arias, E.; Sampayo, V.; Diaz, E.; Oltra, S.; Moiola, C.; Cabrera, S.; Cortegoso, A.; et al. Role of cfDNA and ctDNA to improve the risk stratification and the disease follow-up in patients with endometrial cancer: towards the clinical application. *Journal of experimental & clinical cancer research.* **2024**, *43 (1).* DOI: 10.1186/s13046-024-03158-w.

González-Martínez, S.; Kajabova, V.; Pérez-Mies, B.; Carretero-Barrio, I.; Caniego-Casas, T.; Sarrió, D.; Moreno-Bueno, G.; Gión, M.; Perez-García, J.; Cortés, J.; et al. CDH1 methylation analysis in invasive lobular breast carcinomas with and without gene mutation. *Virchows archiv.* **2024**, *485 (2),* 291-297. DOI: 10.1007/s00428-024-03814-8.

Marugán, C.; Sanz-Gómez, N.; Ortigosa, B.; Monfort-Vengut, A.; Bertinetti, C.; Teijo, A.; González, M.; de la Vega, A.; Lallena, M.; Moreno-Bueno, G.; et al. TPX2 overexpression promotes sensitivity to dasatinib in breast cancer by activating YAP transcriptional signaling. *Molecular oncology.* **2024**, *18 (6),* 1531-1551. DOI: 10.1002/1878-0261.13602.

Moreno-Moreno, E.; Caniego-Casas, T.; Carretero-Barrio, I.; Cortes, A.; Muriel, A.; Dominguez-Rullan, J. A.; Martin-Gromaz, C.; Moreno-Bueno, G.; Matias-Guiu, X.; Palacios, J.; et al. Histologic and Molecular Type Changes in Endometrial Cancer Recurrences in Comparison With Their Corresponding Primary Tumors. *The American journal of surgical pathology.* **2024**. DOI: 10.1097/PAS.0000000000002308.

Morillo-Bernal, J.; Pizarro-García, P.; Moreno-Bueno, G.; Cano, A.; Mazón, M.; Eraso, P.; Portillo, F. HuR (ELAVL1) Stabilizes SOX9 mRNA and Promotes Migration and Invasion in Breast Cancer Cells. *Cancers*. **2024**, *16 (2).* DOI: 10.3390/cancers16020384.

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Rosas-Alonso, R.; Colmenarejo-Fernández, J.; Pernía, O.; Burdiel, M.; Rodríguez-Antolín, C.; Losantos-García, I.; Rubio, T.; Moreno-Velasco, R.; Esteban-Rodríguez, I.; Martínez-Marín, V.; et al. Evaluation of the clinical use of MGMT methylation in extracellular vesicle-based liquid biopsy as a tool for glioblastoma patient management. *Scientific reports.* **2024**, *14 (1).* DOI: 10.1038/s41598-024-62061-8.

Sarrio, D.; Colomo, S.; Moreno-Bueno, G. Gasdermin-B (GSDMB) takes center stage in antibacterial defense, inflammatory diseases, and cancer. *Febs journal.* **2024**, *291 (14),* 3060-3071. DOI: 10.1111/febs.17018.

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DOCTORAL THESES AND OTHER WORKS:

Javier Paulino Sánchez
"Final degree's project: *Estudio de polimorfismos intragénicos y su efecto sobre la expresión diferencial de isoformas de Gasdermina-B (GSDMB) en líneas de cáncer*". Universidad Autónoma de Madrid. Medicina. 2024. Supervisor/s: Gema Moreno Bueno. Grade: Matrícula de Honor

Carolina Silvestre Muniz
"Final degree's project: *Estudio de las interacciones estructurales potencialmente implicadas en la conformación de Gasdermina-B*". Universidad Autónoma de Madrid. Medicina. 2024. Supervisor/s: Gema Moreno Bueno. Grade: Sobresaliente

FUNDING:

"Impact of the functional crosstalk between GSDMB and GSDMD in HER2 breast cancer genesis, progression, and therapy response (CrossGasdermin). PID2022-136854OB-I00". AEI. 2023-2026.

"Validation and valorization of a new gene therapy, nanoGBtox, for the treatment of cancer. PDC2022-133252-I00". AEI. 2023-2025

"Exploring the Feasibility of predictive and pharmacodynamics biomarkers of immunotherapy in solid tumors (Immune4ALL). PMP22/00054" ISCIII. 2022-2025

"Nuevas estrategias de inmunoterapia para el tratamiento del cáncer de ovario (IMCOV). PMPTA22/00076" ISCIII. 2022-2025

"Personalized Clinical Management of Endometrial Cancer using Liquid Biopsy,Genomics and Artificial Intelligence, ECLAI. ERAPERMED2021-076 ECLAI. PERME212426MORE" FCAECC/ Instituto de Salud Carlos III (ISCIII). 2022-2024.

"Targeting the most aggressive type of endometrial carcinoma. GC-TRA18014MATI". Fundación Científica Asociación Española Contra el Cáncer (FCAECC). 2018-2024

"Consortium CIBERONC. Cancer Area. Breast Cancer Program. CB16/12/00295" ISCIII. 2017-

Cellular Senescence in Cancer and Other Pathologies.

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KEYWORDS
Cell senescence, Cancer, Plasticity, Development.

RESEARCH LINES:

Overview

Cellular senescence is a complex program characterized by a stable cell cycle arrest and an exacerbated secretory phenotype, implicated 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 tumor suppressor barrier that prevents tumor initiation, by blocking the proliferation of cells with potentially oncogenic alterations. Conversely, the accumulation of senescent cells in tumors, due to therapeutical interventions or other factors is usually detrimental, being associated to increased tumor 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 to pursue our lines of research.

Senescence in 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 that is linked to mutations in the SIX/EYA regulatory pathway. Considering the key role of SIX1 in adult cellular senescence shown in our lab (Adrados et al, Oncogene, 2016, PMID 26500063, De Lope et al, Sci Rep, 2019, PMID 30723235)) and the relevance of programmed senescence during development, we have used Six1-deficient mice, an animal model of the human BOR syndrome, to explore the link between senescence and developmental disease. Our recent work (De Lope, Development, 2023, PMID 37017267) demonstrated that morphogenic

defects in the inner ear characteristic of this syndrome are associated to the disruption of the physiological senescence program in this organ. These results support the notion that mis-regulation of embryo senescence may lead to genetic developmental disorders. During this period, we have continued the study of normal and aberrant developmental senescence, using a combination of histological and advanced transcriptomics approaches.

Senescence, the 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 or SASP. The SASP plays a central role in the communication of senescent cells with surrounding cells and their physical environment, and is a key mediator of local and systemic cell-extrinsic functions of senescence. Our recent work revealed a link between senescence and the regulation of cell plasticity in fibroblasts, involving dynamic changes in the senescent secretome (Lopez-Antona et al, Aging Cell, 2022, PMID 35266275). During this period, we continued to explore the role of the senescent secretome in cell-extrinsic effects of senescence. To do this, we have established and characterized a set of senescence cellular models, including tumor and non-tumor cells, in order to study the impact in SASP function of cell type, senescence trigger, kinetics and regulatory pathways. We are currently leveraging this resource to dissect the functional role of SASP components, focusing on the cross-talk between tumor and non-tumor cells in cancer.

PUBLICATIONS:

Flores, N. G.; Fernandez-Aroca, D. M.; Garnes-Garcia, C.; Dominguez-Calvo, A.; Jimenez-Suarez, J.; Sabater, S.; Fernandez-Aroca, P.; Andres, I.; Cimas, F. J.; de Carcer, G.; Belandia, B.; Palmero, I.; Huertas, P.; Ruiz-Hidalgo, M. J.; Sanchez-Prieto, R., The CDK12-BRCA1 signaling axis mediates dinaciclib-associated radiosensitivity through p53-mediated cellular senescence. *Mol Oncol.* **2024**.

DOCTORAL THESES AND OTHER WORKS:

Laura Arranz Ortega
"Final degree´s project: *Caracterización de un modelo celular de senescencia inducida por terapia*". Universidad de Alcalá de Henares. 2024. Supervisor: Ignacio Palmero. Grade: Sobresaliente

Alejandro Guerrero Maldonado
"Master´s thesis: *Cell senescence and plasticity in cancer: Studying the link between senescence and cancer stemness*". Universidad Autónoma de Madrid. 2024. Supervisor: Ignacio Palmero. Grade: Sobresaliente

FUNDING:

"Senescencia y plasticidad celular. PID2021-122600OB-I00".
AEI. 2021-2024.

"Senescencia celular en fisiología y enfermedad. P2022/BMD-7393".
Comunidad de Madrid. 2023-2027.

SCIENTIFIC REPORT 2024

department

of Metabolic & Immune Diseases

Cell Compartmentalization, Homeostasis and Inflammation

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TECHNICAL SUPPORT PERSONNEL
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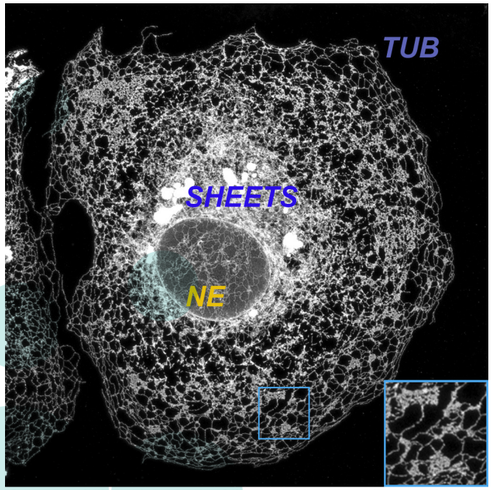
KEYWORDS
Organelles, Secretory Apparatus, Cell stress, Systems biology, Cell defense.



Superresolution micrograph of the endoplasmic reticulum of a COS7 cell, stably expressing a Sec61b-EGFP marker. Three generic domains (NE: nuclear envelope; SHEETS: perinuclear stacked sheets, TUB: peripheral tubular networks) are indicated.

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.

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-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. 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. We also participate in studies focused on caveolae biology and their contribution to cell function and physiopathology.

PUBLICATIONS:

Aboy-Pardal, M. C. M.; Guadamillas, M. C.; Guerrero, C. R.; Català-Montoro, M.; Toledano-Donado, M.; Terrés-Domínguez, S.; Pavón, D. M.; Jiménez-Jiménez, V.; Jimenez-Carretero, D.; Zamai, M.; Folgueira, C.; Cerezo, A.; Lolo, F.-N.; Nogueiras, R.; Sabio, G.; Sánchez-Álvarez, M.; Echarri, A.; Garcia, R.; Del Pozo, M. A. Plasma Membrane Remodeling Determines Adipocyte Expansion and Mechanical Adaptability. *Nat Commun.* **2024**, *15 (1),* 10102. DOI: 10.1038/s41467-024-54224-y.

Terri, M.; Sandoval, P.; Bontempi, G.; Montaldo, C.; Tomero-Sanz, H.; de Turris, V.; Trionfetti, F.; Pascual-Antón, L.; Clares-Pedrero, I.; Battistelli, C.; Valente, S.; Zwergel, C.; Mai, A.; Rosanò, L.; del Pozo, M. Á.; Sánchez-Álvarez, M.; Cabañas, C.; Tripodi, M.; López-Cabrera, M.; Strippoli, R. HDAC1/2 Control Mesothelium/Ovarian Cancer Adhesive Interactions Impacting on Talin-1-A5β1-Integrin-Mediated Actin Cytoskeleton and Extracellular Matrix Protein Remodeling. *J. Exp. Clin. Cancer Res.* **2024, 43 (1),** 27. DOI: 10.1186/s13046-023-02930-8.

DOCTORAL THESES AND OTHER WORKS:

Lucía Valdés Vaquero
"Master´s thesis: *Development of stable cell lines through CRISPR/Cas9 editing for the characterization of novel genes related to the innate immune response*.". Universidad Autónoma de Madrid. Biotechnology. 2024. Supervisor/s: Miguel Sánchez Álvarez, Susana Guerra. Grade: 8.4

Rachele Guerreschi
"Final degree's project: *Cell biology tools for the study of organelle dynamics as regulator immunity and metabolism.*". Università degli Studi di Parma. Biochemistry. 2024. Supervisor/s: Miguel Sánchez Álvarez. Grade: 9.2

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. 2024-2027

"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. 2024-2026

"Novel mechanisms coupling cell secretion with inflammation control: physiopathological relevance (SECRETMMUNE) «Proyectos de Generación de Conocimiento» PID2021-128106NA-I00". MICINN. 2022-2025

"Cell organelles as signaling hubs in disease: novel roles in mechanoadaption and innate immunity «Ramón y Cajal contract» RYC2020-029690-I". MICINN. 2022-2027

PATENTS:

"CXCL8 INHIBITORS FOR USE IN THE TREATMENT OF TUMORS WITH LOW STROMAL CAV1 LEVELS". del Pozo MA, Díez A, Sánchez-Álvarez M, Dompé farmaceutici. PCT/EP2024/083939. 2024

Transcriptional Control of Metabolic Homeostasis

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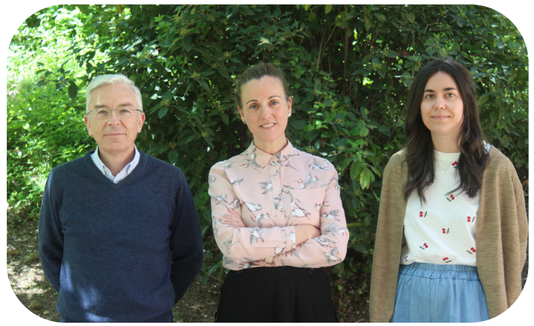
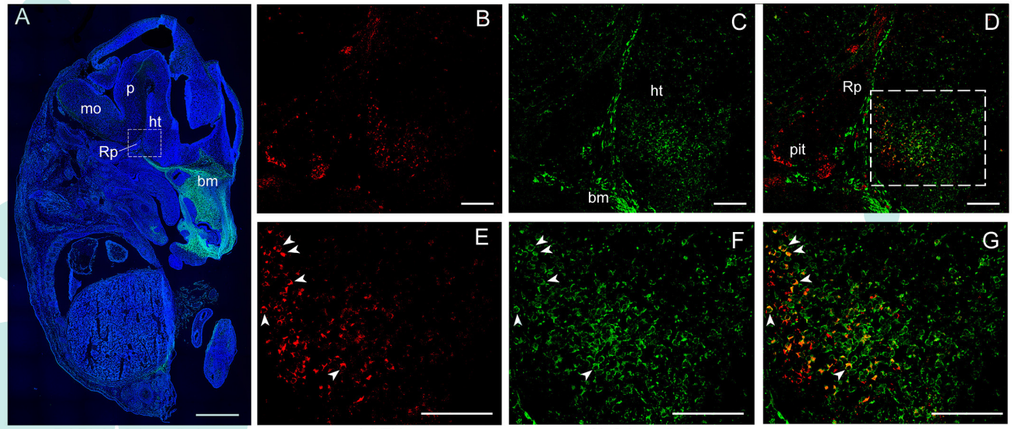
KEYWORDS
Metabolic homeostasis, Diabetes, Pancreatic islets, Hypothalamus, Energy expenditure.





Expression of Alx3 in the hypothalamic primordium during mouse embryonic development.

RESEARCH LINES:

Overview

During the last several years we have been engaged in the study of the mechanisms that maintain metabolic homeostasis. From a functional point of view, these mechanisms require the involvement of peripheral organs such as pancreas, muscle and adipose tissue, as well as brain nuclei mostly located in the hypothalamus, all of them acting in a coordinated manner. Although a great deal of our past work has been focused on pancreatic islets, during 2024 we focused on studies initiated earlier on the regulatory role of Alx3 in hypothalamic regulation of metabolism.

Hypothalamic regulation of energy metabolism by Alx3

We found that Alx3 plays an important role in the regulation of metabolic homeostasis at the systemic level, not only by modulating the responses of pancreatic islets to changes in glucose concentrations, but also by regulating feeding and metabolic partitioning in peripheral organs including muscle and adipocytes from the hypothalamic arcuate nucleus. In the course of studies intended to carry out a comprehensive assessment of metabolic alterations in Alx3 deficiency, we found that 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. To our surprise, 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.

During this period, following on the previous results, we characterized the expression of Alx3 in the arcuate 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.

PUBLICATIONS:

Mirasierra, M., Fernández-Pérez, A., Lizarbe, B., Keiran, N., Ruiz-Cañas, L., Casarejos, M.J., Cerdán, S., Vendrell, J., Fernández-Veledo, S., Vallejo, M. Alx3 deficiency disrupts energy homeostasis, alters body composition, and impairs hypothalamic regulation of food intake. Cell. and Mol. *Life Sci.* **2024,** *81:* 343. DOI: 10.1007/s00018-024-05384-z.

DOCTORAL THESES AND OTHER WORKS:

Paula Pereira Bouzas
"Ph.D. thesis: *Disfunción mitocondrial inducida por diabetes como mecanismo de vulnerabilidad de las neuronas dopaminérgicas nigroestriatales*". Universidad Autónoma de Madrid. Medicina. 2024. Supervisor/s: Mario Vallejo y Mercedes Mirasierra. Grade: Sobresaliente

FUNDING:

"Complicaciones emergentes del desajuste de la homeostasis glucémica. PID2020-117640RB-I00". MICINN. 2021-2024

"Desregulación multinivel de la secreción de insulina como mecanismo patogénico de disfunción de los islotes pancreáticos en la diabetes. PID2023-150719OB-I00". MICIU 2024-2027.

Beta Cell Mass and Pancreatic Islet Development

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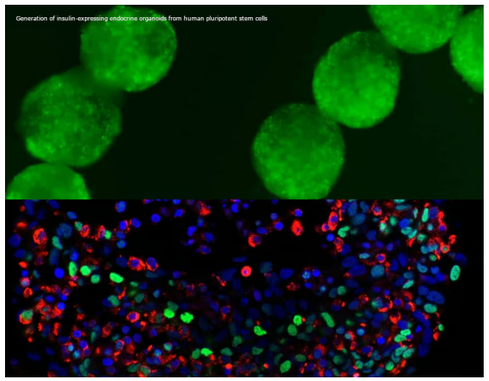
KEYWORDS
Diabetes, Pluripotent stem cells, Differentiation protocols, Pancreatic beta cells, Insulin.



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:

Suda, N.; Bartolomé, A.; Liang, J.; Son, J.; Siebel, C.; Accili, D.; Ding, H.; Pajvani, U.B. β-cell Jagged1 is sufficient but not necessary for islet Notch activity and insulin secretory defects. *Mol Metab.* **2024.** *81*. 101894. DOI: 10.1016/j.molmet.2024.101894

DOCTORAL THESES AND OTHER WORKS:

Mario Hernanz Martín
"Master's thesis: *Characterization of KLF11 R120G mutation in MODY7 appearance during pancreas development*". Universidad Autónoma de Madrid. Master's degree in Molecular Biomedicine. 2024. Supervisor: Alberto Bartolomé.

Andrés Cristian Donoso Osorio
"Final degree's project: "*Diferenciación de células pluripotentes humanas hacia el linaje pancreático y edición genética (CRISPR) para el estudio de genes asociados a diabetes*". Universidad Autónoma de Madrid. Biología. 2024. Supervisor: Alberto Bartolomé.

Saphira Block
"Final degree's project: "*The role of NEUROD1 in monogenic diabetes*". Universidad Autónoma de Madrid. Bioquímica. 2024. Supervisor: Alberto Bartolomé.

FUNDING:

"Programa de atracción de talento 2020/2021. Modalidad 1. Contratación de doctores con experiencia (2020-T1/BMD-20162)". Comunidad Autónoma de Madrid. 2021-2026

"Bases genéticas de la masa de célula beta. PID2021-122284NA-I00". AEI, MICIU. 2022-2025.

"Modelado del desarrollo pancreático en diabetes monogénicas mediante el uso de células madre humanas". Sociedad Española de Diabetes. 2024-2025.

"Impacto de las variantes comunes y raras de NOTCH2 en la masa de célula beta y riesgo de diabetes. CNS2023-145179." AEI, MICIU. 2024-2026

FUNDING:

"Premio a la Mejor Comunicación de Investigación Básica presentada en el XXXV Congreso de la Sociedad Española de Diabetes". Alberto Bartolomé. 2024

Physiopathology and Molecular Mechanisms of Obesity and Comorbidities

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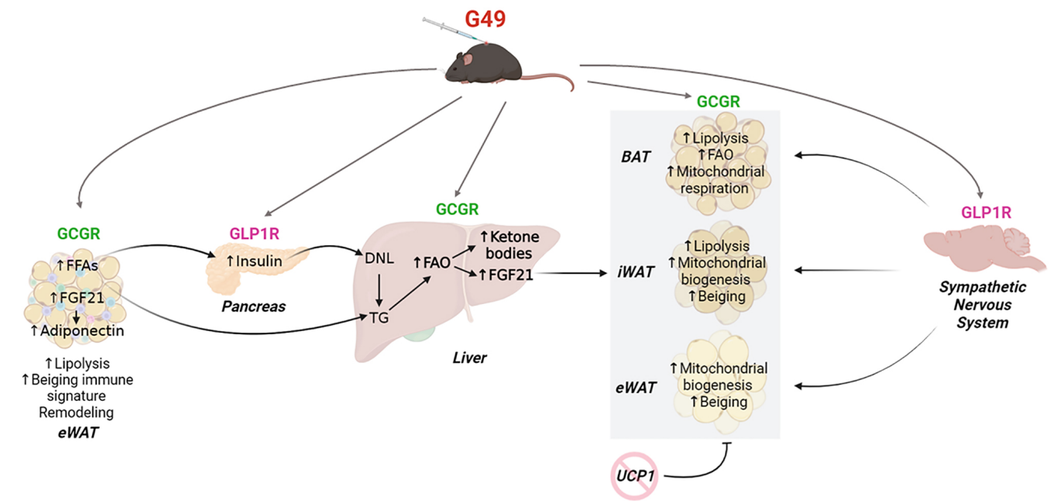
KEYWORDS

Obesity, Type 2 diabetes, Metabolic dysfunction-associated steatotic liver disease, Hepatotoxicity, Extracellular vesicles, Second generation antipsychotics



Schematic representation of the inter-organ crosstalk triggered by G49 that leads to increased ebergy expenditure and weight loss

RESEARCH LINES:

Overview

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) and metabolic dysfunction-associated steatotic liver disease (MAFLD). The changes in the intestinal microbiota that occur in obese individuals are the first trigger of the low-grade chronic inflammation that alters the functions of relevant tissues responsible for controlling whole body glucose and lipid homeostasis. Among them, 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 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), 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 single or dual agonists of the GLP-1 and glucagon 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 insulin sensitivity in the liver: protein kinase D family

Researchers involved: Rada, Patricia; Carceller, Elena; Hitos, Ana B.; Pose-Utrilla, Julia; González Rodríguez, Águeda; Iglesias, Teresa; Martínez Valverde, Ángela.

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 insulin sensitivity has not been fully elucidated and is the focus of this research line. To achieve this, we conducted studies in hepatic cells and also we have generated liver-specific PKD2-deficient mice (PKD2ΔHep), as well as mice overexpressing a constitutive active PKD2 mutant in the liver (EGFP-PKD2-CA). We found that PKD2 silencing enhanced insulin signaling in hepatocytes, an effect also observed in primary hepatocytes from PKD2ΔHep mice. Conversely, EGFP-PKD2-CA overexpression induced the opposite effects. A more in-depth analysis revealed reduced levels of IRS1 serine phosphorylation under basal conditions and increased IRS1 tyrosine phosphorylation in PKD2ΔHep primary hepatocytes upon insulin stimulation and, importantly, we demonstrated that PKD2 interacts with IRS1. In vivo injection of AAV-EGFP-PKD2-CA in male mice resulted in a moderate impairment of glucose homeostasis and reduced insulin signaling. On the contrary, obese PKD2ΔHep male mice displayed improved glucose and pyruvate tolerance, as well as higher insulin sensitivity compared to their controls. This research line has unveiled an unknown role of PKD2 in the control of insulin signaling in the liver at the level of IRS1 and point PKD2 as a therapeutic target for hepatic insulin resistance.

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

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

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 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 interactome in MASLD and T2D with diagnostic potential

Researchers involved: García-Martínez, Irma; Alen, Rosa; Cañete, Héctor; Olivera, Ángela; Izquierdo-Pastor, Manuel; Martínez Valverde, Ángela.

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 hepato-

cyte-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: Valdecantos, M. Pilar; Rada, Patricia; Hitos, Ana B; García-Martínez, Irma; Alén, Rosa; González Rodríguez, Águeda; Montes, Ángela; Martínez Valverde, Ángela.

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 G49, an oxyntomodulin (OXM) analog and GCGR/GLP-1R dual agonist, in preventing diet-induced obesity and its underlying molecular mechanisms. We are particularly focused in the spatio-temporal metabolic rewiring in response to G49 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. Elevation of OXM, under basal and postprandial conditions, and similar metabolic adaptations after G49 treatment were found in plasma from patients with obesity early after metabolic bariatric surgery. These results identify G49 as a potential pharmacological alternative sharing with bariatric surgery hormonal and metabolic pathways.

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: ITN-TREATMENT

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

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. We aim to uncover new mechanistic aspects in the cross-talk between the central nervous system, particularly the hypothalamus, and metabolic tissues in the periphery as well as sex-specific differences. In particular, we addressed OLA i.p. treatment effects in WT and PTP1B-KO female mice. Contrarily to our previous results in WT females receiving OLA orally, the i.p. treatment did not induce weight gain or hyperphagia. Molecularly, in females OLA failed to diminish hypothalamic phospho-AMPK or elevate BAT UCP-1 and energy expenditure (EE) despite the preservation of iWAT browning. Conversely, OLA i.p. treatment in ovariectomized mice reduced hypothalamic phospho-AMPK, increased BAT/iWAT UCP-1 levels and EE, and induced weight loss as occurred in males. Pretreatment of hypothalamic neurons with 17β-estradiol (E2) abolished OLA effects on AMPK activation. Moreover, neither hypothalamic JNK activation nor hepatic FAS upregulation were found in WT and PTP1B-KO females receiving OLA via i.p. importantly, this axis was reestablished upon ovariectomy. In this line, E2 prevented OLA-induced phospho-JNK in hypothalamic neurons. These results support the role of estrogens in sex-related dimorphism in OLA treatment. This study evidenced the benefit of OLA i.p. administration in preventing its obesogenic effects in female mice that could offer clinical value.

Impact of Bone Morphogenetic Proteins (BMPs) on the progression of MASLD

Researchers involved: Hernández, Ivana; Rada, Patricia; Martínez Valverde, Ángela; González Rodríguez, Águeda.

Although MASLD is the major cause of chronic liver disease worldwide, there is no validated non-invasive method to identify patients with MASH, and there are no proven effective treatments. BMPs are growth factors that exert pleiotropic role in different cellular processes, but their involvement in MASLD pathogenesis has poorly been investigated, so this research line

aims to identify the molecular mechanisms involved in the effects of these proteins in the progression of MASLD, and to test whether these proteins, which are soluble factors, can be used as non-invasive biomarkers for the diagnosis/prognosis of the different stages of this disease. In this regard, we have described a significant and progressive increase in serum BMP2 levels in MASLD patients in relation to the histological grade of steatosis and MASLD activity, positioning this protein as a potentially useful non-invasive biomarker for MASH. A phylogenetically analysis conferred high similarity between BMP2 and GDF7, also known as BMP12, sharing 55% amino acid sequence identity, so it has been suggested that BMP12 can exert similar functions as BMP2. However, there are currently no studies exploring BMP12 in the context of MASLD, which could be of great relevance in light of previous studies on other BMPs, particularly BMP2. We have demonstrated that BMP12 expression is enhanced in liver from different preclinical models of fibrosis. Moreover, hepatic levels of BMP12 are increased in MASH fibrotic patients; nevertheless, no difference exits at circulating levels of BMP12. Currently, we are evaluated BMP12 impact on liver fibrogenesis.

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

Researchers involved: Pérez, Carolina; del Fresno, Elena; González Rodríguez, Águeda.

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: Hernández, Miguel Ángel; Pérez, Carolina; González Rodríguez, Águeda.

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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DOCTORAL THESES AND OTHER WORKS:

Beatriz Aldave Orzáiz
"Ph.D. thesis: *Impacto del tratamiento con presión continua positiva en las comorbilidades metabólicas y vasculares vinculadas a la apnea obstructiva del sueño*". Universidad Autónoma de Madrid. Medicina. 2024. Supervisors: Pedro Landete Rodríguez, Águeda González Rodríguez. Grade: Sobresaliente Cum Laude.

Florian Seeger
"Master´s thesis: *Role of the transcription factor GATA4 in liver progenitor cells*". Universidad de Mannheim (Alemania). Supervisors: Laura Villamayor y Ángela Martínez Valverde. Grade: 9

Miguel Hernández García
"Master´s thesis: *Effect of Oxygen-level Normalization on the Progression of Steatotic Liver Disease induced by Intermittent Hypoxia*". Universidad Autónoma de Madrid. 2024. Supervisor/s: Águeda González Rodríguez. Grade: 9.4

Ángela Olivera Rodríguez
"Final degree's project: E*studio del efecto de las vesículas extracelulares lipotóxicas liberadas por hepatocitos en islotes pancreáticos humanos*". Universidad de Alcalá de Henares. Supervisors: Irma García Martínez y Ángela Martínez Valverde. Grade: 9.2

Ivana Henríquez Muñoz
"Final degree's project: *Las proteínas morfogenéticas óseas (BMP) son nuevas dianas moleculares relacionadas con la enfermedad hepática esteatósica asociada a disfunción metabólica (MASLD) y el daño vascular asociado.*" Universidad Autónoma de Madrid. 2024. Supervisor/s: Águeda González Rodríguez. Grade: 9.7

FUNDING:

"Linking Intestinal Bacteria and Host Metabolism to Tackle Type 2 Diabetes with Novel Food (DIBAN)". European Union. Horizon-EIC-Pathfinder-Challenge 2024-2028.

"Role of Protein Kinase D2 in the inflammatory response of the liver (PKD2-INFLIVE)". MICIU 2024-2026

"Avanzando en el conocimiento de nuevos mediadores, mecanismos e interactoma tisular en situaciones de resistencia a la insulina (MOIR-ACTOME-CM)". Comunidad de Madrid. 2023-2026.

"Obesity and comorbidities: advances in the research on tissue/cell interactomes in its development and therapeutic interventions (OBE-INT)". MICIU. 2022-2026

"Identification of new therapeutic targets against β-cell glucolipotoxicity: focus on gut-derived metabolites from flavonoids". CIBER (ISCIII). 2022-2024

"Experimental design for studies on multiagonist peptides in obesity and metabolic diseases". Pep2Tango Therapeutics. 2021-2025

"Impact of bone morphogenetic protein 12 on NAFLD progression and the associated-vascular atherosclerotic damage". ISCIII. 2023-2025.

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Cardiovascular Physiopathology

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Fernández Frías, Lucía
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KEYWORDS
Inflammation, Immunometabolism, Kv1.5, Channelosome, Heart failure, Cardiac remodelling.

RESEARCH LINES:

Line of work: Cardiovascular function
Heart failure (HF) is a complex clinical syndrome that constitutes a major health problem whose 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 in the context of regulating ferroptosis as 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.

Line of work: Immunometabolism and COVID-19
The cytokine storm observed in patients with COVID-19 has emerged as an important factor contributing to the severity of the disease and its associated mortality. Our study was intended to unravel the intricate metabolic alterations that macrophages undergo in response to the COVID-19 cytokine storm, thereby shedding light on the underlying mechanisms that drive the exacerbated immune response. By integrating genomic-scale data and experimental metabolic measurements, a comprehensive workflow was developed. In our study, we applied an innovative workflow to identify the metabolic alterations that occur in human macrophages during the cytokine storm caused by SARS-CoV-2. The methods used include genome-scale metabolic models (GSMM) for the representation of 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 the metabolic reprogramming of macrophages, providing a deeper understanding of their role in the pathogenesis of the COVID-19 cytokine storm.

Line of work: Kv1.5 and Kv4.3 channelosomes

Ion channels are responsible, among other functions, of muscle contraction, cardiac rhythm and synaptic transmission. We focus our research interest in voltage-dependent potassium channels present into the human myocardium, mainly in Kv1.5 (that generate IKur) and in Kv4.3 (that generate Ito), which represent the more important potassium channels responsible of the atrial repolarization and thus they represent pharmacological targets of antiarrhythmic drugs useful 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.

Line of work: 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.

Line of work: Resolution of inflammation in cardiovascular pathologies

The resolution phase following an inflammation process is required for its finalization to avoid 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, or hypertension involve inflammation. Therefore, the knowledge of possible effects of SPMs on different cardiac ion channels may help to the treatment and/or prevention of cardiac diseases.

PUBLICATIONS:

Povo-Retana, A.; Sánchez-García, S.; Alvarez-Lucena, C.; Landauro-Vera, R.; Prieto, P.; Delgado, C.; Martín-Sanz, P.; Boscá, L. Crosstalk between P2Y receptors and cyclooxygenase activity in inflammation and tissue repair. *Purinergic Signal.* **2024,** *20,*145-155. DOI: 10.1007/s11302-023-09938-x.

Povo-Retana, A.; Landauro-Vera, R.; Alvarez-Lucena, C.; Cascante, M.; Boscá L. Trabectedin and Lurbinectedin Modulate the Interplay between Cells in the Tumour Microenvironment-Progresses in Their Use in Combined Cancer Therapy. *Molecules.* **2024,** *29,* e331. DOI: 10.3390/molecules29020331.

Apaza, C.J.; Días, M.; García Tejedor, A.; Boscá, L.; Laparra-Llopis, J.M. Contribution of Nucleotide-Binding Oligomerization Domain-like (NOD) Receptors to the Immune and Metabolic Health. *Biomedicines.* **2024,***12,* e341. DOI: 10.3390/biomedicines12020341.

González-Nicolás, M.Á.; González-Guerrero, C.; Goicoechea, M.; Boscá, L.; Valiño-Rivas, L.; Lázaro, A. Biomarkers in Contrast-Induced Acute Kidney Injury, Towards A New Perspective. I*nt. J. Mol. Sci.* **2024,** *25,* e3438. DOI: 10.3390/ijms25063438.

Vladimir de la Rosa, J.; Tabraue, C.; Huang, Z.; Orizaola, M.C.; Martin-Rodríguez, P.; Steffensen, K.R.; Zapata, J.M.; Boscá, L.; Tontonoz, P.; Alemany, S.; Treuter, E.; Castrillo, A. Reprogramming of the LXRα Transcriptome Sustains Macrophage Secondary Inflammatory Responses. *Adv. Sci.* **2024,** *28,* e2307201. DOI: 10.1002/advs.202307201.

Tébar, D.; Carrillo, X.; García Del Blanco, B.; Gómez-Hospital, J.A.; Nombela, L.; Molina, E.; Galeote, G.; Vilalta, V.; Serra-García, V.; Carol, G.M.; Jiménez-Valero, S.; Fernandez-Nofrerias, E.; Calabuig-Goena, Á.; Jurado-Román, A.; Sánchez-Recalde, Á.; Velasco, M.F.; Bosca, L.; Moreno R. Experience with the ACURATE neo and neo2 transcatheter aortic valves in Spain. The PRECISA (PRospective Evaluation Complementing Investigation with ACURATE devices) registry. Catheter Cardiovasc. *Interv.* **2024,** *103,*1015-1022. DOI: 10.1002/ccd.31032.

Brea, R.; Casanova, N.; Alvarez-Lucena, C.; Fuertes-Agudo, M.; Luque-Tevar, M.; Cucarella, C.; Capitani, M.C.; Marinochi, M.V.; Fusini, M.E.; Lahoz, A.; Nogueroles, M.L.; Fraile, J.; Ronco, M.T.; Boscá, L.; González-

Rodríguez, Á.; García-Monzón, C.; Martín-Sanz, P.; Casado, M.; Francés, D.E. Beneficial effects of hepatic cyclooxygenase-2 expression against cholestatic injury after common bile duct ligation in mice. *Liver Int.* **2024,** *44,* 2409-2423. DOI: 10.1111/liv.16004.

González-Lafuente, L.; Mercado-García, E.; Vázquez-Sánchez, S.; González-Moreno, D.; Boscá, L.; Fernández-Velasco, M.; Segura, J.; Kuro-O, M.; Ruilope, L.M.; Liaño, F.; Ruiz-Hurtado, G. Interleuquina-6 como marcador pronóstico en el fracaso renal agudo y su regulación dependiente de klotho. *Nefrología.* **2024,** *44,* 818-829. DOI: 10.1016/j.nefro.2024.04.002.

Tébar, D.; Jurado-Román, A.; Jiménez-Valero, S.; Galeote, G.; Gonzálvez, A.; Rivero, B.; García, A.; Añón-Elizalde, J.M.; Lorenzo, A.; Fernández-Capitán, C.; Torres, R.; Soto, C.; Alcolea, S.; Rosillo, S.; Caro-Codón, J.; Arbas, E.; Tejera, F.; Plaza, I.; Boscá, L.; Moreno, R. Percutaneous pulmonary thrombectomy with aspiration catheters in patients with high-risk pulmonary embolism and absolute contraindication to systemic thrombolysis. *Cardiovasc. Revasc. Med.* **2024,** *27,* S1553-8389(24)00558-X. DOI: 10.1016/j.carrev.2024.06.020.

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Chakraborty, A.; Sreenivasmurthy, S.G.; Miller, W.; Huai, W.; Biswas, T.; Mandal, S.M.; Boscá, L.; Krishnan, B.; Ghosh, G.; Hazra, T. Fructose-2;6-bisphosphate restores DNA repair activity of PNKP and ameliorates neurodegenerative symptoms in Huntington's disease. Proc. Natl. Acad. Sci. *USA.* **2024,** *121,* e2406308121. DOI: 10.1073/pnas.2406308121.

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Serrano-Novillo, C.; Estadella, I.; Navarro-Perez, M.; Oliveras, A.; de Benito-Bueno, A.; Socuéllamos, P.G.; Bosch, M.; Coronado, M.J.; Sastre, D.; Valenzuela, C.; Soeller, C.; Felipe, A. Routing of Kv7.1 to endoplasmic reticulum plasma membrane junctions. *Acta Physiologica.* **2024,** *240,* e14106. DOI: 10.1111/apha.14106.

Sastre, D.; Colomer-Molera, M.; de Benito-Bueno, A.; Valenzuela, C.; Fernández-Ballester, G.; Felipe, A. KCNE4-dependent modulation of Kv1.3 pharmacology. *Biochem. Pharmacol.* **2024,** *226,* 116368. DOI: 10.1016/j.bcp.2024.116368.

Sastre, D.: Colomer-Molera, M.; Roig, S.R.; de Benito-Bueno, A.; Socuéllamos, P.G.; Fernández-Ballester, G.; Valenzuela, C.; Felipe, A. Molecular mapping of KCNE4-dependent regulation of Kv1.3. *Am. J. Physiol.-Cell. Physiol.* **2024,** *327,* C1497-C1513. DOI: 10.1152/ajpcell.00499.2024.

DOCTORAL THESES AND OTHER WORKS:

Marta Gil Fernández
"Ph.D. thesis: *Role of NOD1 in atrial myopathy associated with heart failure*". Universidad Autónoma de Madrid. Medicina. 2024. Supervisor/s: Lisardo Boscá / María Fernández-Velasco. Grade: Sobresaliente Cum Laude.

Lucía Fernández Frías
"Final degree's project: *Papel de la vitamina D y FICZ en la regulación de la respuesta inmune en cultivos primarios de macrófagos y linfocitos humanos".* Universidad Autónoma de Madrid. Medicina. 2024. Supervisor/s: Adrián Povo / Carlota Alvarez. Grade: Sobresaliente.

Elena Castellví Martínez
"Final degree's project: *Efectos inmunomoduladores de las ecteinascidinas trabectedina, lurbinectedina, ecubectedina y PM54 en cultivos primarios de macrófagos y linfocitos T humanos*". Universidad Autónoma de Madrid. Medicina. 2024. Supervisor/s: Adrián Povo / Rodrigo Landauro. Grade: Sobresaliente.

Pablo José de Lucas Herrero
"Final degree's project: *Estudio de nuevas proteínas integrantes del canalosoma Kv1.5. Estudio del canalosoma Kv1.5*". Universidad Autónoma de Madrid. Medicina. 2024. Supervisor: Carmen Valenzuela Miranda. Grade: Sobresaliente (9,4).

Daniel Ortega Sánchez
"Final degree's project: *Estudio de la electrofisiología del canalosoma Kv4.3. Papel de KChIP2 y Lgi4 en Kv4.3*". Universidad Autónoma de Madrid. Medicina. 2024. Supervisor: Carmen Valenzuela Miranda. Grade: Sobresaliente (9,5).

FUNDING:

"Papel de los receptores nucleares vdr y ahr en la respuesta anti-inflamatoria y proresolutiva frente a la insuficiencia cardiaca. PID2020-113238RB-I00". AEI. 2021-2024.

"Desarrollo de nuevas estrategias terapéuticas basadas en la evidencia para el uso de calcifediol en el tratamiento del Linfoma Difuso de Células B Grandes y el infarto de miocardio. CPP2021-008392". AEI. 2022-2024.

"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.

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

"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.

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

AWARDS:

"Award to the best oral communication presented in the RECI 2024 held in Granada". 13-15 May, 2024.

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

Mitochondrial Function in Health and Disease

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KEYWORDS
Mitochondria, oxidative metabolism, oxidative stress, PGC-1 , CVD, MASLD, T2D, HCC, CRC, Thyroid Cancer, Intestinal dysbiosis.

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, L.; Sastre, M.; Monsalve, M.
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, M.; Monsalve, M.
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, O.; García, R.; Hidalgo, M.; Doblado, L.; Labalde, M.; Ferrero, E.; Monsalve, M.
The third line of research focuses on understanding how the systemic metabolism impacts on CRC development.

Type 2 Diabetes (T2D)
Researchers involved: Gallego, S.; Solla, P.; Plexida, M; Hidalgo, M.; Doblado, L.; Monsalve, M.
The fourth line of research investigates how mitochondrial dysfunction drives the development of CVD in T2D subjects.

Intestinal Inflammatory Disease (IID)
Researchers involved: Bernet, C; Monsalve, M.
The fifth line of research studies how nutrition modifies both systemic and intestinal microbiome oxidative metabolism impacting on intestinal immune-metabolic health.

J. J. Aragón Reyes, O.H. Martinez-Costa Pérez and Alejandro Samhan Arias investigate redox mechanisms and membrane protein interactions relevant to electron transport and disease. We engineer nanocarriers using lipid raft components for drug delivery applications. We also explore mammalian nitrogen-cycle pathways involving molybdenum-dependent enzymes. Additionally, we study post-translational modifications of *b*-type hemoproteins in conditions like cancer and diabetes.

PUBLICATIONS:

Doblado L.; Díaz, L.E.; Nova, E.; Marcos, A.; Monsalve, M. Intestinal Effects of Filtered Alkalinized Water in Lean and Obese Zucker Rats. Microorganisms. **2024**, 12, 316. DOI: 10.3390/microorganisms12020316.

Favero, G.; Golic, I.; Arnaboldi, F.; Cappella, A.; Korac, A.; Monsalve, M.; Stacchiotti, A.; Rezzani, R. Cardiometabolic Changes in Sirtuin1-Heterozygous Mice on High-Fat Diet and Melatonin Supplementation. Int J Mol Sci. **2024**, 25, 860. DOI: 10.3390/ijms25020860.

Selinger Galant, L.; Doblado, L.; Radi, R.; Fabro de Bem, A.; Monsalve, M. Culture of Bovine Aortic Endothelial Cells in galactose media enhances mitochondrial plasticity and changes redox sensing altering Nrf2 and FOXO3 levels. Antioxidants (Basel). **2024**, 13, 873. DOI: 10.3390/antiox13070873.

Kramar, B.; Pirc Marolt, T.; Yilmaz Goler, A.M.; Šuput, D.; Milisav, I.; Monsalve, M. Aripiprazole, but Not Olanzapine, Alters the Response to Oxidative Stress in Fao Cells by Reducing the Activation of Mitogen-Activated Protein Kinases (MAPKs) and Promoting Cell Survival. Int J Mol Sci. **2024**, 25, 11119. DOI: 10.3390/ijms252011119.

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. **2024**, DOI: 10.1101/2024.06.14.599060.

Perdomo Hernández, G.; Liesa Roig, M.; Martín Arribas, M.ª Á.; Martínez Valverde, Á.M.ª; Monsalve Pérez, M.; Sanz Y. Enfermedades metabólicas. S4P CSIC. **2024**. DOI: 10261/361216.

Lopes J, Marques-da-Silva D, Videira PA, Samhan-Arias AK, Lagoa R. Cardiolipin Membranes Promote Cytochrome c Transformation of Polycyclic Aromatic Hydrocarbons and Their In Vivo Metabolites. Molecules. **2024** Mar 3;29(5):1129. doi: 10.3390/molecules29051129.

DOCTORAL THESES AND OTHER WORKS:

Julia Bernal Tirapo
"Ph.D. thesis: *Nuevos biomarcadores del desarrollo tumoral en el cáncer de tiroides: metabolismo oxidativo y resistencia al estrés.*". Universidad Complutense de Madrid. Medicine. 2024. Supervisor/s: Eduardo Ferrero, María Monsalve. Grade: Sobresaliente Cum Laude.

Marta Sastre Arcones
"Final degree's project: *Evaluación del papel jugado por la plasticidad mitocondrial en la mitigación de la mito-toxicidad derivada del tratamiento con ASGs*". Universidad Autónoma de Madrid. Biology. 2024. Supervisor/s: María Monsalve, María Teresa Parra Catalán.

Carolina Ricote Cardena - JAE-intro fellow
(Conexión Nanomedicina CSIC)
"Master's thesis: *Analysis of the interaction of cholesterol and its stereoisomers with Caveolin-2 in lipid bilayers.".* Universidad Autónoma de Madrid (UAM). Master in Biotechnology. 2024. Supervisor/s: Alejandro K. Samhan Arias, Oscar H. Martinez-Costa.

FUNDING:

"Caracterización del papel jugado por el estrés oxidativo mitocondrial en patología humana. PID2021-122765OB-I00". MICIU. 2022-2025.

"Linking Intestinal Bacteria and Host Metabolism to Tackle Type 2 Diabetes with Novel Food (DiBaN). GAP 101162517".
European Innovation Council. 2024-2028.

"Enfermedades metabólicas: Las epidemias del siglo XXI. S4P-2024-01".
CSIC. 2024.

Hypoxia and Angiogenesis

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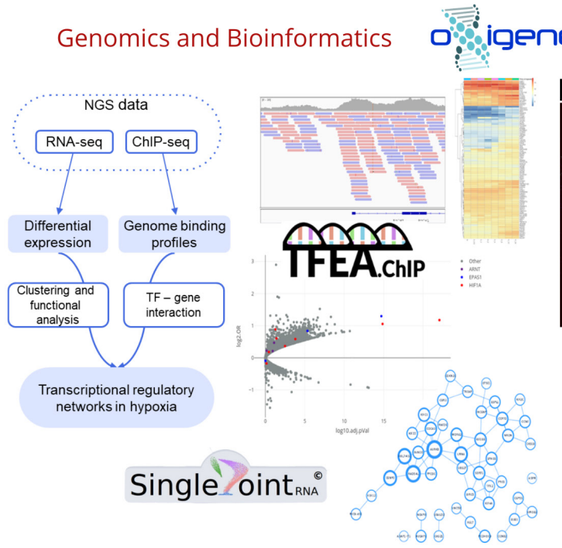
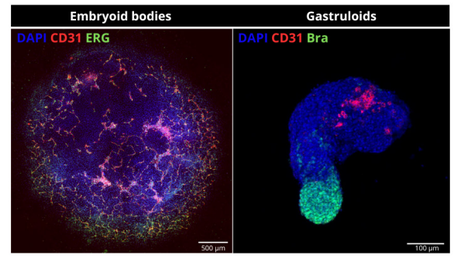
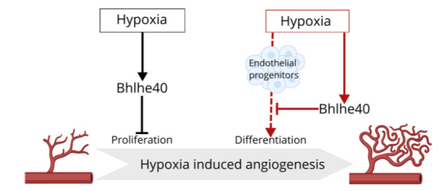
KEYWORDS
Hypoxia, Genomics, Bioinformatics, Angiogenesis.

Genomics and Bioinformatics — Stem Cell Models of angiogenesis







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 overarching objective is to leverage this knowledge to enhance the clinical management of conditions where tissue hypoxia is a common feature.

Transcriptional Response to Hypoxia

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.

The Role of Bhlhe40 in Adipogenesis and Metabolic Syndrome Associated with Obstructive Sleep Apnea

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.

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

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:

Acosta-Iborra, B; Gil-Acero, AI; Sanz-Gómez, M; Berrouayel, Y; Puente-Santamaría, L; Alieva, M; Del Peso, L; Jiménez, B. Bhlhe40 Regulates Proliferation and Angiogenesis in Mouse Embryoid Bodies under Hypoxia. Int J Mol Sci. 2024, 25, 7669-7672. DOI: 10.3390/ijms25147669.

Puente-Santamaría, L; Del Peso, L. SinglePointRNA, an user-friendly application implementing single cell RNA-seq analysis software. PLoS One. 2024, 19, e0300567. DOI: 10.1371/journal.pone.0300567.

DOCTORAL THESES AND OTHER WORKS:

Marta Abanades Salmerón

"Master´s thesis: *Role of Bhlhe40 in the Differentiation and Function of Brown Adipocytes*". Universidad Complutense de Madrid. Medicina. 2024. Supervisor/s: Luis del Peso y Nuria Pescador Sánchez. Grade: Matrícula de Honor

María Fernández Cañizares

"Final degree's project: *Metabolic alterations induced by exposure to intermittent hypoxia, mimicking obstructive sleep apnea, in brown adipocytes*". Universidad Autónoma de Madrid. Medicina. 2024. Supervisor/s: Luis del Peso y Nuria Pescador Sánchez. Grade: Matrícula de Honor

Sara García Bustos

"Final degree's project: *Computational Analysis of Transcriptional Response to Sustained and Intermittent Hypoxia*". Universidad Autónoma de Madrid. Medicina. 2024. Supervisor/s: Luis del Peso y Yosra Berrouayel. Grade: Matrícula de Honor

FUNDING:

"Contribution of BHLHE40 to the transcriptional response to hypoxia and its implication in metabolic and respiratory diseases. PID2020-118821RB-I00". MICINN. 2021-2025

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

"Disfunción vascular en hipertrofia cardiaca isquémica: identificación de nuevos biomarcadores y tratamientos basados en la hiperoxia". Fundación Domingo Martínez. 2023-2024.

AWARDS:

"Mejor comunicación oral presentada en el Congreso Anual de Biotecnología BAC Murcia en la categoría Trabajos de doctorado, postdoctorales y no académicos" 2024.

Oxygen Homeostasis in the Cardiovascular System

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Kennedy Batalla, Rebeca
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Albendea Gómez, Teresa
Mendoza Tamajón, Susana
Urra Balduz, Sonia
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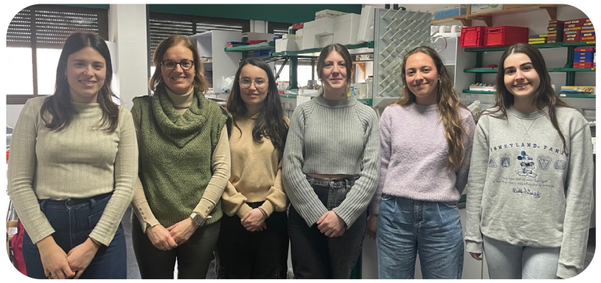
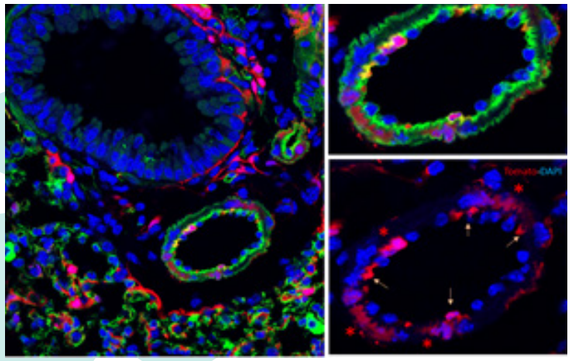
Mateo Rueda, Irene

TECHNICAL SUPPORT PERSONNEL
Novillo Pérez, Miriam

KEYWORDS
Hypoxia, Heart, Oxygen, Cardiovascular System, HIF, VHL





Lineage Tracing Analysis of Wt1 in adult Mouse Lung.

Representative picture of an immunohistochemistry showing a bronchus with an associated pulmonary artery (magnified in right panels). Arrows point to arterial endothelial cells.

Wt1 contribution (red), Nuclei (DAPI, blue), Vasculature (IB4,

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 variety 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 c**ardiovascular 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 essential for myocardial maturation, the correct formation of the ventricular

chambers and the establishment of the conduction system. Our studies reveal the existence of a change in the metabolic programming of the embryonic myocardium, which goes from a glycolytic signature to fatty acid oxidation at intermediate times of cardiogenesis in a HIF1-dependent manner *(Developmental Cell, 2016, STAR Protoc, 2021).* Furthermore, we have determined that in the absence of glycolysis in conditions of loss of HIF1, alternative compensatory mechanisms based on the transport and consumption of amino acids are activated until the correct establishment of an oxidative metabolism of fatty acids (*iScience 2021).* Moreover, our research points to new functions of VHL and HIF2 in the proper formation of cardiac valves that we are currently investigating. 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

Another 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 coronary vessels and cardiac fibroblasts, finding that mutant mice develop several vascular and myocardial alterations. We are currently unraveling the molecular mechanisms behind these dramatic vascular and cardiac defects by RNA sequencing and through the generation of novel mouse models to better dissect the relative contribution of certain cell populations to the phenotype. 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.

Identifying novel molecular mechanisms connecting hypoxia signaling with prevalent CV diseases

In the lab we are also working in the identification of molecular mechanisms that explain how changes in oxygen tension might participate in the development and progression of prevalent CV diseases like cardiac hypertrophy or pulmonary hypertension. On 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. In particular, we are investigating how vascular HIF2 signaling from cell types derived from the Wt1 lineage could influence the successful adaptation to hypoxia with special focus on cardiac and pulmonary performance.

Exploring the impact of hypoxia in redirecting muscle satellite progenitor cells towards cardiomyocyte differentiation with regenerative purposes.

A recent line of research in the lab is focused in investigating the influence of hypoxia in the ability of muscular satellite progenitor cells extracted from the masticatory muscles to differentiate into cardiomyocytes with the final aim to obtain an alternative source of functional cardiac cells able to repair the injured heart. We evaluate the impact of hypoxia exposure of satellite cells at different time points during in vitro differentiation on the expression of cardiac specific markers and metabolic hallmarks. This project is developed in collaboration with a consortium of researchers from the *Comunidad de Madrid* and globally envisions the development of bioengineered patches of satellite cells-derived cardiomyocytes with regeneration capacity.

Understand the importance of hypoxia-mediated signaling in cardiac regeneration

Finally, we are 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 change in cardiac function (measured by echocardiography), scar size or inflammatory resolution after myocardial infarction. In addition, we will explore the impact of temporal activation of HIFs during the onset of myocardial infarction in the regenerative capacity.

PUBLICATIONS:

Martin-Puig, S.; Menendez-Montes, I. Cardiac Metabolism. *Adv Exp Med Biol.* **2024**, *1441*,365-396. DOI: 10.1007/978-3-031-44087-8_19.

DOCTORAL THESES AND OTHER WORKS:

Rebeca Kennedy Batalla
"Doctoral thesis: *Papel de la desregulación inmunológica en pediátricas: ictiosis, enfermedad de Kawasaki y Síndrome multisistémico inflamatorio pediátrico*". Universidad Complutense de Madrid. Facultad de Medicina. 2024. Supervisor/s: José Rafael Correa Rocha y Silvia Martín Puig. Sobresaliente Cum Laude.

Marta Alonso Caubilla
"Master thesis: *Multi-omics characterization of Kawasaki disease versus Multisystem inflammatory syndrome in children (MIS-C)*". Universidad Pompeu Fabra. Facultad de Medicina y Ciencias de la Vida. 2024.Supervisor/s: Silvia Martín Puig y Jorge de la Barrera. Sobresaliente.

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.

"Molecular mechanisms linking hypoxia signaling and cardiopulmonary pathophysiology. HIFPath.PID2020-117629RB-I00". Ministerio de Ciencia e Innovación. 2021-2024 (1 year extension).

"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.

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

Immunity, Immunopathology and Emergent Therapies

PRINCIPAL INVESTIGATOR

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Alemany de la Peña, Susana
Castrillo Viguera, Antonio

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González Castaño, José

STAFF INVESTIGATOR

Pérez Chacón, Gema
Guerrero Espinosa, Erika Marisol

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PRE-DOCTORAL INVESTIGATOR

Navarro Ramírez, Eliezer

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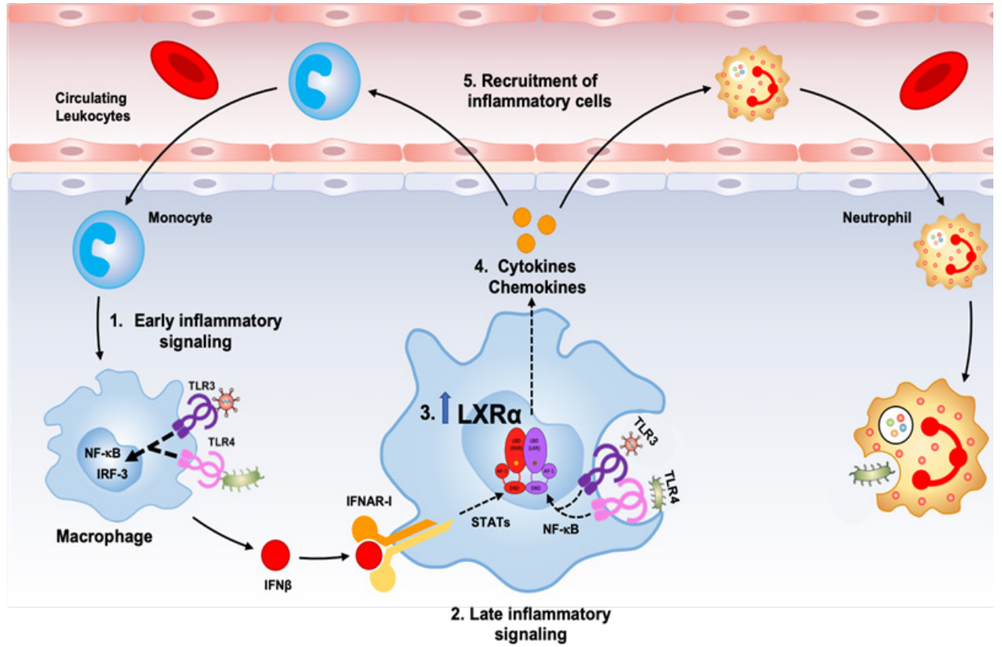
Díaz Guerra, Carolina
López Reyes, Ximena

KEYWORDS

Leukemia, lymphoma, immunotherapy, CAR T, Macrophage, Liver X Receptors, LXR, Inflammation, Transcriptional Regulation, Innate Immunity.



LXRα as a key factor in the transcriptional regulation of secondary-inflammatory response.

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 identification and characterization of new therapeutic drugs to combat these diseases. Furthermore, we also work in the development of new approaches and immunotherapeutic tools against cancer.

Functional activity of Indole-3-Carbinol derivates in B cell lymphoma y leukemia

Researchers involved: Zapata, Juan Manuel; Pérez Chacón, Gema; Aldea, Marcos; Gaibar, María; Lanza, José María; Aramberri, Ane.

Indole-3-carbinol (I3C) is a natural compound present in plants of the Brassica genus that has antitumor action in a wide variety of cancer. Its dimer, diindolylmethane (DIM), which is formed in the acidic environment of the stomach from I3C, also has antitumor activity. Previously, our group has shown that I3C and DIM induce apoptosis in Epstein-Barr virus (EBV)-positive Burkitt lymphoma (LB) cells in vitro and in vivo. We are characterizing the cytotoxic activity of new synthetic DIM derivatives with different functional groups in Burkitt´s lymphoma cell lines. These studies are done in collaboration with Ana M. Estévez Braun, Ángel Amesty Arrieta and Jorge Suárez Ortega (Instituto Universitario de Bio-Orgánica Antonio González, Universidad de la Laguna). All the DIM-derivatives studied cause cell death by apoptosis in both cell lines without altering the cell cycle and are potential drug candidates against BL. Among all of them, one compound stands out, M19, with an LD50 lower than that of DIM in both BL60.2 and Ramos, showing greater effectiveness in inducing the breakdown of PARP and apoptosis and, like DIM, causing a reduction in the expression of key anti-apoptotic proteins such as XIAP and cIAP1/2, as well as the proto-oncogene c-MYC. These results suggest that DIM and M19 have a similar mechanism of action. Additional studies are necessary to verify the cytotoxic potential of these compounds in in vivo models of the various variants of Burkitt's lymphoma.

Development of a new CAR targeting B cell neoplasias

Researchers involved: Zapata, Juan Manuel; Pérez Chacón, Gema; Aldea, Marcos; Gaibar, María.

CD45 is a membrane glycoprotein with phosphotyrosine phosphatase activity that is specifically expressed in nucleated hematopoietic cells. CD45 is the most abundant protein on the surface of lymphocytes. Several isoforms of this molecule have been described that are generated by alternative splicing of exons 4, 5 and 6 (also called exons A, B and C, respectively) and whose expression is regulated during the development, differentiation and activation of lymphocytes. Furthermore, glycosylation processes confer additional antigenic variability to CD45 molecules.

We identified one mAb, Lia1/11, that recognized an epitope on exon A of CD45 (Zapata et al., 1995, Blood 86:1861). The epitope that is recognized by this antibody was expressed in B lymphocytes, but was not expressed in activated NK or in T lymphocytes, regardless of their level of activation, due to the specific masking of this epitope by glycosylation in these cell types. Further studies on the expression of this CD45 epitope using tumor microarrays representing all types of B cell lymphoma and also cytometry analyses of leukemia samples confirmed the broad expression of this epitope on pre- and post-germinal center B cell malignancies. We have developed a CAR based on the ScFv region of the CD45 lia1/11 mAb. This CAR will target most B cell malignancies but would lack of fratricidal activity since it would not recognize resting and activated T cells and NK cells. In collaboration with Javier Ruiz Navarro and Manuel Izquierdo (IIBM), we have shown that this CD45-CAR readily forms immune synapsis with the target cell. We will continue with the in vivo characterization of the functionality of this CAR.

Role of LXR Nuclear Receptors In Macrophage function

Researchers involved: Castrillo, Antonio; Guerrero, Erika

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. LXR are master controllers of cholesterol physiology working in a transcriptional program promoting cholesterol utilization. However, they also play important roles in inflammatory macrophages in response to injury or infection. The molecular mechanisms that activate innate immune pathways and their connections with endogenous LXRα or LXRβ activities have not been explored in depth, and this is one of the main interests of our group.

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 LXR in macrophages, through in vitro and in vivo studies with mouse models of LXR_{α} deficiency, and knockin transgenics of conditional absence or reporter mice. Our recent results suggest that LXR_{α} 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 LXR_{α} in the differentiation and transcriptional activity of tissue-resident macrophages. Using genomic strategies we search for the genome-wide localization of LXR_{α}, and we will analyze by proteomic approaches the molecular interactions of LXR_{α} 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 LXR_{α} 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:

Domínguez-Luis, MJ; Castro-Hernández, J; Santos-Concepción, S; Díaz-Martín, A; Arce-Franco, M; Pérez-González, N; Díaz, M; Castrillo, A; Salido, E; Machado, JD; Gumá, M; Corr, M; Díaz-González, F. Modulation of the K/BxN arthritis mouse model and the effector functions of human fibroblast-like synoviocytes by liver X receptors. *Eur. J. Immunol.* **2024**, *54(11)*:e2451136. DOI:10.1002/eji.202451136.

Hernández-Hernández, I; De La Rosa, JV; Martín-Rodríguez, P; Díaz-Sarmiento, M; Recio, C; Guerra, B; Fernández-Pérez, L; León, TE; Torres, R; Font-Díaz, J; Roig, A; de Mora, F; Boscá, L; Díaz, M; Valledor, AF; Castrillo, A ; Tabraue, C. Endogenous LXR signaling controls pulmonary surfactant homeostasis and prevents lung inflammation. *Cell. Mol. Life Sci.* **2024,** *81 (1)*:287 DOI:10.1007/s00018-024-05310-3.

de la Rosa, JV; Tabraue, C; Huang, Z; Orizaola, MC; Martin-Rodríguez, P; Steffensen, KR; Zapata, JM; Boscá, L; Tontonoz, P; Alemany, S; Treuter, E; Castrillo, A. Reprogramming of the LXRα Transcriptome Sustains Macrophage Secondary Inflammatory Responses. *Adv. Sci.* **2024** (Weinh) DOI:10.1002/advs.202307201.

Sánchez-García S, Castrillo A, Boscá L, Prieto P. Potential Beneficial Role of Nitric Oxide in SARS-CoV-2 Infection: Beyond Spike-Binding Inhibition. *Antioxidants.* **2024** Oct 26;13(11):1301. DOI: 10.3390/antiox13111301.

Perez-Chacon, G; Vincent-Fabert, C; Zapata, JM. Editorial: Community series in mouse models of B cell malignancies, volume II. *Front. Immunol.* **2024**. 15:1488601. DOI:10.3389/fimmu.2024.1488601.

Martín-Antonio B; Blanco B; González-Murillo Á; Hidalgo L; Minguillón J; Pérez-Chacón G; Next Generation CART MAD Consortium. Newer generations of multi-target CAR and STAb-T immunotherapeutics: NEXT CART Consortium as a cooperative effort to overcome current limitations. *Front. Immunol.* **2024**, *15:*1386856. DOI: 10.3389/fimmu.2024.1386856.

DOCTORAL THESES AND OTHER WORKS:

Ane Aramberri Iribarren
"Master thesis: *Studies on the cytotoxic activity of diindolylmethane derivatives in Burkitt́ s lymphoma cell lines*". Universidad Complutense de Madrid. Facultad de Ciencias Biológicas. 2024. Supervisor/s: Juan Manuel Zapata Hernández and Gema Pérez Chacón Grade: 8,6

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. P2022/BMD-7274". Consorcios en Biomedicina CAM. 2023-2026.

"New Functions of TRAF1 in T Lymphocyte immune Responses, Homeostasis and Aging. PID2022-136909OB-I00.". MICINN. PID2022-136909OB-I00. 2023-2026. IP. Juan M Zapata.

"Next Generation multitarget STAB and CART Immunotherapies. P2022/BMD-7225". Consorcios en Biomedicina CAM
2023-2026. IP. Juan M Zapata.

Immunobiology of platelets research group

PRINCIPAL INVESTIGATOR
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Fernández Fernández, María

KEYWORDS
Platelets, Immunotherapy, Cancer Immunology, Myeloid Suppressor Cells, Microenvironment.

RESEARCH LINES:

Overview

Our lab is dedicated to understanding the intricate interactions between cancer cells and their surrounding environment, known as the tumor microenvironment (TME). While traditionally recognized for their role in blood clotting, platelets have emerged as key players in tumor progression by actively communicating with cancer cells and other components of the TME. Upon activation, platelets release a variety of bioactive molecules that can support tumor growth, enhance metastatic potential, and modulate the immune response. Targeting platelet activity has shown promise as a potential strategy to improve the efficacy of conventional cancer therapies, including chemotherapy and immunotherapy.

Our research also extends to chronic inflammatory diseases such as atherosclerosis, obesity, and autoimmune disorders, where persistent inflammation leads to platelet hyperactivity even before other disease-related complications arise. We explore how this preconditioned heightened platelet activation might reshape the TME in the event of tumor development, potentially suppressing the anti-tumor immune response and supporting tumor survival. We are particularly interested in how platelet-driven inflammatory pathways contribute to a tumor-promoting environment, ultimately impacting patient outcomes.

By investigating the mechanisms through which platelets shape the TME, we aim to identify novel therapeutic strategies to reprogram platelet activity, thereby enhancing cancer treatment responses.

FUNDING:

"Atherosclerosis Associated Chronic inflammation in the progression and response of cancer to immunotherapies: MDSC Modulation by platelets. Ref: PID2021-126811OB-I00". Ministerio de Ciencia e Invovacion 2022-2025.

"Associated Chronic inflammation and response to immunotherapy. Ref: 2020-T1/BMD-20365". Comunidad Autonoma de Madrid. 2022-2027

Precision Medicine in Diseases Caused by Alterations in Lipid Metabolism

PRINCIPAL INVESTIGATOR
Lacal Sanjuán, Juan Carlos

KEYWORDS
Precision medicine, Lipid metabolism, Choline kinase, NSCLC, PDAC.

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.

PUBLICATIONS:

Rio-Vilariño, A; Cenigaonandia-Campillo, A; García-Bautista, A; Mateos-Gómez, PA; Schlaepfer, MI; Del Puerto-Nevado, L; Aguilera, O; García-García, L; Galeano, C; de Miguel, I,;Serrano-López, J; Baños, N; Fernández-Aceñero, MJ; Lacal ,JC; Medico,E; García-Foncillas, J,;Cebrián, A. Inhibition of the AURKA/YAP1 axis is a promising therapeutic option for overcoming cetuximab resistance in colorectal cancer stem cells. *Br J Cancer.* **2024** Mar 11. DOI: 10.1038/s41416-024-02649-z.

Lacal, JC; Ibrahim, SA; Zimmerman, T. Is choline kinase alpha a drug target for obesity? *Front Endocrinol.* **2024** Nov 6;15,1492753. DOI: 10.3389/fendo. 2024.1492753.

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).

"Tratamiento de modelos de patient-derived xenografts (PDX) y patient-derived organoids (PDO) con fármacos experimentales". CSIC. 2022-2024.

"Material transfer agreement between CSIC, M.P. and Gossamer Bio Services". 2022-2025.

"Targeting choline metabolism in high-risk hepatoblastoma with a novel release-controlled drug. **2024** LLAV 00109 ". Fundació Institut d'Investigació en Ciències de la Salut Germans Trias i Pujol. 2024-2025.

PATENTS:

1.- INVENTORS: T. Zimmerman, S. Ibrahim, JC Lacal. PATENT NUMBER: USSN 63/567,849-2024

MicroRNAs in immune tolerance, autoimmunity and cancer

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González Molina, María del Pilar

Jiménez Sánchez, Ana

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TECHNICAL SUPPORT PERSONNEL
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Moreno Jerez, Alba

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

RESEARCH LINES:

Overview

Our laboratory is interested in understanding the role of microRNAs (miRNAs) in health and disease, with a primary focus on immune tolerance, autoimmune diseases and cancer. We have previously identified miR-148a and the miR-17-92 miRNA cluster as essential regulators of B cell tolerance. Additionally, we demonstrated the causal role of miR-148a in lethal autoimmunity and elucidated the molecular mechanisms through which miR-148a and miR-17-92 exert their effects. Overall, we established miRNAs as critical regulators of B cell tolerance and autoimmunity.

Our current research is focused on: (1) identifying novel regulators of B cell tolerance and T cell responses in autoimmune diseases and cancer, (2) establishing the cellular and molecular mechanisms underlying their regulatory function, and (3) determining their potential role in the development and progression of these diseases. This research might uncover new therapeutic targets for the treatment of autoimmune diseases and cancer.

MicroRNA control of immune tolerance and autoimmunity

Researchers involved: Rocío Bartolomé Cabrero, Laura Gámez Reche, Javier Sanz Gallardo, Leticia Villadangos Reyes, Tania Gonzalo Santana and Alicia González-Martín.

A main research direction in our laboratory is to explore the function of miRNAs and their target genes in immune tolerance and autoimmune diseases. During 2023 and 2024, we studied the mechanisms of action of a novel miRNA that regulate B cell tolerance previously identified by us. We are currently exploring its potential role in murine models of autoimmune diseases. Furthermore, we have identified another miRNA that, when expressed at increased levels, promotes spontaneous autoimmunity in mice. This is currently being investigated in-depth from both a mechanistic and therapeutic perspective.

Cellular and molecular mechanisms of antitumor immunity

Researchers involved: Eleftheria Papaioannou, María del Pilar González Molina, Ana María Prieto Muñoz, Laura Mañas Cordero, Manuel dos Santos Matias, Jesús Adrián Gómez García and Alicia González-Martín.

Another key research focus of our group is the identification of novel cellular and molecular mechanisms that contribute to antitumor immunity. Using a preclinical model developed by us, we have performed mechanistic studies of a miRNA that enhances T cell-mediated responses against tumors. We also identified additional miRNAs that improve immune responses to melanoma through an *in vivo* functional screen platform previously setup in our lab. We expect that this research direction will contribute advance our current knowledge on tumor immunology and potentially provide promising new therapeutic targets to improve cancer immunotherapy.

DOCTORAL THESES AND OTHER WORKS:

María del Pilar González Molina
"Ph.D. thesis: Deciphering the role of microRNAs in tumor immunology". Universidad Autónoma de Madrid. Medicina. 2024. Supervisor: Alicia González Martín. Grade: Sobresaliente Cum Laude with International Mention.

Rocío Bartolomé Cabrero
"Ph.D. thesis: MicroRNA control of neurite growth and polarization by regulating the stability of the axon initial segment". Universidad Autónoma de Madrid. Medicina. 2024. Supervisores: Alicia González Martín and Sergio Gascón Jiménez. Grade: Sobresaliente Cum Laude with International Mention.

Nikola Arsik
"Masteŕ s thesis: Identification of novel regulators of B cell tolerance". Universidad Autónoma de Barcelona (UAB), Universidad de Barcelona (UB), Universidad de Amberes (Belgica), Universidad Claude Bernard Lyon 1 (UCBL, Francia) y Universidad Jean Monnet (UJM, Francia). 2024. Supervisor: Alicia González Martín. Grade: Sobresaliente.

Tania Gonzalo Santana
"Final degree's project: Functional analysis of microRNAs and its target genes in B cell tolerance". Universidad Autónoma de Madrid. Medicina. 2024. Supervisor: Alicia González Martín. Grade: Sobresaliente (9.8).

FUNDING

"Systematic analysis of tumor-specific B cell immunity. CNS2022-136069". Ministry of Science and Innovation. 2023-2025.

"Use of miRNAs for cancer immunotherapy. XXI National Call for Research Grants in Life Sciences". Ramón Areces Foundation. 2023-2026.

"MicroRNA regulatory networks in B cell tolerance and autoimmunity. PID2021-128244OB-I00". Ministry of Science and Innovation. 2022-2025.

"Harnessing microRNAs for lung cancer immunotherapy. XXIII Beca FERO". *FERO* Foundation. 2022-2024.

"Identifying novel targets for cancer immunotherapy. LAB AECC-2020". Spanish Association Against Cancer (AECC), 2020-2024.

"Achieving Long-Term Humoral Protection Against HIV and other Antibody Resistant Pathogens". Bill and Melinda Gates Foundation. 2019-2024.

PATENTS:

"MicroRNAs for use in the stimulation of T cell antitumor responses". González-Martín A, Gonzalez-Molina P. European patent EP23382510.8. Universidad Autónoma de Madrid. 2024.

Nanoimmunology of T Lymphocyte Activation and Apoptosis

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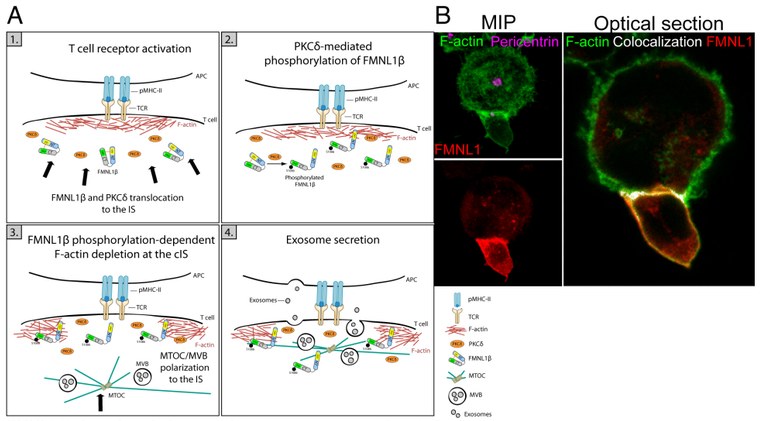
KEYWORDS
T lymphocytes, Immunological synapse, Exosomes, Multivesicular bodies, Actin cytoskeleton, Protein kinase C delta, FMNL1.



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) Confocal image of a Tlymphoblast (bottom) forming immune synapse with a Raji cell (top). 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 non-oriented, fundamental 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, 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 T lymphocytes.

Role of FMNL1 formin in polarized secretory traffic towards the 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 b (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.

PUBLICATIONS:

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Ruiz-Navarro, J.; Calvo, V.; Izquierdo, M., Extracellular vesicles and microvilli in the immune synapse. *Frontiers in immunology.* **2024**, *14*. DOI: 10.3389/fimmu.2023.1324557

Ruiz-Navarro, J.; Fernández-Hermira, S.; Sanz-Fernández, I.; Barbeito, P.; Navarro-Zapata, A.; Pérez-Martínez, A.; Garcia-Gonzalo, F. R.; Calvo, V.; Izquierdo Pastor, M., Formin-like 1β phosphorylation at S1086 is necessary for secretory polarized traffic of exosomes at the immune synapse in Jurkat T lymphocytes. *eLife.* **2024**, *13*, RP96942. DOI: 10.7554/eLife.96942

Izquierdo, M.; Ruiz-Navarro, J.; Baldari, C. T.; Roda-Navarro, P., Editorial: Structure and function of the immunological and redirecting artificial synapses and their clinical implications. *Frontiers in immunology.* **2024**, *15*. DOI: 10.3389/fimmu.2024.1497118

Martín-Antonio, B.; Blanco, B.; González-Murillo, Á.; Hidalgo, L.; Minguillón, J.; Pérez-Chacón, G.; , N. G. C. M. C.; Minguillón, J.; Fernández, L.; Bareke, H.; París-Muñoz, A.; Mañas, A.; Aguirre-Portolés, C.; Navarro-Zapata, A.; Mestre-Durán, C.; Ibáñez-Navarro, M.; Clares-Villa, L.; Naharro, S.; Perez-Martinez, A.; Aldea, M.; Gaibar, M.; Ruiz-Navarro, J.; Izquierdo, M.; Zapata, J. M.; Murillo, Á. G.; Sánchez, E. G.; Martínez, J. G.; Simón, A. R. S.; Herrero, B.; Aguilera, G. G.; Morera, B. H.; Orellana, M. R.; Blanco-Durango, B.; Jiménez-Reinoso, A.; Lázaro-Gorines, R.; Zagorac, I.; Galisteo, A. T.; Ramirez-Fernández, Á.; Díez-Alonso, L.; Dominguez-Alonso, C.; Erce-Llamazares, A.; Hangiu, O.; Pérez, L. R.; Rosel, M. G.; Tudela, A. S.; Arroyo-Ródena, J.; Álvarez-Vallina, L.; Milla, M. Á. R.; Rodríguez, P. G.; Crespo, B. S.; García-Castro, J.; Tarifa, E. E.; Serrano-López, J.; Llamas-Sillero, P.; Martin-Antonio, B., Newer generations of multi-target CAR and STAb-T immunotherapeutics: NEXT CART Consortium as a cooperative effort to overcome current limitations. *Frontiers in immunology.* **2024**, *15*. DOI: 10.3389/fimmu.2024.1386856

DOCTORAL THESES AND OTHER WORKS:

Alberto Jarabo Briones
"Master´s thesis: E*studio del control de tráfico polarizado en la sinapsis inmunitaria: función de FMNL1¨*. Universidad Complutense de Madrid. Facultad de Medicina. 2024. Supervisor/s: Manuel Izquierdo, Javier Ruiz-Navarro. Grade: Sobresaliente 9,8.

Elena Fernández San Juan
"Final degree's project: *Estudio de la formina reguladora de la f-actina FMNL1 en el proceso de secreción polarizada de exosomas en la sinapsis inmune de linfocitos t humanos*". Universidad Politécnica de Madrid. Escuela Técnica Superior De Ingeniería Agronómica, Alimentaria y de Biosistemas. 2024. Supervisor/s: Manuel Izquierdo, Javier Ruiz-Navarro. Grade: Sobresaliente 8,8.

Gonzalo Rodrigo Albert
"Final degree's project: *Estudio de la formina reguladora de la f-actina FMNL1 en el proceso de secreción polarizada de exosomas en la sinapsis inmune de linfocitos t humanos*". Universidad Autónoma de Madrid. Facultad de Medicina. 2024. Supervisor/s: Manuel Izquierdo, Javier Ruiz-Navarro. Grade: Sobresaliente 9,6.

Paula Suárez Distasio
"Final degree's project: *Estudio del tráfico polarizado en la sinapsis inmunitaria: papel de FMNL1¨*. Universidad Autónoma de Madrid. Facultad de Biología. 2024. Supervisor/s: Manuel Izquierdo, Javier Ruiz-Navarro. Grade: Notable 8.

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.

SCIENTIFIC REPORT 2024

department

of Neurological Diseases & Aging

Biomedical Imaging Analysis and Multi-Omics Integration

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TECHNICAL SUPPORT PERSONNEL
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KEYWORDS
Computational biology, Live imaging, Artificial intelligence, Single cell, Immunotherapy, Cancer, Invasion

RESEARCH LINES:

Overview

The group of Dr Alieva (imAIgene-lab) is a **computational laboratory** that leverages advanced artificial intelligence tools, including computer vision, single-cell technologies, and data analytics. We extract biologically relevant insights from microscopy data to gain better **understanding of cancer nature and develop novel therapeutic strategies.** Our **strategic collaboration with leading labs specializing in state-of-the-art imaging and sequencing technologies** amplifies our impact, emphasizing the **synergy of expertise in advancing scientific understanding.**

Our research is focused on understanding cellular complex behavior and function in two key topics within oncology: (1) understanding immunotherapy mode-of action and heterogeneity in tumor response, and (2) investigating the complex behavior of tumor invasion. We utilize computational methodologies to extract meaningful insights from imaging data, dissecting it to understand the functional outcomes of cellular behaviors, the corresponding molecular characteristics, and observe the diversity of cancer responses to treatment.

Decoding tumor resistance to cellular Immunotherapy against cancer.

Researchers involved: Hernández De Roca, Miguel; Zamora Berna, Jorge Aurelio; Fernández Archidona, Sandra; Alieva Krasheninnikova, María

Despite the revolutionary success of cancer T-Cell Immunotherapies their efficacy in treating solid tumours, is still very limited. These widely exploited 'living' drugs are inherently dynamic and our previous results have shown that dynamic live imaging can unravel functional differences between T cells. In this research line I am exploiting a new paradigm for improving engineered T cell anti-tumor function: that heterogeneous T cell dynamics displayed by the T cells upon attack of PDOs is predictive of diverse molecular and phenotypic mechanisms of immunotherapy resistance. Here, we aim to utilize AI and multi-omics integration of T cell imaging and organoid sequencing data to therapeutically exploit this unique concept, by mapping the efficacy of combinatorial drug treatments for their ability to counteract ineffective T cell dynamics.

Understanding microenvironmental drivers of tumor cell invasion.

Researchers involved: Rios Jiménez, Emilio; Muñoz Rubio, Alejandra; Fernández Archidona, Sandra; Alieva Krasheninnikova, María.

Modern technologies allow to study the distinct aspects involved in tumor progression from different angles, such as live imaging for tumor cell invasion studies; multiplexed imaging for tumor microenvironment (TME) characterization or single cell transcriptomics to quantitively dissect tumor heterogeneity and complexity. While separately each of these single omics approaches can mainly reflect one aspect of tumor biology, here we aim to use computational integrative approaches to acquire systematic understanding of how these different aspects interact and define predictive paths to invasion, providing a workflow to bridge phenotypic, molecular and contextual networks driving tumor cell invasion in the brain.

Software development for imaging based immune oncology assay.

Researchers involved: Redondo, Jose Carlos; Moustaine, Zaineb; Fernández Archidona, Sandra; Villegas López, Lupe Ivette; Alieva Krasheninnikova, María.

Recognizing patient variability as a key factor in cancer resistance, including in immunotherapy, emphasizes the importance of screening assays, capable of effectively evaluating the heterogenous response and mode-of-action of the wide panel of cellular immunotherapy products currently in available. Our newly developed live cell imaging platform, BEHAV3D, has not only proven its ability to identify significant cell populations and functional states that result in tumor elimination but has also exhibited potential for enhancing cancer targeting in a patient-specific fashion. Nonetheless, its current architecture is restricted in its ability to handle high-throughput screening of large patient cohorts. Our goal is to leverage the latest advances in AI to adapt our analytical pipeline to large scale screening imaging assays.

PUBLICATIONS:

Jeremiasse, B.; Van Ineveld, R.; Bok, V.; Kleinnijenhuis, M.; de Blank, S.; Alieva, M.; Johnson, H.; Vn vliet, E.; Zeeman, A.; Wellens, L.; Llibre-Palomar, G.; Barrera, M.; Di Maggio, A.; Dekkers, J.; Oliveira, S.; Vahrmeijer, A.; Molenaar, J.; Wijnen, M.; Van der Steeg, A; Wehrens, E.; Ríos, A. A multispectral 3D live organoid imaging platform to screen probes for fluorescence guided surgery. *EMBO Mol Med.* **2024**, *16(7),* 1495-1514. DOI: 10.1038/s44321-024-00084-4.

Alieva, M.; Barrera, M.; de Blank, S.; Petcu, D.; Zeeman, A.; Dautzenberg, N.; Cornel, A.; Van de ven, C.; Pieters, R.; den Boer, M.; Nierkens, S.; Calkoen, F.; Clevers, H.; Kuball, J.; Sebestyén, Z.; Wehrens, E.; Dekkers, J.; Ríos, A. BEHAV3D: a 3D live imaging platform for comprehensive analysis of engineered T cell behavior and tumor response. *Nat. Protoc.* **2024**, *19(7),* 2052-2084. DOI: 10.1038/s41596-024-00972-6.

Ríos, E.; Zomer, A.; Collot, R.; Barrera, M.; Ariese, H.; Van Ineveld, R.; Kleinnijenhuis, M.; Bessler, N.; Johnson, H.; Ríos, A.; Alieva, M. BEHAV3D Tumor Profiler to map heterogeneous cancer cell behavior in the tumor microenvironment. *Elife*. **2024**. DOI: 10.7554/eLife.102097.1.

DOCTORAL THESES AND OTHER WORKS:

Miguel Muñoz Silva
"Master´s thesis: *Uncovering microenvironmental drivers of tumor cell invasion by live and multiplex imaging integration"*. Instituto de Salud Carlos III. CNIO. 2024. Supervisor/s: Maria Alieva Krasheninnikova. Grade: 7.5

Alejandra Muñoz Rubio
"Master´s thesis: *Integration of different image modalities to identify tumour cell behavioral phenotypes in relation to the tumour microenvironment"*. Universidad Autónoma de Madrid. 2024. Supervisor/s: Maria Alieva Krasheninnikova. Grade: 9.25

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:

"ASEICA-FERO Vth award.". 2024

Protective Strategies for Non-Communicable Diseases

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KEYWORDS
NRF2, Oxidative Stress, Inflammation, Neurodegeneration, Chronic Diseases.

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; 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 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:

Fernández-Ginés, R.; Encinar, JA.; Escoll, M.; Carnicero-Senabre, D.; Jiménez-Villegas, J.; García-Yagüe, ÁJ.; González-Rodríguez, Á.; Garcia-Martinez, I.; Valverde, ÁM.; Rojo, AI.; Cuadrado, A. Specific targeting of the NRF2/β-TrCP axis promotes beneficial effects in NASH. *Redox Biol.* **2024**, DOI: 10.1016/j.redox.2024.103027

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Daniel 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.* **2024**. DOI: 10.1016/j.freeradbiomed.2024.11.048

DOCTORAL THESES AND OTHER WORKS:

Raquel Míguez Rodríguez
"Master´s thesis: P*reclinical evaluation of novel NRF2 activators in neurodegenerative diseases*". Universidad Autónoma de Madrid. Medicina. 2024. Supervisor/s: Raquel Fernández y Antonio Cuadrado. Grade: Sobresaliente

FUNDING:

Optimización de un nuevo activador del factor de transcripción NRF2 para frenar la progresión de NASH. PDC2022-1337665-I00". MICINN. 2022-2024

"NRF2 as a novel therapeutic target in early and intermediate age-related macular degeneration". La Caixa. 2022-2025

"Optimización y validación in vivo de fármacos innovadores para el tratamiento de taupatías. S2022/BMD-7230". Comunidad Autónoma de Madrid. 2023-2026

"Papel del factor de transcripción NRF2 en protección sináptica en las Taupatías. PID2022-141786OB-I00". MICINN. 2023-2026

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

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

Neuroprotective Peptides in Excitotoxicity and Stroke

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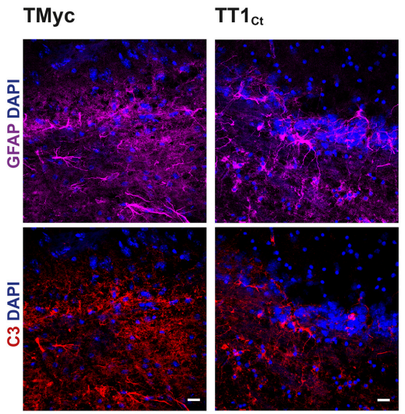
KEYWORDS
Cell-penetrating Peptides, Excitotoxicity, Neuroprotection, PSD-95, Stroke, TrkB.



Neuroprotective peptide $TT1_{Ct}$ reduces the number of inflammatory GFAP+/C3+ astrocytes after stroke

RESEARCH LINES:

Overview

Ischemic stroke is a leading cause of death, disability and dementia with limited therapies available to restrict brain damage or improve functional recovery after ischemia. A promising approach is restriction of neuronal death by excitotoxicity occurring in the infarct penumbra, a potentially recoverable area. Two alternative strategies are currently under exploration: interference of death signaling downstream overactivation of the N-methyl-D-aspartate type of glutamate receptors (NMDARs), main cause of excitotoxicity, or protection of survival pathways negatively affected 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.

Our work is focused in the characterization of the mechanisms of excitotoxicity, the identification of molecules of therapeutic and diagnostic interest, and the development of relevant neuroprotective peptides to treat stroke but also other acute or chronic CNS pathologies associated with excitotoxicity.

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. Therefore, interference of this PSD-95 processing might be a therapeutic target for stroke and other excitotoxicity-associated pathologies. To investigate this hypothesis, we started by analyzing the nature and stability of the PSD-95 fragments produced by calpain using a combination of in vitro assays with purified enzyme or excitotoxic conditions, induced in rat primary neuronal cultures by NMDAR overactivation or a mouse model of permanent stroke. We then sequenced PSD-95 cleavage-sites and demonstrated that calpain processes three interdomain linker regions in this protein and produces stable fragments corresponding to previously described PSD-95 supramodules (PDZ1-2 and PDZ3-SH3-GK) as well as a truncated form SH3-GK. These results allowed rationally design three cell-penetrating peptides (CPPs) containing the PSD-95 cleavage sequences. CPPs are very promising molecules for treatment of CNS diseases since they can cross the blood-brain barrier and have low toxicity. The effects of the generated peptides on PSD-95 stability and neuronal viability were investigated in cultured neurons subjected to excitotoxicity. Only MTP95414, containing the cleavage site in the PDZ3-SH3 linker, was able to interfere PSD-95 downregulation and reduce neuronal death by in vitro excitotoxicity. This peptide has also great potential for ischemia therapy since it is efficiently delivered to mice cortex after intravenous injection and significantly improves neurological outcome in a preclinical model of stroke after brain damage. We are also interested in a possible improvement of MTP95414 neuroprotection by combined use with a different PSD-95-targeted CPP, nerinetide, a peptide currently in Phase 3 clinical trials for acute stroke.

Interference of TrkB-FL retrograde transport as a novel target for stroke treatment

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 and neurodegenerative diseases, 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 prevents excitotoxicity-induced receptor processing and neuronal death by a PLC_{γ}-dependent mechanism. In the stroke model, $MTFL_{457}$ decreases the infarct size and improves the neurological outcome. Our results show that receptor endocytosis induced by excitotoxicity is 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 interferes TrkB-FL/Hrs interaction and receptor trafficking, required for excitotoxic GA fragmentation and TrkB-FL cleavage. Thus, TrkB-FL has 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 neurodegenerative diseases.

We are also investigating $MTFL_{457}$ potential to promote oligodendrocyte survival in models of multiple sclerosis (in collaboration with C. Dreyfus) or prevent cochlear synaptopathy (in collaboration with I. Varela's group), induced by excitotoxicity or decreased neurotrophic support in the inner ear. Recent results highlight the extraordinary therapeutic potential of peptide MTFL457 for treatment of noise-induced hearing loss and, probably, other types of sensorineural hearing loss similarly associated to excitotoxicity.

TrkB-T1 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, TT1Ct, results in prevention of reactive gliosis and strongly decrease excitotoxicity-induced damage in cellular and mouse models of stroke. In collaboration with M. Concepción Serrano (ICMM, CSIC), we have started to load peptide TT1Ct into natural hydrogels and nanoparticles for local administration, with improved biodistribution and stability properties, in models of stroke and spinal cord injury. In addition. a biotinylated form of TT1Ct has proven to be 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. Finally, we have designed additional TrkB-T1-containing CPPs resembling excitotoxicity-induced TrkB-T1-ICD to model changes in transcriptional activity and viability induced by RIP in neurons and astrocytes.

PUBLICATIONS:

Esteban-Ortega, G.M.; Díaz-Guerra, M. Retrograde transport of neurotrophin receptor TrkB-FL induced by excitotoxicity regulates Golgi stability and is a target for stroke neuroprotection. *bioRxiv*. **2024**. DOI 10.1101/2024.10.29.620835.

Ugalde-Triviño, L.; Tejeda, G.S.; Esteban-Ortega, G.M.; Díaz-Guerra, M. Interactions established by isoform-specific TrkB-T1 sequences govern inflammatory response and neurotoxicity in stroke. *bioRxiv*. **2024**. DOI:10.1101/2024.11.13.623346

DOCTORAL THESES AND OTHER WORKS:

Sara Ruiz Cabrera

"JAE Intro ICU fellowship: Exploración del procesamiento del receptor de neurotrofinas TrkB-T1 por metaloproteinasas y γ-secretasas en cultivos primarios de astrocitos". Conexión de Nanomedicina CSIC. Supervisor/s: Margarita Díaz-Guerra González

Beatriz Maldonado Vega
"Final degree's project: Efecto de la administración combinada de dos péptidos neuroprotectores con PSD-95 como diana terapéutica frente a la excitotoxicidad". Universidad Autónoma de Madrid. Ciencias. 2024.
Supervisor: Margarita Díaz-Guerra González. Grade: Sobresaliente

Candela Chovas Fuentes
"Final degree's project: Exploration of NMDAR overstimulation and TrkB-T1-derived peptide Bio-TLT1 as models of reactive astrogliosis.".
Universitat Rovira i Virgili. Bioquímica y Biología Molecular. 2024.
Supervisor: Margarita Díaz-Guerra González. Grade: Sobresaliente

FUNDING:

"Pérdida auditiva neurosensorial y sinaptopatía coclear: estudio de vías no invasivas de tratamiento con otoprotectores y exploración de TrkB como nueva diana terapéutica. IND2020/BMD-17454". Comunidad de Madrid. Co-IP with Isabel Varela Nieto and the participation of Alodia Farmacéutica, S.L. 2021-2024

"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". MICINN. 2023-2026

Thyroid Hormones and Central Nervous System

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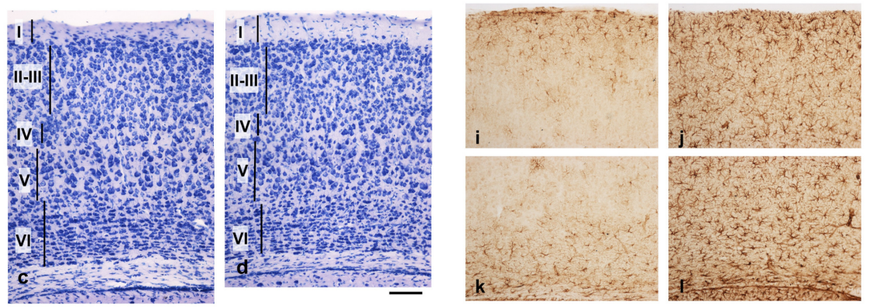
KEYWORDS
Thyroid hormones, Thyroid hormone transport, MCT8 deficiency, Brain, Blood-brain barrier, Thyromimetics



Nissl staining (left) and GFAP immunolabeling (right) in the developing visual cortex of WT and *Mct8/Dio2*KO mice at postnatal day 15. Note the different cortical thickness between genotypes. *Mct8/Dio2*KO mice show a pronounced increase of GFAP+ astrocytes as compared to WT suggesting astrogliosis. *Guillén-Yunta et al 2024 Neurobiol Dis.*

RESEARCH LINES:

Overview

Our research group is committed to advancing the understanding of the pathophysiology and disease mechanisms in the central nervous system (CNS) related to rare disorders associated with thyroid hormone signaling defects. 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 thyroid hormones play in 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 thyroid hormone 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.

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 models include animals with congenital hypothyroidism, mice deficient in MCT8, the main transporter of thyroid hormones in brain barriers and neural cells, and mice deficient in proteins involved in thyroid hormone metabolism and action. We have analyzed the impact of the lack of Th signaling on CNS plasticity processes, such as glial plasticity and adult neurogenesis.
- Additionally, we conduct preclinical studies using AHDS animal models to evaluate the effects of different thyroid hormone analogs on thyroid hormone target neural cells under MCT8-deficient conditions. More recently, we have also been evaluating the effects of various gene therapy approaches on the MCT8-deficient brain.

Exploring the pathophysiology underlying MCT8 deficiency

Researchers involved: Bárez-López, S; García-Aldea, A; Guadaño-Ferraz, A; Grijota-Martínez, C; Guillén-Yunta, M; Montero-Pedrazuela, A; Sanz-Bógalo A; Valcárcel-Hernández, V.

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 explored potential alterations in astrocyte populations and astrocyte plasticity under MCT8 deficiency, in AHDS patients and in an AHDS mouse model. Our results revealed changes in brain cytoarchitecture through magnetic resonance imaging and immunohistochemical analyses. The findings indicate widespread astrocytic dysfunction, affecting metabolism and mitochondrial function, with persistent astrogliosis-like features. These results suggest that astrocytes could be potential therapeutic targets and propose Apparent Diffusion Coefficient (ADC) imaging, a type of magnetic resonance imaging, as a tool for monitoring disease progression and treatment response in AHDS.

As thyroid hormones play a crucial role in maintaining adult brain function, particularly in regulating neurogliogenesis from neural stem cells, we also investigated the impact of MCT8 and DIO2 (an enzyme that generates the transcriptionally active hormone T3) on adult neurogliogenesis in the subventricular zone (SVZ) using Mct8/Dio2 knockout mice. Single-cell RNA sequencing revealed that Mct8 is widely expressed in SVZ cells, while Dio2 is enriched in neurons, astrocytes, and quiescent neural stem cells. The lack of MCT8 and DIO2 activity dysregulated gene expression, an increased neuroblast/OPC ratio, impaired OPC differentiation, and disrupted neuroblast migration. These changes led to defective interneuron formation and impaired odor discrimination, highlighting MCT8 and DIO2 as key regulators of neurogenesis and myelination.

We have also published a review on the role of thyroid hormones in sensory system development and function, emphasizing MCT8's role in neurosensory pathways. While most AHDS patients exhibit neuromotor and cognitive deficits, sensory impairments remain unclear. By examining animal models, the review seeks to better understand neurosensory alterations in AHDS, which could aid in identifying and addressing sensory deficits in affected individuals.

To develop therapies to alleviate the neurological alterations due to MCT8 deficiency

Researchers involved: Bárez-López, S.; Guadaño-Ferraz, A.; Grijota-Martínez, C.; Guillén-Yunta, M.; Montero-Pedrazuela, A.; Valcárcel-Hernández, V.

MCT8 deficiency, a rare disorder causing 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 dedicated significant efforts to developing therapeutic strategies to address these issues. The brain impairments in the AHDS mainly result from impaired thyroid hormone transport to the brain across the blood-brain barrier. In view of this, we have conducted preclinical studies using thyroid hormone analogs that can be transported across plasma membranes in the absence of MCT8, and more recently, we are investigating the potential of gene therapy to palliate the neurological alterations due to MCT8 deficiency.

One of the most plastic processes of the adult brain is adult neurogliogenesis. We reviewed the role of thyroid hormones in adult neurogliogenesis, focusing on neural stem cell niches in the SVZ, hippocampus, hypothalamus, striatum, and cerebral cortex. In this review it is discussed particularly how the regulation of thyroid hormone availability trough MCT8 and DIO2 influences neural lineage decisions and its potential for stimulating brain repair in neurodegenerative diseases like Alzheimer's, Parkinson's, multiple sclerosis, and stroke. Future research aims to use thyroid hormone signaling to promote neuron and oligodendrocyte regeneration as therapeutic strategies.

PUBLICATIONS:

Valcárcel-Hernández, V.; Mayerl, S.; Guadaño-Ferraz, A.; Remaud, S. Thyroid hormone action in adult neurogliogenic niches: the known and unknown. *Front Endocrinol (Lausanne)* **2024**, *15*, 1347802. DOI: 10.3389/fendo.2024.1347802.

Bárez-López, S.; Bishop, P.; Searby, D.; Murphy, D.; Greenwood, M.P. Male rat hypothalamic extraretinal photoreceptor Opsin3 is sensitive to osmotic stimuli and light. J *Neuroendocrinol*. **2024**, *36(2)*, e13363. DOI: 10.1111/jne.13363.

García-Aldea, Á.; Guillén-Yunta, M.; Valcárcel-Hernández, V.; Montero-Pedrazuela, A.; Guadaño-Ferraz, A.; Bárez-López, S. Insights on the role of thyroid hormone transport in neurosensory organs and implication for the Allan-Herndon-Dudley syndrome. *Eur Thyroid J* **2024**, *13(2)*, e230241. DOI: 10.1530/ETJ-23-0241.

Valcárcel-Hernández, V.; Vancamp, P.; Butruille, L.; Remaud, S.; Guadaño-Ferraz, A. Combined deletion of Mct8 and Dio2 impairs SVZ neurogliogenesis and olfactory function in adult mice. *Neurobiol Dis* **2024**, *199*, 106572. DOI: 10.1016/j.nbd.2024.106572

Guillén-Yunta, M.; García-Aldea, Á.; Valcárcel-Hernández, V.; Sanz-Bógalo, A.; Muñoz-Moreno, E.; Matheus, MG.; Grijota-Martínez. C.; Montero-Pedrazuela, A.; Guadaño-Ferraz, A.*; Bárez-López, S.* Defective thyroid hormone transport to the brain leads to astroglial alterations. *Neurobiol Dis* **2024**, *200*, 106621. DOI: 10.1016/j.nbd.2024.106621

DOCTORAL THESES AND OTHER WORKS:

Marina Guillén Yunta
"Ph.D. thesis: *Alteraciones de la integridad de la barrera hematoencefálica y del desarrollo y función de células gliales en la deficiencia de MCT8*". Universidad Autónoma de Madrid. Medicina. 2024. Supervisors: Ana Guadaño & Ana Montero-Pedrazuela. Grade: Sobresaliente Cum Laude

Beatriz Muñoz Falder
"Master's thesis: *Exploring the pathophysiology and potential therapeutic strategies for Allan-Herndon-Dudley syndrome*". Universidad Autónoma de Madrid. Medicina. 2024. Supervisor: Soledad Bárez. Grade: Notable.

Teresa Lara Cerezo
"Final degree's project: *Explorando el papel de la hormona tiroidea en la regulación de oxitocina en Mus musculus"* Universidad Politécnica de Madrid. Biotecnología. 2024-2025. Supervisor: Soledad Bárez. Grade: ongoing

FUNDING:

"Exploring a personalized gene replacement therapy approach for the Allan-Herndon-Dudley Syndrome OTR10255". Sherman Foundation. 2023-2025

"Allan-Herndon-Dudley Syndrome: pathological studies and development of a novel pharmacological strategy at the preclinical level. PID2020-113139RB-I00". MICINN. 2021-2025.

"Ayudas para contratos Juan de la Cierva-incorporación 2020. IJC2020-043543-I". MCIU. 2022-2025.

"Ayudas extraordinarias para la preparación de proyectos a realizar en el marco del plan estatal de I+D+I.2024AEP100". CSIC. 2024.

"Regulation of Thyroid Hormone Availability during Neurodevelopment in Health and Disease. PID2023-152523OB-I00". MICIU. 2024-2027.

"Ayudas de atracción de talento investigador César Nombela. 2024-T1/SAL-GL-31247". Comunidad de Madrid. 2024-2028.

PATENTS:

"Registration of the Allan-Herndon-Dudley Syndrome Avatar Mouse Model as Designated Biological Material." Guadaño-Ferraz A, Bárez-López S, Valcárcel-Hernández V, Montero Pedrazuela A. CSIC Technology Transfer Department (Registration Number: 262/2024). 2024.

AWARDS:

"Jacques Dumont Poster Prize (basic)" to Víctor Valcárcel Hernández. 46th Annual Meeting of the European Thyroid Association" 2024

Novel Targets in Neurodegeneration and Cancer

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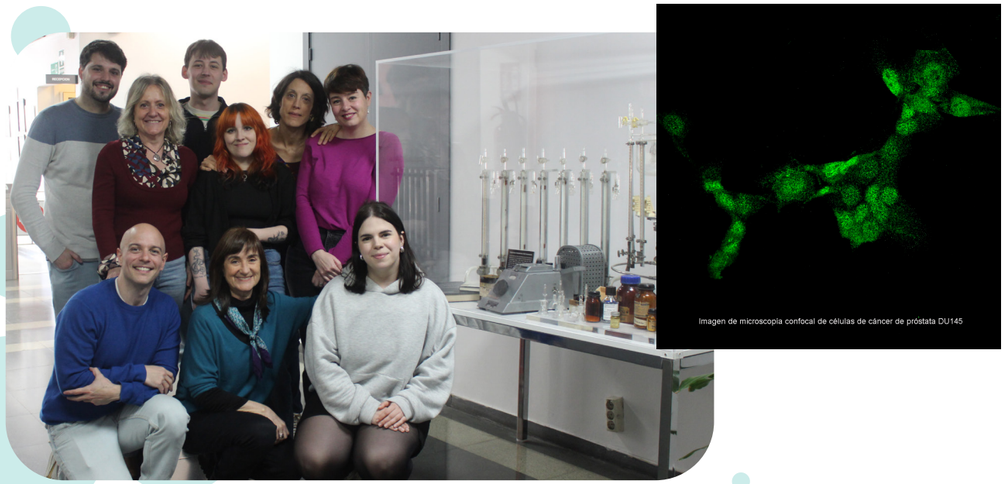
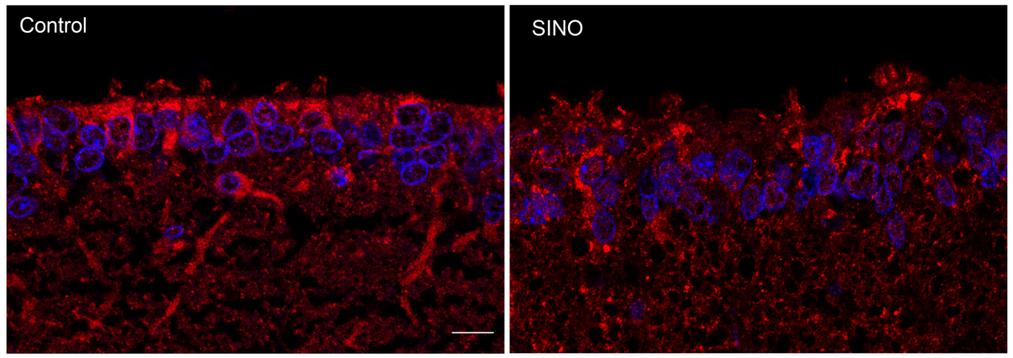
KEYWORDS

SINO Syndrome, Stroke, Hydrocephalus, Prostate Cancer, Kidins220, Protein Kinase D





SINO pathogenic variant generates KIDINS220 truncated forms with a distinct immunostaining pattern at the ependymal barrier of brain lateral ventricles. KIDINS220 immunofluorescence (red) and nuclear staining (blue) at the ependymal barrier of lateral ventricles in the brain of a control foetus (left panel) or a SINO syndrome foetus carrying a KIDINS220 pathogenic variant (right panel). Truncated forms of KIDINS220 in SINO syndrome brain induce the disorganization of ependymal cells and altered KIDINS220 distribution. Scale bar: 10 µm.

RESEARCH LINES:

Overview

Our group is dedicated to investigate 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. **Prostate 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. 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; 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. 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) **(Figure 1)**. 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, D.

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 tumours 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 tumours, 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.

PUBLICATIONS:

Cilleros-Rodríguez, D.; Toledo-Lobo, M.V.; Martínez-Martínez, D.; Baquero, P.; Angulo, J.C.; Chiloeches, A.; Iglesias, T.; Lasa, M. Protein kinase D activity is a risk biomarker in prostate cancer that drives cell invasion by a Snail/ERK dependent mechanism. *Biochim Biophys Acta Mol. Basis Dis.* **2024**, *(1870)*. DOI. 10.1016/j.bbadis.2023.166851

Díaz-Gago, S.; Vicente-Gutiérrez, J.; Ruiz-Rodríguez, J.M.; Calafell, J.; Álvarez-Álvarez, A.; Lasa, M.; Chiloeches, A.; Baquero, P. Autophagy sustains mitochondrial respiration and determines resistance to BRAFV600E inhibition in thyroid carcinoma cells. *Autophagy*. **2024**, *(20)*. DOI. 10.1080/15548627.2024.2312790)

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Rada, P.; Carceller-López, E.; Hitos, A.B.; Gómez-Santos, B.; Fernández-Hernández, C.; Rey, E.; Pose-Utrilla, J.; García-Monzón, C.; González-Rodríguez, Á.; Sabio, G.; García, A.; Aspichueta, P.; Iglesias, T.; Valverde, Á.M. Protein Kinase D2 modulates hepatic insulin sensitivity in male mice. *Mol. Metab.* **2024**, *(90)*. DOI. 10.1016/j.molmet.2024.102045

FUNDING:

"Neuroprotection Strategies and Molecular Mechanisms Related to Kidins220 and Protein Kinase D Dysfunction. PID2020-115218RB-I00" MICINN. 2021-2024

"Analysis of Thiamine Deficiency in Huntington's Disease as a Biomarker of Progression and for Evaluation of Therapeutic Response. CIBERNED 2022/03" CIBERNED. 2023-2024

"PRKD1- and KIDINS220-related Neurological Disorders: Novel Disease Models to Identify Pathological Mechanisms and Therapeutic Targets and Strategies. PID2023-153284OB-I00" MICIU. 2024-2028

"Nuevas estrategias terapéuticas basadas en la modulación del metabolismo oxidativo para el tratamiento del cáncer de tiroides V600EBRAF-positivo. PID2023-147040NB-I00" MICIU. 2024-2027

PATENTS:

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Parkinson, ALS and Tauopathies: New Insights

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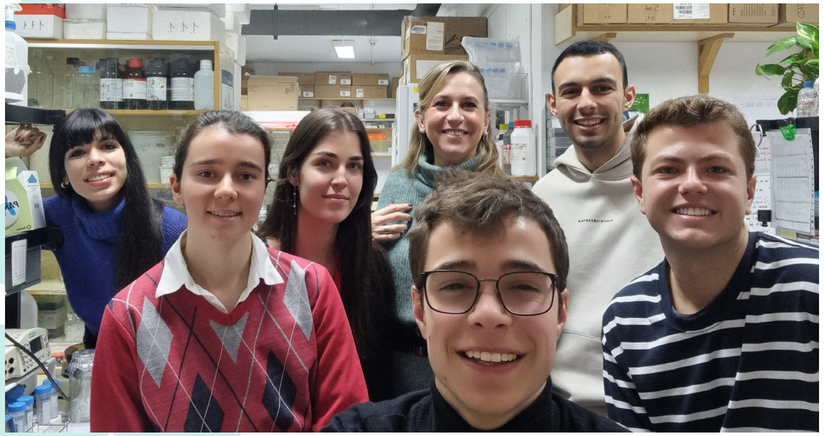
KEYWORDS
Parkinson's disease, ALS, TAU, Neuroinflammation/ pyroptosis, Oxidative stress, Drug discovery.



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; Smith, L; Kaminski Santamaría, S; 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; 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; Abdelkader Guillén, A; 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; Smith, L; 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.

PUBLICATIONS:

Morgenstern C, Lastres-Becker I, Demirdöğen BC, Costa VM, Daiber A, Foresti R, Motterlini R, Kalyoncu S, Arioz BI, Genc S, Jakubowska M, Trougakos IP, Piechota-Polanczyk A, Mickael M, Santos M, Kensler TW, Cuadrado A, Copple IM. Biomarkers of NRF2 signalling: Current status and future challenges. *Redox Biol.* **2024**, Jun;72:103134.DOI: 10.1016/j.redox.2024.103134.

Silva-Llanes I, Martín-Baquero R, Berrojo-Armisen A, Rodríguez-Cueto C, Fernández-Ruiz J, De Lago E, Lastres-Becker I. Beneficial Effect of Dimethyl Fumarate Drug Repositioning in a Mouse Model of TDP-43-Dependent Frontotemporal Dementia. Antioxidants (Basel). **2024**, *Sep 2;13(9),* 1072. DOi: 10.3390/antiox13091072.

Burgaz S, Navarro E, Rodríguez-Carreiro S, Navarrete C, Garrido-Rodríguez M, Lastres-Becker I, Chocarro J, Lanciego JL, Muñoz E, Fernández-Ruiz J. Investigation in the cannabigerol derivative VCE-003.2 as a disease-modifying agent in a mouse model of experimental synucleinopathy. *Behav Brain Funct.* **2024** *Nov 1;20(1),* 28. DOI: 10.1186/s12993-024-00256-9.

DOCTORAL THESES AND OTHER WORKS:

Sara Kaminski Santamaría
"Master´s thesis: *Analysis of the role of GASDERMIN D in a TAU- dependent Frontotemporal Dementia mouse model*". Universidad Autónoma de Madrid. Medicina. 2019. Supervisor/s: Isabel Lastres-Becker, Ignacio Silva-Llanes. Grade: 9.1.

Pablo Baceiredo Macho
"Final degree's project: *Análisis de la implicación de proteínas de unión al RNA en la neurodegeneración asociada a la demencia frontotemporal TAU-dependiente*". Universidad Autónoma de Madrid. Medicina. 2024. Supervisor/s: Isabel Lastres Becker. Grade: 9.8.

FUNDING:

"Aging and neurodegeneration targeting by protein kinase small molecules inhibitors pid2019-105600rb-i00". Ministerio de Ciencia, Innovación y Universidades. 2020-2024

"Modulación del receptor cannabinoide CB2 como nueva estrategia terapéutica para proteger contra la neurodegeneración inducida por TAU ". Fundación Tatiana Pérez de Guzmán el Bueno. 2021-2024

"Luchando contra la enfermedad de Parkinson con inhibidores de sgk1. pdc2022-133774-i00". Ministerio de Ciencia, Innovación y Universidades. 2022-2024.

"Targeting SGK1: Bridging Therapies for Parkinson and Cardiovascular Diseases (SGK1-4PDCar)". Joint call Centre for Network Biomedical Research (Ciber) of Cardiovascular Diseases and Ciber Neurodegenerative diseases. 2024-2026.

"α-Synuclein-dependent neuron-astrocyte differential function of mitochondria in Parkinson's disease (MitAsNeu4PD)". Ministerio de Ciencia, Innovación y Universidades. 01/09/2023-31/08/2026.

"Receptor cannabinoide CB2: Validación como Biomarcador en Demencias TAU-dependientes". Beca Dr. Luis Álvarez 2023 de grupos emergentes o clínicos asociados (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 García-Rubia, Isabel Lastres-Becker. ESP202430463. España. 07/06/2024. CSIC.

Biomedical Magnetic Resonance

PRINCIPAL INVESTIGATOR
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KEYWORDS
Preclinical magnetic resonance, Neuroimaging, Glioblastoma, Obesity, Multimodal imaging, Nanomedicine

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TECHNICAL SUPPORT PERSONNEL
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Holgado Pordomingo, Maya

RESEARCH LINES:

Overview
Our laboratory is aimed in applying biomedical imaging to personalize medicine, particularly within the areas of oncology and neurology. We wanted to develop novel non-invasive biomarker that allow to understand the evolution of different diseases, as obesity or cancer, and how to use this information to tailor therapy.

We are also interested in developing theragnostic agents. These are functionalized nanoparticles acting as platforms that combine the selective delivery of a therapeutic agent with the ability to be detected by medical imaging, offering information about the site to be treated.

Neuroinflammation in Glioblastoma and Other Neurological Disorders
Researchers involved: Lopez Larrubia, Pilar.
Neuroinflammation plays a critical role in the progression of various neurological disorders, including glioblastoma (GBM), the most aggressive primary brain tumor in adults. Understanding the underlying mechanisms of neuroinflammation in these conditions is crucial for developing effective therapeutic strategies.

This research line employs a multi-pronged approach to investigate the critical role of neuroinflammation in the progression of glioblastoma and other neurological disorders, such as obesity and brain trauma. Utilizing MRI, the team meticulously characterizes neuroinflammatory responses in both wild-type and transgenic animal models. By meticulously analyzing MRI data from human glioblastoma patients, the research aims to translate these findings into clinically relevant applications, ultimately paving the way for the development of novel therapeutic targets.

To evaluate and characterize the contribution and role of NI in obesity
Researchers involved: Lopez Larrubia, Pilar; Lizarbe Serra, Blanca.
Obesity has become a major global health concern, and recent evidence strongly suggests a link between obesity and chronic low-grade neuroinflammation. Understanding the role of neuroinflammation in obesity is crucial for developing effective strategies to mitigate its associated health risks.
To address this challenge, this research line focuses on evaluating and characterizing the crucial role of neuroinflammation in obesity through a multi-disciplinary approach. The team has conducted comprehensive MRI studies on both wild-type and transgenic animal models with obesity induced at 10 and 20 weeks. While the acquisition of PET imaging data is currently pending due to un-

foreseen delays in equipment installation, the team has diligently completed histological preparations of all animal samples, with ongoing immunohistochemical studies. To mitigate potential challenges in obtaining specific knockout models, the research team is actively exploring alternative approaches, such as acquiring other relevant animal lines or expanding the scope to investigate other related pathologies.

Immunotherapy in glioblastoma: a multimodal approach.

Researchers involved: Pacheco Torres, Jesús; Lopez Larrubia, Pilar.

Glioblastoma is an aggressive brain tumor with a poor prognosis. Despite the remarkable success of immunotherapy (IMT) in treating certain cancers, its efficacy in GBM remains limited. Understanding the mechanisms underlying GBM's resistance to immunotherapy is crucial for developing more effective treatment strategies.

This research line employs a sophisticated multimodal imaging approach to investigate the challenges of IMT in GBM. By integrating MRI, MRS, and hybrid MRI-PET with advanced molecular biology techniques, the team aims to gain a deeper understanding of the mechanisms underlying GBM's resistance to immunotherapy. This integrated approach will be instrumental in developing innovative, image-based biomarkers that can be used to design, test, and personalize combinatorial therapies, ultimately improving treatment outcomes and enhancing patient survival for individuals with GBM.

Neurological Interplay of Cancer, and Physical Exercise: Advancing Personalized Biomarkers and Therapeutic Strategies

Researchers involved: Pacheco Torres, Jesús; Lopez Larrubia, Pilar

Understanding the intricate interplay between cancer and physical exercise on the central nervous system is crucial for developing personalized interventions to improve patient outcomes.

This research line utilizes a multifaceted approach to investigate the profound neurological implications of the intricate interplay between cancer, obesity, and physical exercise. By employing advanced preclinical models, including a glioblastoma mouse model, and integrating cutting-edge MRI, PET, and mass spectrometry imaging techniques with sophisticated AI-powered data analysis, the team aims to gain a comprehensive understanding of how these crucial factors exert a significant influence on the central nervous system. This interdisciplinary approach will enable the discovery of novel biomarkers that can be used to personalize physical exercise and dietary interventions, advancing the field of precision oncology and supporting the development of non-invasive strategies for monitoring and enhancing therapeutic efficacy.

Biophysical models of diffusion MRI

Researchers involved: Lizarbe Serra, Blanca

Diffusion magnetic resonance imaging (dMRI), provides evidence of cerebral inflammation in several pathologies. dMRI measures the Brownian motion or diffusion of water molecules, which reflects their random translational movements due to thermal energy. When water molecules interact with cell membranes, macromolecules, or fibers, their diffusion properties change. Quantifying diffusion across biological tissues in vivo can reveal underlying microstructural and geometric features and their changes during pathology. In diffusion-weighted MRI (DWI) experiments, the observed signal results from the combination of all microscopic displacements of water molecules in each voxel, with the macroscopic diffusing behavior defined as the apparent diffusion coefficient. Diffusion tensor imaging (DTI) measures 3D diffusion components, including mean diffusivity, fractional anisotropy, axial and radial diffusivities. Beyond the DWI or DTI strategies, in which data analysis does not rely on assumptions of the underlying tissues, the development of advanced models of dMRI data analysis as a function of biophysically meaningful parameters, is currently providing new insights on the microstructural changes underlying pathophysiological processes. We investigate the implementation of new diffusion mathematical models that join biophysical and neurobiological knowledge exhibit the specificity of distinguishing cell types within the grey matter, or even the specific signatures of microglia and astrocytes.

Cerebral mechanisms underlying obesity development and treatment.

Researchers involved: Lizarbe Serra, Blanca; López Larrubia, Pilar.

Obesity is a chronic condition associated with various comorbidities, such as type 2 diabetes, cardiovascular disease, hypertension, certain cancers, and an increased risk of neurodegenerative disorders. Bariatric surgery has been highly effective in achieving non-obese body mass indexes and induces changes in overall metabolism, gut microbiota, and brain function. Simultaneously, research into anti-obesity drugs is yielding promising preclinical results. Notably, celastrol has emerged as a promising anti-obesity and anti-inflammatory agent. Both bariatric surgery and celastrol reduce inflammation in the hypothalamus, a crucial brain region that regulates appetite and energy balance, although the exact mechanisms are not fully understood. Other brain regions, such as the mesocorticolimbic and reward centers, also play roles in controlling food intake and show inflammation during obesity. Recent evidence suggests that the initial level of inflammation may directly impact the success of anti-obesity treatments. Our research de-

velops and applies MRI and spectroscopy methods to demonstrate that these techniques can detect appetite and obeisty-induced changes in the mouse hypothalamus in vivo. We aim to uncover the mechanisms of obesity onset and reversal through MRI, histology, and blood sampling. The hypothesis is that the identified biomarkers will have predictive value, potentially determining the most effective anti-obesity therapy on an individual basis.

PUBLICATIONS:

Knopf, P.; Pacheco-Torres, J.; Zizmare, L.; Mori, N.; Wildes, F.; Zhou, B.; Krishnamachary, B.; Mironchik, Y.; Kneilling, M.; Trautwein, C.; Pichler, B. J.; Bhujwalla, Z. M., Metabolic fingerprinting by nuclear magnetic resonance of hepatocellular carcinoma cells during p53 reactivation-induced senescence. NMR Biomed. 2024, 37(9), e5157. DOI: 10.1002/nbm.5157

Pacheco-Torres, J.; Sharma, R. K.; Mironchik, Y.; Wildes, F.; Brennen, W. N.; Artemov, D.; Krishnamachary, B.; Bhujwalla, Z. M. Prostate fibroblasts and prostate cancer associated fibroblasts exhibit different metabolic, matrix degradation and PD-L1 expression responses to hypoxia. Front Mol Biosci. 2024, 11, 1354076. DOI: 10.3389/fmolb.2024.1354076

Herraiz, A.; Morales, M. P.; Martinez-Parra, L.; Arias-Ramos, N.; Lopez-Larrubia, P.; Gutierrez, L.; Mejias, J.; Diaz-Ufano, C.; Ruiz-Cabello, J.; Herranz, F. Periodic table screening for enhanced positive contrast in MRI and in vivo uptake in glioblastoma. Chem Sci. 2024, 15 (22), 8578-8590. DOI: 10.1039/d4sc01069h

Arias-Ramos, N.; Vieira, C.; Perez-Carro, R.; Lopez-Larrubia, P. Integrative Magnetic Resonance Imaging and Metabolomic Characterization of a Glioblastoma Rat Model. Brain Sci. 2024, 14 (5), 409. DOI: 10.3390/brainsci14050409

Ruiz-Garcia, C.; Lassaletta, L.; Lopez-Larrubia, P.; Varela-Nieto, I.; Murillo-Cuesta, S. Tumors of the nervous system and hearing loss: Beyond vestibular schwannomas. Hear Res. 2024, 447, 109012. DOI: 10.1016/j.heares.2024.109012

Mirasierra, M.; Fernández-Pérez, A. Lizarbe, B.; Keiran, N.; Ruiz-Cañas, L.; Casarejos, M.J.; Cerdán, S.; Vendrell, J.; Fernández-Veledo, S.; Vallejo, M. Alx3 deficiency disrupts energy homeostasis, alters body composition, and impairs hypothalamic regulation of food intake. Cell Mol Life Sci. 2024, 81(1), 343. DOI: 10.1007/s00018-024-05384-z.

DOCTORAL THESES AND OTHER WORKS:

Sofía Rodríguez Serrano
"Final degree's project: *Exercise-induced neurological changes assessed by Magnetic Resonance Imaging".* Universidad Autónoma de Madrid. Medicina. 2024. Supervisor/s: Jesús Pacheco Torres. Grade: Sobresaliente

Fadhel Mohcen Boumeddiene
"Final degree's project: *Connection between tumor metabolism and resistance to immunotherapies in glioblastoma multiforme models".* Universidad Europea de Madrid. Biotecnología. 2024. Supervisor/s: Jesús Pacheco Torres. Grade: Sobresaliente

Carla Dávila Yagüe
"Final degree's project: *Assessment of a preclinical neuroinflammation model using multiparametric magnetic resonance imaging".* Universidad Autónoma de Madrid. Licenciatura de Bioquímica. 2024. Supervisor/s: Pilar López Larrubia and Nuria Arias Ramos. Grade: Sobresaliente.

Irene González-Ortega Villena
"Final degree's project: *Resonancia magnética de la respuesta cerebral al tratamiento contra la obesidad en ratones".* Universidad Autónoma de Madrid. Grado en Bioquímica. 2024. Supervisor/s: Blanca Lizarbe. Grade: Sobresaliente.

Pablo Tirado García
"Final master's project: *"Role of aquaporin-4 in brain function in a magnetic resonance imaging model of obesity".* Universidad Autónoma de Madrid. Máster en Biomedicina Molecular. 2024. Supervisor/s: Blanca Lizarbe and Pilar López Larrubia. Grade: Sobresaliente.

FUNDING:

"Personalized immunotherapy for glioblastoma: interaction between immune checkpoints, tumor microenvironment and tumor metabolism. PID2022-137572OA-I00" Ministerio de Ciencia e Innovación. 2023-2026

"Selective Imaging of Neuroinflammation by Multiparametric MRI/PET Technologies" Ministerio de Ciencia e Innovación. 2022-2026

"The cerebral changes that underly obesity development and treatment: multimodal imaging and magnetic resonance spectroscopy (PhotOBrains). PID2021-126888OA-I00" Ministerio de Ciencia e Innovación 2022-2025

"Selective Imaging of Neuroinflammation by Multiparametric MRI/PET Technologies. PID2021-122528OB-I00" Ministerio de Ciencia e Innovación. 2022-2026

"Hybrid MRI/PET scanner for small animals. EQC2021-006909-P".
Ministerio de Ciencia e Innovación 2022-2023

"Diagnóstico por imagen molecular: investigación básica y desarrollo traslacional. RED2022-134299-T" Ministerio de Ciencia e Innovación 2023-2025

"Red de Enfermedades Raras CSIC (RER-CSIC) 202420E019". Consejo Superior de Investigaciones Científicas. 2024-2025

"Preclinical Optical Imaging System: Fluorescence, bioluminescence and 3D X-rays. EQC2024-008496-P". Ministerio de Ciencia e Innovación 2024-2025

"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

"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.

SCIENTIFIC REPORT 2024

department of **Rare Diseases**

Cilia and Ciliopathies

PRINCIPAL INVESTIGATOR
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KEYWORDS
Cilia, Ciliopathy, Joubert syndrome, INPP5E, Hedhehog signaling.

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.

To perform their sensory functions, these ciliary "antennae" must first be tuned: all receptors and transducers needed for reception and transmission of a given signal must accumulate within cilia. The main focus of our lab is how these cellular sorting of ciliary proteins occurs, and its physiopathological implications.

Primera línea de investigación

Researchers involved: Martin-Morales, R; Parrondo-Tamayo, C; Garcia-Gonzalo, FR.

Ciliary targeting and functions of INPP5E, a phosphoinositide phosphatase implicated in Joubert syndrome.

¿ESTOS TITULOS VAN EN ESPAÑOL?

Segunda línea de investigación

Researchers involved: Martin-Morales, R; Zhuo, J; Garcia-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: Martin-Morales, R; Garcia-Gonzalo, FR.

Characterization of ciliary targeting and functions of EVC-EVC2, a protein complex involved in Ellis van Creveld syndrome.

PUBLICATIONS:

Ruiz-Navarro, J.; Fernández-Hermira, S.; Sanz-Fernández, I.; Barbeito, P.; Navarro-Zapata, A.; Pérez-Martínez, A.; Garcia-Gonzalo, FR.; Calvo, V.; Izquierdo Pastor, M. Formin-like 1β phosphorylation at S1086 is necessary for secretory polarized traffic of exosomes at the immune synapse in Jurkat T lymphocytes. *eLife.* **2024,** 13, RP96942. DOI: 10.7554/eLife.96942.

DOCTORAL THESES AND OTHER WORKS:

Carolina Parrondo Tamayo

"Final degree's project: *Caracterización del fenotipo ciliar de las células INPP5E-KO".* Universidad de Alcalá. Biología. 2023. Supervisor/s: Francesc García Gonzalo. Grade: Sobresaliente

JiaJia Zhuo

"Final degree's project: *Identificación de CLSs en GPCRs ciliares".* Universidad Autónoma de Madrid. Bioquímica. 2023. Supervisor/s: Francesc García Gonzalo. Grade: Sobresaliente

FUNDING:

"Sintonizando la Antena Celular: Mecanismos Moleculares de Control de la Composición de Cilios Primarios. PID2019-104941RB-I00". MICINN. 2020-2024

"Sintonizando la Antena Celular: Mecanismos Moleculares de Control de la Composición de Cilios Primarios. PID2023-149472NB-I00". MICINN. 2024-2028

Rare Diseases Associated to Defects in Autophagy

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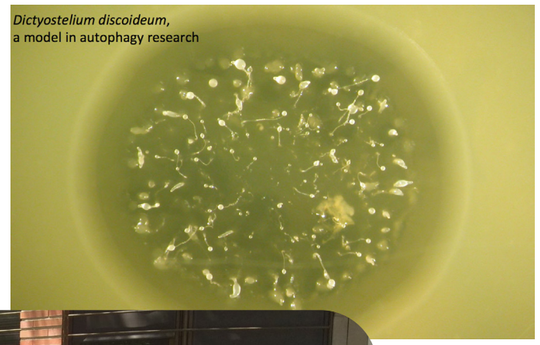
KEYWORDS
Autophagy, BPAN, ChAc, Rare diseases.



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
Vincent, Olivier; Escalante, Ricardo; Navas, María de los Ángeles; Antón, Laura.
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
Researchers involved: Escalante, Ricardo; Vincent, Olivier; Navas, María de los Ángeles; Antón, Laura.
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

Researchers involved: Escalante, Ricardo; Vincent, Olivier; Navas, María de los Ángeles; Antón, Laura.

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 membrane 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:

Antón-Esteban, L.; Tornero-Écija, A.; Vincent, O.; Escalante, R. Methods to assess autophagic activity in Dictyostelium. *Methods Mol Biol.* **2024**, *2814*, 97-106. DOI: 10.1007/978-1-0716-3894-1_7.

DOCTORAL THESES AND OTHER WORKS:

Lucía Lorenzo Parodi

"Master´s thesis: *Modelling rare diseases in the social amoeba Dictysotelium".* Universidad Autónoma de Madrid. 2024. Supervisor/s: Ricardo Escalante. Grade: Sobresaliente

Beatriz Monforte Martínez

"Master´s thesis: *Caracterización de interactores de las proteínas VPS13/ATG2 implicadas en el transporte de lípidos entre orgánulos"*. Universidad Complutense de Madrid. 2024. Supervisor/s: Olivier Vincent. Grade: Sobresaliente.

FUNDING:

""Bases moleculares y celulares de enfermedades raras asociadas a autofagia. PID2021-127355OB-100". MICINN. 2022-2025.

Telomeric Diseases and Experimental Therapie

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KEYWORDS
Telomeres, Telomere Biology Disorders, Telomeropathies, Cutaneous squamous cell carcinoma, DUSP1.

RESEARCH LINES:

Telomere Biology Disorders

Researchers involved: Fernández Varas, B.; Guillen Morales, P.; Manguán-García, C.; Guerrero López, R.; Perona, R.; Sastre, L.

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: Fernández Varas, B.; Manguán-García, C.; García Castro, L.; Seoane Botella, A.; Acero Riaguas, L.; Sastre-Perona, A.; Guerrero López, R.; Perona, R.; 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 dual 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:

Fernandez-Varas, B.; Manguan-Garcia, C.; Rodriguez-Centeno, J.; Mendoza-Lupianez, L.; Calatayud, J.; Perona, R.; Martin-Martinez, M.; Gutierrez-Rodriguez, M.; Benitez-Buelga, C.; Sastre, L. Clinical mutations in the TERT and TERC genes coding for telomerase components induced oxidative stress, DNA damage at telomeres and cell apoptosis besides decreased telomerase activity. *Hum Mol Genet* **2024**, *33 (9)*, 818-834. DOI: 10.1093/hmg/ddae015

Acero-Riaguas, L.; Griso-Acevedo, A. B.; SanLorenzo-Vaquero, A. ; Ibanez-Herrera, B.; Fernandez-Diaz, S. M.; Mascaraque, M.; Sanchez-Siles, R.; Lopez-Garcia, I.; Benitez-Buelga, C.; Bravo-Burguillos, E. R.; Castelo, B.; Cebrian-Carretero, J. L.; Perona, R.; Sastre, L.; Sastre-Perona, A. DUSP1 and SOX2 expression determine squamous cell carcinoma of the salivary gland progression. *Sci Rep* **2024**, *14 (1),* 15007. DOI: 10.1038/s41598-024-65945-x

DOCTORAL THESES AND OTHER WORKS:

Laura García Castro
"Master´s thesis: *Function of MAAP kinases ERK and JNK in cutaneous squamous cell carcinoma*". Universidad Complutense de Madrid. Ciencias Biológicas. 2024. Supervisor/s: Rosa Guerrero López and Leandro Sastre Garzón.

Adriana Seoane Botella
"Final degree's project: *Ruta de las MAPKs en células de carcinoma escamoso de piel*". Universidad de Alcalá. Ciencias. 2024. Supervisor/s: Leandro Sastre Garzón

FUNDING:

"Dual-specificity protein phosphatase 1, DUSP1: a regulator of the interplay between cutaneous squamous cell carcinoma and the immune system? PI23/00677". Instituto de Salud Carlos III. 2024-2026.

"Validación de una terapia basada en P3, un péptido derivado de disquerina, encapsulado en nanopartículas biomiméticas para ser utilizadas en el tratamiento de fibrosis pulmonar idiopática. DTS24/00130". Instituto de Salud Carlos III. 2025-2026.

Genetics and Pathophysiological Mechanisms of Congenital Anomalies

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KEYWORDS
Ellis-van Creveld syndrome, Primary cilia, Hedgehog signaling, Osteogenesis imperfecta, Skeletal Dysplasias.

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 pro-collagen 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:

Tenorio-Castano, J.; Mansilla Aparicio, E.; Garcia Santiago, F. A.; Klotz, C. M.; Regojo, R. M.; Anguita, E.; Ryan, E.; Juusola, J.; Herrero, B.; Arias, P.; et al. Non-immune hydrops fetalis is associated with bi-allelic pathogenic variants in the MYB Binding Protein 1a (MYBBP1A) gene. *Clin Genet.* **2024**, *106* (6), 713-720. DOI: 10.1111/cge.14601.

Altunoglu, U.; Palencia-Campos, A.; Gunes, N.; Turgut, G. T.; Nevado, J.; Lapunzina, P.; Valencia, M.; Iturrate, A.; Otaify, G.; Elhossini, R.; et al. Variant characterisation and clinical profile in a large cohort of patients with Ellis-van Creveld syndrome and a family with Weyers acrofacial dysostosis. *J Med Genet.* **2024**, *61* (7), 633-644. DOI: 10.1136/jmg-2023-109546.

DOCTORAL THESES AND OTHER WORKS:

Juan Andrés Jimenez Estrada
"Ph.D. thesis: *Caracterización de KDELR2 como un nuevo gen asociado a osteogénesis imperfecta y determinación de los mecanismos que lo relacionan con esta enfermedad".* Universidad Autónoma de Madrid. Medicina. 2024. Supervisor/s: Víctor Luis Ruiz Pérez y Carmen Lisset Flores Mauriz. Grade: Sobresaliente Cum Laude

FUNDING:

"Deciphering pathological mechanisms behind ciliopathies and uncovering new genes responsible for developmental disorders. PID2022-139565OB-I00". MICINN. 2023-2026

Molecular Mechanisms of Mitochondrial Pathophysiology

PRINCIPAL INVESTIGATOR
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KEYWORDS
Mitochondria, OXPHOS, mtDNA, Mitochondrial Diseases, Animal Models.

RESEARCH LINES:

Overview

The main function of mitochondria is the production of most 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 in the mitochondrial ribosome.

Given their 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 new genes involved in OXPHOS function using genomic data mining.

P.I. Miguel A. Fernández Moreno.

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 roteins involved in the synthesis of singular mitochondrial tRNAs.

P.I. Miguel A. Fernández Moreno.

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 pathway for the synthesis of the mitochon-

drial 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.

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.

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

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.

PUBLICATIONS:

Alcalá, S.; Villarino, L.; Ruiz-Cañas, L.; Couceiro, J. R.; Martínez-Calvo, M.; Palencia-Campos, A.; Navarro, D.; Cabezas-Sainz, P.; Rodriguez-Arabaolaza, I.; Cordero-Barreal, A.; Trilla-Fuertes, L.; Rubiolo, J. A.; Batres-Ramos, S.; Vallespinos, M.; González-Páramos, C.; Rodríguez, J.; Gámez-Pozo, A.; Vara, J. Á. F.; Fernández, S. F.; Berlinches, A. B.; Moreno-Mata, N.; Redondo, A. M. T.; Carrato, A.; Hermann, P. C.; Sánchez, L.; Torrente, S.; Fernández-Moreno, M. Á.; Mascareñas, J. L.; Sainz, B. Targeting Cancer Stem Cell OXPHOS with Tailored Ruthenium Complexes as a New Anti-Cancer Strategy. *J. Exp. Clin. Cancer Res.* **2024**, *43 (1),* 33. DOI: 10.1186/s13046-023-02931-7.

González, C.; Martínez□Sánchez, L.; Clemente, P.; Toivonen, J. M.; Arredondo, J. J.; Fernández□Moreno, M. Á.; Carrodeguas, J. A. Dysfunction of Drosophila Mitochondrial Carrier Homolog (Mtch) Alters Apoptosis and Disturbs Development. *FEBS Open Bio.* **2024**, DOI: 10.1002/2211-5463.13742.

Antolínez-Fernández, Á.; Esteban-Ramos, P.; Fernández-Moreno, M. Á.; Clemente, P. Molecular Pathways in Mitochondrial Disorders Due to a Defective Mitochondrial Protein Synthesis. Front. *Cell Dev. Biol.* **2024**, 12, 1410245. DOI: 10.3389/fcell.2024.1410245.

DOCTORAL THESES AND OTHER WORKS:

Paula Esteban Ramos

"Master's thesis: *Investigating the function of genes involved in RNA processing and maturation in mitochondria*". Universidad Autónoma de Madrid. Facultad de Medicina. 2024. Supervisors: Paula Clemente Pérez and Miguel A. Fernández Moreno. Grade: 9,9. Sobresaliente.

Patricia Ramos Salido
"Final degree project: Hacia un modelo de reversión de defectos en la síntesis de un tRNA mitocondrial singular". Supervisors: Miguel A. Fernández Moreno and Paula Clemente Pérez. Universidad Autónoma de Madrid. Facultad de Ciencias. 2024. Grade: 8,5. Notable.

FUNDING:

Miguel Ángel Fernández Moreno
"Nuevos genes implicados en la función OXPHOS: desarrollo de modelos experimentales para el diagnóstico, estudio y tratamiento de las enfermedades mitocondriales. PID2019-110320RB-I00". Ministerio de Ciencia, Innovación y Universidades. 2020-2024

"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. 2022-2024.

"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. 2024-2027.

Paula Clemente Pérez
"Contrato y dotación adicional programa Ramón y Cajal. RYC2022-037640-I". Ministerio de Ciencia, Innovación y Universidades. 2024-2028.

Neuropathology of Hearing and Myelinopathies

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KEYWORDS
Hearing, Hearing loss, Vestibular schwannoma, IGFs, Cellular senescence, Inflammation.

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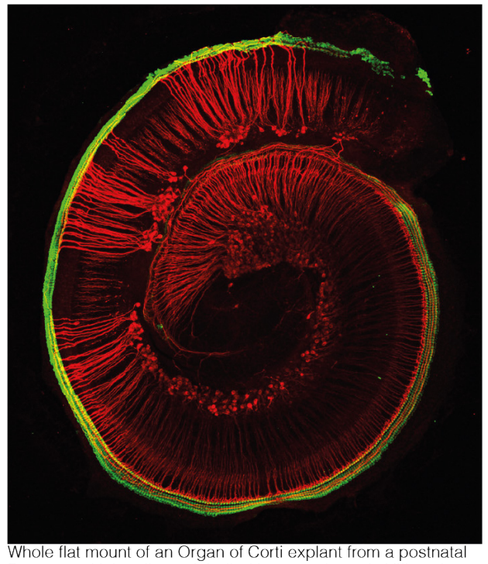
RESEARCH LINES:

Overview

Hearing loss is pathological condition that, beyond its individual implications, holds a profound social importance in contemporary society and has become a global public health challenge that demands top priority. Hearing loss can have a profound impact on the quality of life for those who experience it. Communication is affected, which can lead to social isolation, difficulties in family and workplace interactions, and a decrease in participation in social activities. This can have a negative effect on the mental and emotional well-being of affected individuals.



Whole flat mount of an Organ of Corti explant from a postnatal

Apart from the increasingly prevalent age-related or injury-induced hearing loss, a significant number of diseases, and especially rare diseases, are associated with hearing loss of genetic origin, both in a syndromic or non-syndromic way.

We are interested in a multifaceted view of hearing pathophysiology, covering basically all the relevant aspects that contribute to normal hearing and, therefore, whose malfunction can lead to hearing disabilities, starting from the development of the inner ear, its normal function, and the different pathological conditions that could affect it.

To do so, we use different approaches, such as the genetic analysis of different hearing loss conditions, in vitro molecular and cellular biology studies analyzing the physiology of the major cell types implicated in hearing (hair cells, neurons and glial cells), ex vivo models using organotypic cultures and, finally, in vivo models using small rodents to pinpoint the causes of different hearing loss conditions and trying to find new ways of preserving or restoring normal hearing.

IGF-1 as a cochlear hair cell pro-survival factor.

IGF-1 is a peptide hormone belonging to the family of the insulin family of growth factors. IGF-1 is essential for normal embryonic and

postnatal inner ear development and its absence or defective signaling leads to severe hearing loss in men and mouse models.

During this period, we have continued our studies about the mechanisms of action of IGF-1 and its implications on hearing.

Hair cells are the specialized cells in charge of the mechano-transduction of the sound waves into electrical inputs in the cochlea. HEI-OC1 cell line can be manipulated to acquire a proliferative progenitor or a quiescent differentiated hair cell phenotype, constituting the sole established cellular model for this cell type. We have found that IGF-1 signaling is present in this cell type and that it plays a role as a central regulator of metabolic and oxidative homeostasis in sensory hair cells, acting as pro-survival autocrine factor.

We have found that IGF-1 signaling is present in this cell type and that it plays a role as a central regulator of metabolic and oxidative homeostasis in sensory hair cells, acting as pro-survival autocrine factor.

Cochlear redox homeostasis, oxidative stress and inflammation.

Several ototoxic substances and conditions have in common the induction of a disbalance in cochlear redox homeostasis, the generation of an oxidative stress situation and the induction of a cochlear inflammatory response, which ultimately produces the death of distinct cell types in the inner ear, mainly hair cells as well as the spiral ganglion neurons.

We are interested in determine how different ototoxic insults induce oxidative stress and/or inflammation and, specially, in finding new ways to prevent or revert this homeostasis imbalance, as well as to minimize and repair the cellular damage that it could have been already caused.

1. **DUSP-1 as a model of accelerated presbycusis: implications on cochlear redox homeostasis.** DUSP1, a MAP kinase phosphatase induced by stress stimuli to control the magnitude and extent of JUNK and p38 activation, appears to be a converging node for stress-related inflammation and oxidative stress pathways in the cochlea. DUSP1 contributes to redox homeostasis, inflammatory response and, consequently, to hearing preservation. More interestingly, the administration of antioxidants to Dusp1 knockout mice mitigates the onset of hearing loss. We have continued to analyze the mechanisms of hearing loss in the DUSP-1 deficient mice as a model of accelerated presbycusis.

2. **Age-related hearing loss and gut microbiota.** As other age-related disorders, presbycusis share common mechanisms which often converge on low-grade chronic inflammation known as "inflammaging". Gut microbiota plays a central role in inflammaging because it can release inflammatory mediators and crosstalk with other organ systems. A proinflammatory gut environment associated with ageing could result in a leaky gut and the translocation of bacterial metabolites and inflammatory mediators to distant organs via the systemic circulation.

New therapies for hearing loss.

All our studies are focused in finding new targets that could potentially be druggable in order to prevent or reduce hearing loss, either by generating new molecules, repurposing already know drugs or by exploring new materials for drug delivery. In this regard, we are currently focused in:

1. **Antioxidants as therapeutical agents in injury-induced hearing loss:** We have already shown that nitrones, a family of small antioxidant molecules able to reduce oxidative stress by trapping reactive oxygen and nitrogen species, are able to reduce auditory injury after exposure to noise. In collaboration with Jose Luis Marco Contelles (ICOG-CSIC) we have analyzed the ability of newly synthesized nitrones to act as potential therapeutical agents in hearing loss.
2. **Newly formulated dexamethasone as a therapy for Lipopolysaccharide-induced** ototoxicity: Bacterial lipopolysaccharide induces a strong inflammatory reaction in the cochlea and disrupts the blood-labyrinth barrier (BLB), leading to an increased cochlear permeability and hearing loss. We have explored the effect of a novel dexamethasone formulation for local (intratympanic) administration, in a rat model of lipopolysaccharide ototoxicity. We have found that single local administration of dexamethasone formulated as SPT-2101 protects BLB functional integrity during endotoxemia, providing a novel therapeutic opportunity to treat diseases related to BLB dysfunction.
3. **Small penetrating peptides as therapeutic agents in injury-related hearing loss:** Several injuries can produce hearing loss by a mechanism that involves neuronal excitotoxicity. We have addressed the possibility that small penetrating peptides that have proven to reduce excitotoxic neuronal cell death in other contexts could also be capable of reducing cellular damage in the inner ear. We have found that these peptides can prevent the severe noise damage-induced synaptopathy, providing, thus, a new potential therapeutic approach.

Genetic causes of syndromic and non-syndromic hearing loss.

Apart from the increasingly prevalent age-related or injury-induced hearing loss, a significant number of hearing loss cases are of genetic origin. In addition, other diseases, and especially rare diseases, are associated with hearing loss, both in a syndromic or non-syndromic way.

The specific cellular and molecular mechanisms associated to each genetic hearing loss form are poorly understood. There are still many genes not identified and there are no sufficient experimental models for the study of those identified.

During this period, we have focused our efforts on collaborative studies to understand the relationship between gene mutations and hearing impairment:

1. **Generation of a new animal model for the study of DFNB1:** Non-syndromic hearing loss and deafness (DFNB1) is the most frequent type of autosomal recessive non-syndromic hearing impairment in most populations. DFNB1 is caused by different types of pathogenic variants or large deletions that remove an upstream regulatory element essential for the expression of GJB2, the gene encoding connexin-26. By using CRISPR-Cas9 genetic edition, we have generated a murine model that reproduces the most frequent of those deletions. These homozygous mutant mice are viable, bypassing the embryonic lethality of the Gjb2 knockout, and present a phenotype of profound hearing loss that correlates with specific structural abnormalities in the cochlea. This model, thus, constitutes a valuable tool to study the pathological mechanisms of DFNB1 and to assay therapies for this disease.
2. **Finding new genes involved in FMD:** Familial Meniere's disease (FMD) is a rare polygenic disorder of the inner ear. Through exon sequencing from a large cohort of 94 Meniere disease patients, our collaborators found an enrichment of rare missense variants in the GJD3 gene when comparing allelic frequencies in FMD with the Spanish reference population. GJD3 encodes the human connexin 31.9 (Cx31.9), whose mouse homolog Cx30.2 is expressed in the organ of Corti and vestibular organs, particularly in the tectorial membrane, the base of inner and outer hair cells and the nerve fibers.
3. **Generation of a new animal model for the study of autoimmune/autoinflammatory hearing loss DFNA34:** DFNA34 is an autosomal dominant form of postlingual, slowly progressive sensorineural hearing loss with variable severity and variable additional features. Some patients have pure hearing loss without significant additional features, whereas some patients have features of an autoinflammatory disorder with systemic manifestations, including periodic fevers, arthralgias, and episodic urticaria. The disorder results from abnormally increased activation of the inflammatory pathway due to gain-of-function mutations in the NLRP3 gene. By using CRISPR-Cas9 genetic edition, we have generated a murine model that reproduces the heterozygous missense mutation described by Nakanishi et al. (2017).

Vestibular schwannomas and cellular senescence

Vestibular schwannomas are tumors of the peripheral nervous system originating from the Schwann cells that myelinate the cochleovestibular nerve. They can be bilateral, usually linked to the NF2-related-Schwannomatosis (formerly known as neurofibromatosis type 2 or NF2 syndrome) or, in the majority of instances, appear as sporadic unilateral schwannomas. Although they are benign and many are slow growing, they can provoke increasing nerve function loss and are usually accompanied with hearing loss. If not removed, over time, they will provoke deafness and even become life threatening. Due to their slow growth rate, typical chemotherapy treatments are ineffective and surgical removal or radiotherapy are the only interventions available. Due to their nature and localization, one of the collateral effects of this kind of interventions is the severing of the auditory nerve and, therefore, inducing deafness.

In collaboration with the department of Otology in Hospital LaPaz, we have generated a collection of tumors, including frozen samples for genomic, transcriptomic or morphological analysis, together with fresh samples to generate primary cultures. This is the first step in the characterization of tumors´ individual variability in search of potential therapies that could hinder tumor growth or prevent their deleterious effects on hearing.

We are currently focused on the following aspects:

1. **Cellular senescence as a target for schwannoma tumor growth:** We have described that cellular senescence naturally occurs on vestibular schwannomas and that it could potentially be a target to impede its growth.
2. **Deciphering the molecular signature of vestibular schwannomas:** We are using a multiomic approach to better characterize the nature of vestibular schwannomas and trying to understand the molecular basis of their different clinical manifestations. In this regard, we have already performed transcriptomic and methylomic epigenetic analysis from a large cohort of vestibular schwannomas. Currently, we are exploring the implication of the different pathways that we have found that could be involved in the different clinical manifestations of vestibular schwannomas, focusing on the molecular basis of hearing damage and tumor recurrence.
3. **Generation of organoid models for vestibular schwannoma:** We have generated spheroids from primary cultures of vestibular schwannomas. Further studies will be needed to validate their potential to test therapeutical agents.

PUBLICATIONS:

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DOCTORAL THESES AND OTHER WORKS:

Jose Luis Pérez Troncoso
"Master´s thesis: *Estudio del papel de la NADPH oxidasa 4 (NOX4) en la función auditiva y su evolución con la edad*". Universidad Autónoma de Madrid. Medicina. 2024. Supervisor/s: Silvia Murillo Cuesta. Grade: Sobresaliente

Carla Arraiza Shakirova
"Master´s thesis: *Caracterización de cultivos primarios de esferoides de Schwannoma vestibular humano*". Universidad CEU San Pablo. Medicina. 2024. Supervisor/s: Jose Miguel Cosgaya Manrique. Grade: Notable

FUNDING:

"THEARPY: bases genéticas y moleculares de la sordera neurosensorial y del daño auditivo: exploración de nuevas dianas y estrategias terapéuticas (10.13039/501100011033)". MCIN/AEI. 2021-2024

"Bench to bedside transition for pharmacological regulation of NRF2 in noncommunicable diseases (CA20121)". COST-H2020. 2021-2025

"A sound proteome for a sound body: targeting proteolysis for proteome remodeling Proteocure (CA20113)". COST-H2020. 2021-2025

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"Ayudas extraordinarias a realizar en el marco del plan estatal de i+d+i. 2024AEP115". CSIC. 2024

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PATENTS:

"Quinolylnitrone derivatives for use in the prevention and/or treatment of hearing loss". Marco Contelles J.L., Varela Nieto I., Murillo Cuesta S., Rodríguez de la Rosa L., Alcazar Gonzalez A. EP22382706.4". European Patent application EP 4 309 655 A1. International publication number WO 2024/018073 A1 (25/01/2024)

AWARDS:

"Fuga en Corti Menor. CAJALXMAS Award. 1st prize Concurso Neurociencia y Arte (Michael David Espitia Arias, Elena Torres campos, Victor Paleo García" XII CAJALXMAS Meeting. CSIC. 2024

New Mechanisms and New Models of DNA Replication and Repair

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KEYWORDS
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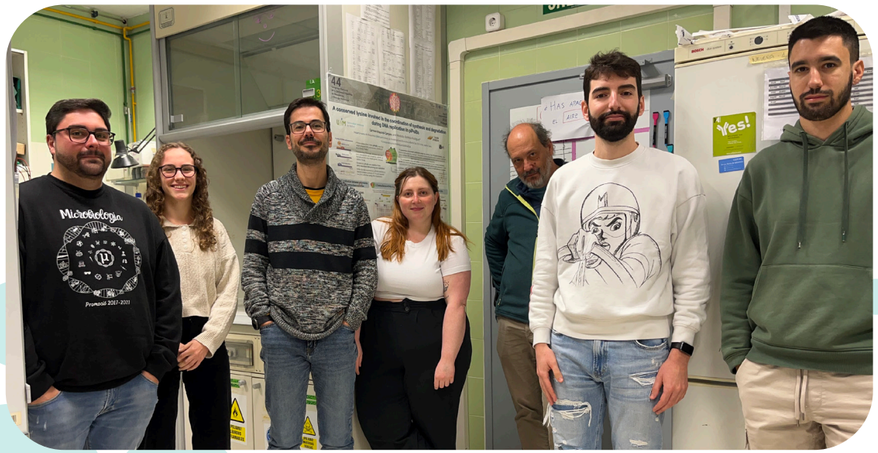

RESEARCH LINES:

Overview

Our primary focus lies in characterizing the molecular mechanisms that ensure genetic stability. Our research philosophy aims to advance both the biochemical understanding and the diversity and evolution of the proteins and mechanisms we study, along with their potential biotechnological applications. To achieve this, we concentrate on simple models, such as bacteriophages or bacterial genomic mobile elements, using a multidisciplinary approach that combines bioinformatics, biochemistry, molecular biology, and microbiology.

We are particularly interested in the biochemical characterization of enzymes involved in alternative mechanisms of DNA replication initiation or priming, independent of DNA primases. In the last years, we have made significant progress in understanding a new subfamily of PolB, known as piPolBs ("primer-independent PolBs"), which we described in 2017. The piPolBs are likely the origin of replicative DNA polymerases from family B, such as human polymerases pol α, pol δ, and pol ε, among others. However, they exhibit unexpected properties, such as the ability to initiate replication de novo, without a pre-existing primer. In recent years, we have concentrated on structure-function studies to dissect the molecular mechanisms underlying each of the piPolB activities and harness our findings to develop new DNA amplification methods. Moreover, as detailed below, we have analyzed in detail the diversity of cargo genes in pipolins, identifying a new role as a toolbox of bacterial defense systems.

The Genetic Plasticity and Repertoire of Pipolins Suggest Their Role in the Maintenance and Diversification of Bacterial Defense Systems

Researchers involved: Mateo Cáceres, V; Redrejo Rodríguez, M

The detailed analysis of bacterial pipolins revealed a key role of pipolins in the maintenance of the arsenal of antiviral defense systems by frequent exchange with other mobile genetic elements (MGEs). Thus, pipolins serve as an orthogonal reservoir of adaptive traits, primarily related to defense functions. Pipolins are characterized by a bimodular structure, with a wide array of cargo defense genes and a minimalist set of core genes, with a primer-independent DNA polymerase (piPolB) being the only universal hallmark. Moreover, analysis of the weighted gene repertoire relatedness (wGRR) revealed that many of these defense factors are actively exchanged with other mobile elements, mainly plasmids (Mateo-Cáceres & Redrejo-Rodríguez, 2024).

Overall, our findings suggest that pipolins act as bimodular defense units with a minimalist genetic core and a dynamic module for building a variable defensive arsenal. Pipolins, along with similar integrative defense elements, maintain a reservoir of defense factors and a reduced set of core genes. These MGEs likely represent a new superfamily dedicated to hosting a dynamic catalog of defense systems. This dynamic reservoir is eventually incorporated by other MGEs, plasmids, and elements with gene transfer capabilities, benefiting from an orthogonal supply of defense genes. A detailed analysis of gene exchange rates in MGEs may further elucidate the dynamics of defense systems among these elements.

Engineering thermostable variants of piPolB

Researchers involved: Mateo Cáceres, V; Mayoral Campos, C; Redrejo Rodríguez M.

In collaboration with Andrew Ellington's laboratory (UT, USA), we employed a machine learning-based approach to develop new piPolB variants that exhibit enhanced DNA amplification capacity and thermostability. We established an efficient protocol for the batch purification of his-tagged piPolB variants and assessed their DNA amplification capacity by measuring DNA concentration at both the endpoint reaction and in real time using EvaGreen DNA binding dye. Various combinations of single and multiple mutants were analyzed to identify the top-performing variants in both piPolB-MDA and piMDA, utilizing a commercially available Φ29 DNA polymerase variant with enhanced thermostability (EquiPhi29TM). The new variants are capable of amplifying DNA at temperatures up to approximately 40 ºC in both protocols, with negligible ab initio DNA synthesis observed in negative controls lacking input DNA.

PUBLICATIONS:

Mateo-Cáceres, V.; Redrejo-Rodríguez, M., N. Pipolins are bimodular platforms that maintain a reservoir of defense systems exchangeable with various bacterial genetic mobile elements. Nucleic Acids Res. 2024, 52, 12498-12516. DOI. 10.1093/nar/gkae891

DOCTORAL THESES AND OTHER WORKS:

Eduardo D. Lozano Escobar

"Master´s thesis: *Análisis genómico de cepas bacterianas de interés en la industria alimentaria*". Universidad Autónoma de Madrid. Master's Degree in Bioinformatics and Computational Biology. 2024. Supervisor/s: Modesto Redrejo Rodríguez. Grade: 9

Rodrigo de la Llave Castro

"Final degree's project: *Papel biológico de las ADN polimerasas independientes de primer en Escherichia coli en el contexto de tratamiento con agentes genotóxicos*". Universidad Autónoma de Madrid. BSc Degree in Biochemistry. 2024. Supervisor/s: Juan J. Arredondo & Modesto Redrejo Rodríguez. Grade: 9

FUNDING:

Caracterización funcional de DNA polimerasas independientes de primer en el contexto de estrés genotóxico en bacterias. PID2021-123403NB-I00". MCIN/AEI/10.13039/501100011033 and ERDF A way of making Europe. 2022-2025