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SCIENTIFIC REPORT 2023

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 pleasure to present this report as a summary of the activities of the IIBM-CSIC/UAM during the year 2023.

Perhaps the most important event during this period has been the official renewal of the Joint Institute Agreement between the CSIC and the Universidad Autónoma de Madrid (UAM), which was pending given the difficulties to match the administrative complexities that regulate the functional intricacies in each institution, and that have been happily solved. The renewal of the IIBM CSIC-UAM agreement provides a solid base to regulate and promote the collaborative efforts of all the personnel of the IIBM, regardless of the institution to which they belong (either CSIC or the Department of Biochemistry of the School of Medicine of the UAM), to function as a single joint institute, as has been the case since the initiation of the first collaborative CSIC-UAM agreement in 1988.

The signing of the agreement has made it possible to officially implement important structural changes that were needed to give an impulse to the IIBM to adapt to the rapidly changing challenges of modern Biomedical research. Thus, the reorganization of research groups and the definition of new strategic research lines allowed us to re-design the departmental structure of the institute, eliminating three of the four departments that were substituted by others, reflecting more accurately our goal to continuously pursue excellent science in Biomedical research. Specifically, we have reinforced our commitment to study the molecular mechanisms of disease from basic science to translational approaches, focusing primarily on cancer, rare diseases, metabolic diseases, immune response, cardiovascular pathologies and neurodegenerative diseases.

Coinciding with these changes, we launched our new Web page to expand our visibility and reinforce our compromise to translate to the public and society at large all the information about our discoveries and the activities of the Institute.

During 2023 we have seen how our institute has kept growing both in scientific outputs and in our capacity to raise competitive funding. In addition, new scientists have joined the IIBM through junior programs such as Ramón y Cajal and Talento de la Comunidad de Madrid or as staff scientists appointed by the CSIC or by the UAM. As I welcome them, I would also like to express my gratitude to the personnel who retired in 2023, recognizing their valuable contribution to the activities of the IIBM for many years.

My mandate as Director of the IIBM is due to finish in January 2024. It has been a privilege and an honor to serve the IIBM during these four years. I would like to thank all the people in the IIBM who helped me on this endeavor, and particularly two individuals without whose help my task as a director would have been a lot more complex. On the one hand, the IIBM Vice-director Aurora Sánchez, and on the other hand, the IIBM executive manager Isabel Ocaña, both of whom provided continuous support to handle many of the managerial issues encountered on a day-to-day basis. My extended gratitude as well to all the personnel in administration and to the staff of general and core services and maintenance, whose dedication is essential to keep the IIBM running.

Mario Vallejo. Director, IIBM

Key Metrics

Publications

- **2** Books
- **65** Internationally co-authored publications
- **7** Medium impact
- **2** Low impact
- **119** High impact

Fundraising

- **5.660.946€** Funding
- **347.358€** R&D Contracts

Supervision activities

- **19** Doctoral theses
- **21** Master´s theses
- **32** Undergraduate theses

Outreach activities

- **26** Materials
- **115** Events

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:

Diaz de Greñu, B; Fernández-Aroca, DM; Organero, JA; Durá ,G; Jalón, FA; Sánchez-Prieto, R; Ruiz-Hidalgo, MJ; Rodríguez ,AM; Santos, L; Albasanz, JL; Manzano, BR;. Ferrozoles: Ferrocenyl derivatives of letrozole with dual effects as potent aromatase inhibitors and cytostatic agents. J Biol Inorg Chem. **2023**, Sep;28(6), 531-547. DOI: 10.1007/s00775-023-02006-0.

Fernández-Aroca, DM; García-Flores, N; Frost, S; Jiménez-Suárez ,J; Rodríguez-González, A; Fernández-Aroca, P; Sabater ,S; Andrés; I,; Garnés-García, C; Belandia, B; Cimas ,FJ; Villar, D; Ruiz-Hidalgo; MJ; Sánchez-Prieto R. MAPK11 (p38β) is a major determinant of cellular radiosensitivity by controlling ionizing radiation-associated senescence: An in vitro study. Clin Transl Radiat Oncol. **2023**, Jun 2;41:100649. DOI: 10.1016/j.ctro.2023.100649.

Sánchez-Fdez, A; Matilla-Almazán, S; Del Carmen, S; Abad, M; Arconada-Luque, E; Jiménez-Suárez,J; Chinchilla-Tábora, LM; Ruíz-Hidalgo, MJ; Sánchez-Prieto, R; Pandiella, A; Esparís-Ogando, A. Etiopathogenic role of ERK5 signaling in sarcoma: prognostic and therapeutic implications. Exp Mol Med. **2023** Jun;55(6):1247-1257. DOI: 10.1038/s12276-023-01008-x

Jimenez-Garcia, IE; Sabater, S; Martinez-Gutierrez, R; Sanchez-Galiano, P; Berenguer-Serrano, R; Castro-Larefors, S; Rey-Lopez, I; Ruiz-Herrero, B; Sánchez-Prieto, R; Rovirosa, A; Arenas, M; Gonzalez-Suarez, HA. LDR brachytherapy offers superior tumor control to single-fraction HDR prostate brachytherapy: A prospective study. Prostate. **2023** Aug;83(11):1068-1075. DOI: 10.1002/pros.24548.

García-Flores, N; Jiménez-Suárez, J; Garnés-García, C; Fernández-Aroca, DM; Sabater, S; Andrés, I; Fernández-Aramburo, A; Ruiz-Hidalgo, MJ; Belandia, B; Sanchez-Prieto, R; Cimas, FJ. P38 MAPK and Radiotherapy: Foes or Friends? Cancers (Basel). **2023** Jan 30;15(3):861. DOI:10.3390/cancers15030861.

DOCTORAL THESES AND OTHER WORKS:

Elena Arconada Luque
"Ph,D Thesis: ERK5 pathway is a determinant in 3-METHYLCHOLANTHRENE- induced sarcoma"' Universidad de Castilla-La Mancha.
Medicina. 2023. Supervisor Ricardo Sanchez Prieto / Maria Jose Ruiz Hidalgo, Grade: Sobresaliente cum laude.

Alba Garcia Ferrer
"Master´s thesis: *Dinaciclib induces a TP53-associated senescence-dependent radiosensitising effect: implications for lung carcinoma therapy".*
Universidad de Castilla la Mancha. 2023.
Medicina. Supervisor/s: Ricardo Sanchez Prieto. Grade: 8,9/19.

David Morcillo Corominas
"Final degree's project: *Study of the prognostic value of mapk7/erk5 in cancer by in silico analysis".* Universidad de Castilla la Mancha. 2023.
Medicina. Supervisor/s: Ricardo Sanchez Prieto. Grade 9.7/10.

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

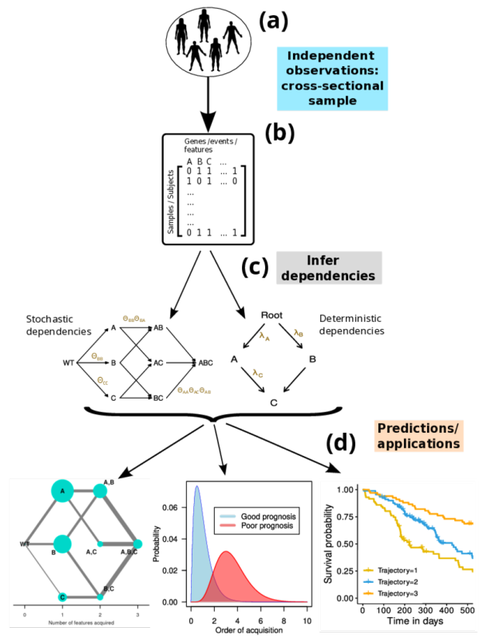
"Nuevos determinantes de radiorresistencia" Research Contracts, as PI, with the Association Comarcal contra el cancer de Motilla del Palancar. 2023-2025

Bioinformatics and Computational Biology of Cancer Evolution

PRINCIPAL INVESTIGATOR
Díaz Uriarte, Ramón

UNDERGRADUATE STUDENT
Fontaneda Arenas, David

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



Cancer progression and evolutionary accumulation models: main steps.(a) Features of relevance (e.g., mutations in genes) are measured on some subjects (e.g., patients). (b) Data are arranged as a binary matrix of samples by features. For cancer data, each row in (b) is a mutational profile. (c) The researcher uses a method to infer the dependencies in the order of accumulation of the events (or mutations) from the data in (b). Some methods (left) model stochastic dependencies for the transitions between the combinations of events, other methods (right) model deterministic relationships encoded as graphs; in both figures, letters denote events or gene alterations (the column names of the matrix in (b)). Each method finds the parameters of the model (interaction and spontaneous rates or transition probabilities for models with stochastic dependencies; trees/graphs, type of dependencies, and rates/conditional probabilities for models with deterministic dependencies) that allow that model to provide the closest match between predicted and observed frequencies of mutational profiles (the data in (b)). (d) Some uses of cancer progression and evolutionary models. Left: predicting the next genotype and predicting the paths of progression of the disease; edge weights give predicted probabilities. Centre: distribution of ordering of some feature (e.g., a mutation in a gene) between patients with different prognosis, facilitating stratification. Right: patients are stratified based on evolutionary trajectory, and the survival of the different groups is compared.

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:

Diaz-Colunga, J.; Skwara, A.; Gowda, K.; Diaz-Uriarte, R.; Tikhonov, M.; Bajic, D.; Sanchez, A. Global epistasis on fitness landscapes. Phil. Trans. Roc. Soc. B. 2023, 378 (1877), 20220053. https://doi.org/10.1098/rstb.2022.0053.

DOCTORAL THESES AND OTHER WORKS:

David Fontaneda Arenas
"Final degree's project: *An agent-based model of tumor growth to predict adaptive therapy success*". Universidad Autónoma de Madrid. Medicina. 2023. Supervisor/s: Ramón Díaz Uriarte.

Laurentiu Mihai Adetu
"Final degree's project: *Modelos evolutivos en cáncer: mejoras y extensiones a web apps interactivas. Automatización de pruebas con Selenium WebDriver*". Universidad Autónoma de Madrid. Escuela Politécnica Superior. 2023. Supervisor/s: Ramón Díaz Uriarte.

Sergio García-Vaquero
"Final degree's project. *Interfaz de Usuario para ejecución de simulaciones tumorales*". Universidad Autónoma de Madrid. Escuela Politécnica Superior. 2023. Supervisor/s: Ramón Díaz Uriarte.

FUNDING

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

Colon Cancer: Organoids, Microenvironment and Vitamin D

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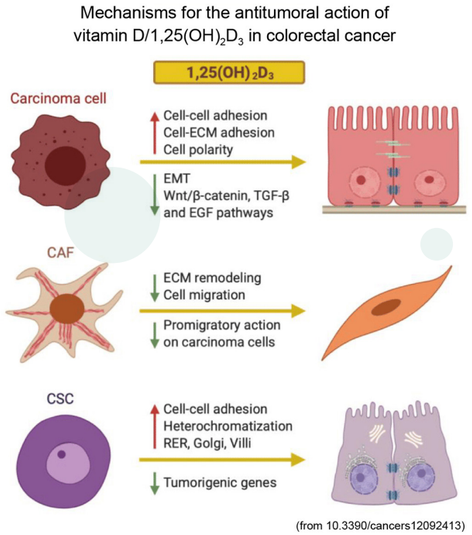
Albandea Rodríguez, David

KEYWORDS

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



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 collaborative efforts that lead to identify the epigenetic regulator SIRT1 as a vitamin D target gene in colon cancer and to characterize the mechanism responsible for the beneficial effects of vitamin D in pulmonary hypertension. We have also contributed to describe that an antibody-drug conjugate that targets the amino acid transporter subunit CD98hc has antitumor effects in colon cancer by analyzing its activity in colon organoids.

PUBLICATIONS:

Prieto, I.; Barbáchano, A.; Rodríguez-Salas, N.; Viñal, D.; Cortés-Guiral, D.; Muñoz, A.; Fernández-Barral, A. Tailored chemotherapy for colorectal cancer peritoneal metastases based on a drug-screening platform in patient-derived organoids: a case report. *J. Gastrointest. Oncol.* **2023**, 14(1), 442-449. DOI: 10.21037/jgo-22-599.

Martínez-Pérez, D.; Viñal, D.; Peña-López, J.; Jiménez-Bou, D.; Ruiz-Gutiérrez, I.; Martínez-Recio, S.; Alameda-Guijarro, M.; Rueda-Lara, A.; Martín-Montalvo, G.; Ghanem, I.; Custodio, A.B.; Trilla-Fuertes, L.; Gámez-Pozo, A.; Barbáchano, A.; Rodríguez-Cobos, J.; Bustamante-Madrid, P.; Fernández-Barral, A.; Burgos, A.; Prieto-Nieto, M.I.; Pastrian, L.G.; González-Sancho, J.M.; Muñoz, A.; Feliu, J.; Rodríguez-Salas N. Clinico-pathological features, outcomes and impacts of COVID-19 pandemic on patients with early-onset colorectal cancer: A single-institution experience. *Cancers.* **2023**, 15(17), 4242. DOI: 10.3390/cancers15174242.

García-Martínez, J.M.; Chocarro-Calvo, A.; Martínez-Useros, J.; Fernández-Aceñero, M.J.; Fiuza, M.C.; Cáceres-Rentero, J.: De la Vieja, A.; Barbáchano, A.; Muñoz, A.; Larriba, M.J.; García-Jiménez, C. Vitamin D induces SIRT1 activation through K610 deacetylation in colon cancer. *eLife.* **2023**, 12, RP86913. DOI: 10.7554/eLife.86913

Olivencia, M.A.; Villegas-Esguevillas, M.; Sancho, M.; Barreira, B.; Paternoster, E.; Adão, R.; Larriba, M.J.; Cogolludo, A.; Pérez-Vizcaino, F. Vitamin D receptor deficiency upregulates pulmonary artery Kv7 channel activity. *Int. J. Mol. Sci.* **2023**, 24(15), 12350. 10.3390/ijms241512350.

Montero, J.C.; Del Carmen, S.; Abad, M.; Sayagués, J.M.; Barbáchano, A.; Fernández-Barral, A.; Muñoz, A.; Pandiella, A. An amino acid transporter subunit as an antibody-drug conjugate target in colorectal cancer. J. Exp. Clin. *Cancer Res.* **2023,** 42(1), 200. 10.1186/s13046-023-02784-0.

Martínez, N.; Gragera, T.; de Lucas, M.P.; Cámara, A.B.; Ballester, A.; Anta, B.; Fernández-Medarde, A.; López-Briones, T.; Ortega, J.; Peña-Jiménez, D.; Barbáchano, A.; Montero-Calle, A.; Cordero, V.; Barderas, R.; Iglesias, T.; Yunta, M.; Oliva, J.L.; Muñoz, A.; Santos, E.; Zarich, N.; Rojas-Cabañeros, J.M. PKD phosphorylation and COP9/Signalosome modulate intracellular Spry2 protein stability. *Oncogenesis* **2023**, 12(1), 20. 10.1038/s41389-023-00465-3.

Agudo-Ibáñez, L.; Morante, M.; García-Gutiérrez, L.; Quintanilla, A.; Rodríguez, J.; Muñoz, A.; León, J.; Crespo, P. ERK2 stimulates MYC transcription by anchoring CDK9 to the MYC promoter in a kinase activity-independent manner.
Sci. Signal. **2023**, 16(794), eadg4193. 10.1126/scisignal.adg4193.

FUNDING

"Patient-derived organoids and primary fibroblast cultures to study the effects of vitamin D on human colon physiology and pathology. PID2019-104867RB-I00". MICINN. 2020-2023

"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

Cancer Stem Cells and Fibroinflammatory Microenvironment

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

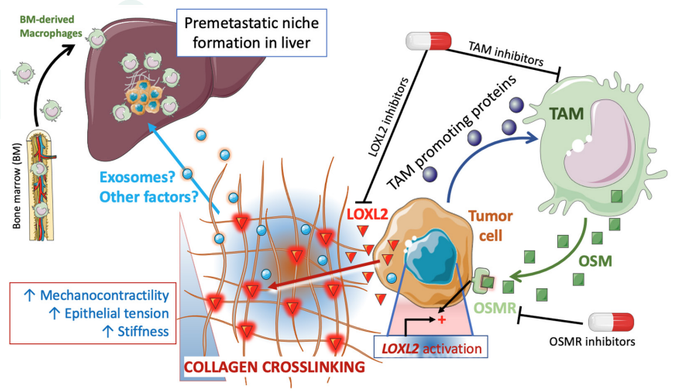

Figure. Role of LOXL2 in PDAC. PDAC tumor cells secrete factors that convert macrophages into tumor-associated macrophages (TAMs), which then release Oncostatin M (OSM). OSM activates LOXL2 transcription via OSM receptor (OSMR). Secreted LOXL2 crosslinks collagen fibers, increasing ECM stiffness, epithelial tension, and mechanocontractility. This organized ECM promotes exosome-mediated communication with secondary organs, fostering premetastatic niches and aiding tumor cell metastasis. Targeting LOXL2, TAMs, or OSM signaling could offer new therapeutic strategies to reduce PDAC metastasis.

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.

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:

Alonso-Nocelo, M.; Ruiz-Cañas, L.; Sancho, P.; Görgülü, K.; Alcalá, S.; Perdero, C.; Vallespinos, M.; López-Gil, J.C.; Ochando, M.; García-García, E.; Trabulo, S.; Martinelli, P.; Sánchez-Tomero, P.; Sánchez Palomo, C.; Santamaría, P.G.; Yuste, L.; Wörmann, S.M.; Kabacaoglu, D.; Earl, J.; Martin, A.; Salvador, F.; Valle, S.; Martin-Hijano, L.; Carrato, A.; Erkan, M.; Garcia-Bermejo, L.; Hermann, P.C.; Algül, H.; Moreno-Bueno, G.; Heeschen, C.; Portillo, F.; Cano, A.; Sainz Jr., B. Macrophages direct cancer cells through a LOXL2-mediated metastatic cascade in pancreatic ductal adenocarcinoma. *Gut.* **2023**, 72(2), 345-359. DOI: 10.1136/gutjnl-2021-325564.

Carrión-Marchante, R.; Pinto-Díez, C.; Klett-Mingo, J.I.; Palacios, E.; Barragán-Usero, M.; Pérez-Morgado, M.I.; Pascual-Mellado, M.; Alcalá, S.; Ruiz-Cañas, L.; Sainz Jr., B.; González, V.M.; Martín, M.E. An Aptamer against MNK1 for Non-Small Cell Lung Cancer Treatment. *Pharmaceutics.* **2023**, 15(4), 1273. DOI: 10.3390/pharmaceutics15041273.

Díaz-Alejo, J.F.; April-Monn, S.; Cihova, M.; Buocikova, V.; Villalón López, J.; Urbanova, M.; Lechuga, C.G.; Tomas, M.; Dubovan, P.; Sánchez, B.L.; Páez, S.C.; Sanjuanbenito, A.; Lobo, E.; Romio de la Heras, E.; Guerra, C.; de la Pinta, C.; Barreto Melian, E.; Rodríguez Garrote, M.; Carrato, A.; Ruiz-Cañas, L.; Sainz Jr., B.; Torres, A.; Smolkova, B.; Earl, J. Establishment of Pancreatic Cancer-Derived Tumor Organoids and Fibroblasts From Fresh Tissue. *J Vis Exp.* **2023**, 195. DOI: 10.3791/65229.

Urbanova, M.; Cihova, M.; Buocikova, V.; Slopovsky, J.; Dubovan, P.; Pindak, D.; Tomas, M.; García-Bermejo, L.; Rodríguez-Garrote, M.; Earl, J.; Kohl, Y.; Kataki, A.; Dusinska, M.; Sainz Jr., B.; Smolkova, B.; Gabelova, A. Nanomedicine and Epigenetics: New Alliances to Increase the Odds in Pancreatic Cancer Survival. Biomed Pharmacother. **2023**, *165*, 115179. DOI: 10.1016/j.biopha.2023.115179.

Alors-Pérez E.; Pedraza-Arevalo S.; Blázquez-Encinas R.; Moreno-Montilla M.T.; García-Vioque V.; Berbel I.; Luque R.M.; Sainz Jr., B.; Ibáñez-Costa A.; Castaño J.P. Splicing Alterations in Pancreatic Ductal Adenocarcinoma: A New Molecular Landscape with Translational Potential. J Exp Clin Can Res, **2023**, 42(1), 282. DOI: 10.1186/s13046-023-02858-z.

Zheng, Q.; Tang, J.; Aicher, A.; Bou Kheir, T.; Sabanovic, B.; Anantha-narayanan, P.; Reina, C.; Chen, M.; Gu, J.M.; He, B.; Alcala, S.; Behrens, D.; Lawlo, R.T.; Scarpa, A.; Hidalgo, M.; Sainz Jr. B.; Sancho, P.; Heeschen, C. Inhibiting NR5A2 targets stemness in pancreatic cancer by disrupting SOX2/MYC signaling and restoring chemosensitivity. J Exp Clin Can Res, **2023**, *42(1)*, 323. DOI: 10.1186/s13046-023-02883-y.

FUNDING:

"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

"Preclinical Development of an Aptamer for Cancer Treatment. RTC2019-007227-1". MCIU. 2020-2023

Cell Cycle & Cancer Biomarkers

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Cambón Hernández, Aitana
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UNDERGRADUATE STUDENTS
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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.

Finally, we are also interested in determining the physiological role of cell cycle genes that serve as therapeutic targets in cancer, with the intention of defining possible biological side effects of drugs related to the cell cycle.

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

Researchers Involved: Sanz, Natalia; Monfort, Ana; Bertinetti, Cristina.

Cell division and CIN genes are often overexpressed in tumors, and this commonly confers poor prognosis to the patients. We want to evaluate if the expression of CIN genes can predict sensitivity to different pharmacologic drugs. For this, we have a collaboration with the pharma company Lilly (SPAIN), where we have 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; Ballesteros, Sandra; Cambón, Aitana; Escribano, María.

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

To this end, we have created a platform for genome-wide genetic screens using CRISPR-Cas9 technology in breast cancer cell lines. In addition, we also use in silico databases of drug response and genetic variation (Cancerxgene.org and DepMap portal) to identify potential correlations for further experimental validation in the laboratory.

Mitotic Regulators: Oncogenes or Tumor Suppressors?

Researchers involved: Sanz, Natalia; Marcos, Guillermo; Escribano, María.

An interesting feature of cell division genes is that they are often overexpressed in cancer, and this confers poor prognosis to the patients. This is typically symbolized by the master mitotic regulator Plk1 (Polo-Like Kinase 1). Plk1 has been considered an oncogene for decades. Surprisingly, in recent years, solid data emerged indicating that Plk1 can also have a role as a tumor suppressor. The logical and immediate question that then arises is: When can Plk1 act as a tumor suppressor or as an oncogene? To this end, we have been able to transform primary fibroblasts in the presence of high Plk1 levels, being Plk1-tolerant cells. These transformed cells are the biological base to find the genetic determinants that overcome the tumor suppressor barrier. We also have generated the genetically engineered mouse models (GEMMs) that will serve as a physiological platform to test our hypothesis.

The physiological role of the mitotic gene PLK1 in endothelial homeostasis

Researchers involved: González, María

Strategies targeting the cell cycle have been successful in cancer treatment, and many drugs targeting cell cycle genes are currently in the clinic or in advanced clinical trials. However, there is still a need to define the associated toxicities and physiological effects to better understand the use of these cell cycle-targeting drugs. On the other hand, the endothelium is a poorly proliferative tissue and therefore anti-cell cycle drugs should not have a major impact on endothelial biology. However, during tissue regeneration and tumor growth, there is a need for new blood vessels (angiogenesis) to provide oxygen and nutrients to the new tissue. Therefore, understanding the physiological impact of these new drugs on endothelial homeostasis and vascular physiology may reveal new mechanisms of toxicity and consequently new biomarkers to define the right treatment for the right patient. In summary, how mitotic and cell cycle genes interfere with endothelial physiology is, to date, completely unknown, and may open new avenues to understand the process of angiogenesis, and subsequently find new and better therapeutic opportunities. To address this question we have generated a genetically engineered mouse model (GEMM) for specifically depleting the mitotic gene PLK1 in the mouse endothelial cells, and also work with mouse stem cells to simulate angiogenesis in vitro and determine the effect of cell cycle inhibition.

PUBLICATIONS:

Baldrighi, M.; Doreth, C.; Li, Y.; Zhao, X.; Warner, E.; Chenoweth, H.; Kishore, K.; Umrania, Y.; Minde, DP.; Thome, S.; Yu, X.; Lu, Y.; Knapton, A.; Harrison, J.; Clarke, M.; Latz, E.; de Cárcer, G.; Malumbres, M.; Ryffel, B.; Bryant, C.; Liu, J.; Lilley, K. S.; Mallat, Z.; Li, X. PLK1 inhibition dampens NLRP3 inflammasome-elicited response in inflammatory disease models. *J Clin Invest.* **2023**, *133(21)*, e162129. DOI: 10.1172/JCI162129.

Sanz-Gómez, N.; González-Álvarez, M.; De Las Rivas, J.; de Cárcer G. Whole-Genome Doubling as a source of cancer: how, when, where, and why? Front Cell Dev Biol., **2023**, *11*, 1209136. DOI: 10.3389/fcell.2023.1209136.

Monfort-Vengut, A.; de Cárcer, G. Lights and Shadows on the Cancer Multi-Target Inhibitor Rigosertib (ON-01910.Na). Pharmaceutics, **2023**, *15(4)*, 1232. DOI: 10.3390/pharmaceutics15041232.

Marugán, C.; Ortigosa, B.; Sanz-Gómez, N.; Monfort-Vengut, Ana.; Bertinetti, C.; Teijo, Ana.; González, M.; Alonso de la Vega, A.; Lallena, M. J.; Moreno-Bueno, G.; de Cárcer, G. TPX2 expression promotes sensitivity to dasatinib in breast cancer by ativating the YAP transcriptional signaling. *bioRxiv*, **2023**. DOI: 10.1101/2023.09.04.556165

DOCTORAL THESES AND OTHER WORKS:

Sandra Ballesteros Sanchez
"Master´s thesis: *The osmostress WNK1 kinase as a modulator of cancer chemotherapy*". Universidad Autónoma de Madrid. Medicina. 2023. Supervisor/s: Guillermo de Cárcer. Grade: Sobresaliente

Guillermo Marcos Zapatero
"Master´s thesis: *Unraveling the Mechanisms of Whole Genome Doubling modulation in cell transformation*". Universidad Autónoma de Madrid. Medicina. 2023. Supervisor/s: Guillermo de Cárcer and Natalia Sanz. Grade: Sobresaliente

Cristina Bertinetti del Hierro
"Final degree's project: *Finding new therapeutic opportunities for CIN positive breast tumoral cells*". Bath University. UK. 2023. 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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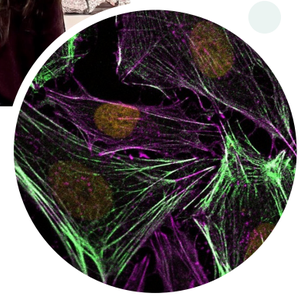
KEYWORDS
Melanoma, Myosin, Cytoskeleton, Therapy Resistance, Metastasis.



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.; Orgaz, J.

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

PUBLICATIONS:

Samain, R.; Maiques, O.; Monger, J.; Lam, H.; Candido, J.; George, S.; Ferrari, N.; Kohlhammer, L.; Lunetto, S.; Varela, A.; Orgaz, J.L.; Vilardell, F.; Olsina, J.J.; Matias-Guiu, X.; Sarker, D.; Biddle, A.; Balkwill, F.R.; Eyles, J.; Wilkinson, R.W.; Kocher, H.M.; Calvo, F.; Wells, C.M.; Sanz-Moreno, V. CD73 controls Myosin II-driven invasion, metastasis, and immunosuppression in amoeboid pancreatic cancer cells. *Sci Adv*. **2023**, *9(42) eadi0244*, 1-19. DOI: 10.1126/sciadv.adi0244.

FUNDING:

"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

Emerging Genes in Thyroid Cancer

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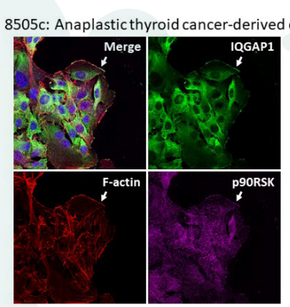
KEYWORDS
Oncogenes, Signaling, RNA editing, MiRNAs, Thyroid cancer



The scaffolding protein IQGAP1 colocalizes with p90RSK in the membrane ruffles of migrating cells

RESEARCH LINES:

Overview

In recent years, our work has focused on identifying new genes involved in thyroid cancer and functionally evaluating their role in tumor progression. The main idea is unraveling the main molecular events that lead to the transformation of the normal thyroid follicular cells into an invasive thyroid carcinoma. Thyroid differentiation is characterized by the presence of iodide metabolizing genes required for thyroid hormone biosynthesis. Expression of these genes is essential for beneficial radioiodine treatment of thyroid tumors. However, some tumors progress to radioiodine-refractory metastatic disease due to loss of differentiation. Currently, the comprehension of how genetic alterations and impaired pathways induce the loss of thyroid differentiation and metastasis formation is a hot topic that remains unresolved.

The main mutations in thyroid cancer are in BRAF, RAS and RET/PTC genes, that lead to aberrant MAPK activation, driving progression through a process of dedifferentiation. However, although significant, these advances have not yet allowed definitive translation to the clinic of curative therapies or reliable diagnostic markers. It is now increasingly clear that this loss of differentiation is a multifactorial process in which a multiplicity of mechanisms are ultimately responsible for tumor progression concomitant with a loss of differentiation.

Our main goal has been to study new diagnostic and therapeutic targets for metastatic thyroid cancer by studying, in an integrative and technically innovative manner, a variety of new genes and mechanisms.

In the 2021 and 2022 memoire, all newly identified emerging genes and their role in thyroid differentiation were described. Here, we will explain the advance in a couple of genes and mechanisms important both for thyroid differentiation and for thyroid progression.

1. Role of the Trancription factor GLIS3 in thyroid differentiation

Santisteban, Pilar.

In the course of our studies, we identified a new transcription factor, GLIS3, which controls thyroid differentiation, which it is able to bind to the promoters of thyroid differentiation genes (Thyroglobulin (Tg), and Sodium Iodide Symporter (NIS)). We have shown that this factor is important in another thyroid pathology, congenital hypothyroidism. Future studies will be aimed at studying the role of GLIS3 in thyroid cancer.

2. New insights into the role of IQGAP scaffolding proteins 1 and 2 in MAPK signaling,

Zaballos, Miguel A; Carrasco-López, Carlos; Makiadi, Jennifer; Santisteban, Pilar.

These scaffold proteins IQGAP serve as dimerization platforms in which ERK dimers are assembled. As the MAPK signaling plays a critical role in thyroid cancer, we have studied what function do these scaffolding proteins in thyroid cancer. Using public databases, we have confirmed that IQGAP1 expression is higher in thyroid tumors than in healthy tissue and it is associated with BRAF mutations and with tumors aggressively. On the other hand, IQGAP2 expression is lower in BRAF tumors and its decrease is associated with an increase in tumor malignancy. Since these scaffolding proteins are involved in multiple signaling pathways, we have analyzed them, and have observed that both IQGAP1 silencing and IQGAP2 overexpression despite not preventing ERK phosphorylation, produces a reduction in the levels of active RSK (p90-ribosomal subunit kinase). RSK is the main cytoplasmic effector of MAPK signaling, involved in cell proliferation, migration and invasion.

Our work would suggest that IQGAP1 and IQGAP2 play different roles in the pathogenesis of thyroid cancer, as IQGAP1 acts as an oncogenic protein while IQGAP2 has a tumor suppressor activity.

PUBLICATIONS

Acuña-Ruiz, A.; Carrasco-López, C.; Santisteban, P. Genomic and epigenomic profile of thyroid cancer. *Best Pract Res Clin Endocrinol Metab.* **2023**, 37 (1),101656. DOI.10.1016/j.beem.2022.101656

Kang, H.S.; Sara. A.; Grimm, S.A.; Jothi R, Santisteban, P.; Jetten, A.M. GLIS3 regulates transcription of thyroid hormone biosynthetic genes in coordination with other thyroid transcription factors. *Cell Biosci*. **2023**, 13(1),32 DOI 10.1186/s13578-023-00979-8

DOCTORAL THESES AND OTHER WORKS

Carlos Carrasco López

"Ph.D. thesis: *Role of the scaffolding proteins IQGAP1 and IQGAP2 in thyroid cancer*". Universidad Autónoma de Madrid. Medicina. 2023. Supervisors: Pilar Santisteban and Miguel A. Zaballos. Calificación: Sobresaliente Cum Laude.

FUNDING

"Análisis molecular integral para el estudio de la diferenciación en el cáncer de tiroides" MICINN. 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

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 **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 theWrapies.

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:

Bargiela-Iparraguirre, J.; Herrero, J. M.; Pajuelo-Lozano, N.; Perez, M.; Perona, R.; Quiroga, A.; Calés, C.; Sanchez-Perez, I., Regulatory effects of miR-19a on MAD2 expression and tumorigenesis in gastric cancer. Genes & Diseases **2023,** *10 (4)*, 1180-1182. DOI: 10.1016/j.gendis.2023.02.025

Herrero, J. M.; Fabra, D.; Matesanz, A. I.; Hernandez, C.; Sanchez-Perez, I.; Quiroga, A. G., Dithiobiureas Palladium(II) complexes' studies: From their synthesis to their biological action. J Inorg Biochem. **2023,** *246*, 112261. DOI: 10.1016/j.jinorgbio.2023.112261.

DOCTORAL THESES AND OTHER WORKS:

Patricia Delgado Aliseda
"Master´s thesis: *Estudio del efecto de complejos metálicos derivados de platino en la hematopoiesis".* Universidad Complutense de Madrid. 2023. Supervisor/s: Carmela Calés e Isabel Sánchez Pérez. Grade: Sobresaliente

Javier Montero Gutierrez
"Master´s thesis: *Efficient and selective thiosemicarbazones for heavy metal sequestration from water. Aqueus Stability studies in water of their stable nontoxic materials".* Máster en Nanociencia Molecular y Nanotecnología, Universidad Autónoma de Madrid. 2023. Supervisor/s: Adoración Gomez Quiroga e Isabel Sánchez Pérez. Grade: Sobresaliente

Paula Giménez Meliá
"Master´s thesis: *Evaluación in vitro y ex vivo del potencial antitumoral de nuevos metalofármacos".* Máster de Investigación en Medicina Traslacional. Universidad Complutense de Madrid 2023. Supervisor/s: Carmela Calés y Jorge Melones Herrero. Grade: Notable

Gabriel Rivas Hernández
"Final degree's project: *Estudio del rol de los microARNs en el efecto anti-senescente yanti-inflamatorio vascular de la angiotensina (1-7)er".* Instituto Tecnológico de Costa Riica & Universidad Autónoma de Madrid. 2023. Supervisor/s: Concepción Peiró e Isabel Sánchez 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

"Metallic drugs with alternative structures to explore their potential in Biological Chemistry and induce cell death and specific damage in tumors. PID2019-106220RB-I00". Ministry of Science and Innovation (MICINN). 2019- 2023

"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.**

AWARDS:

"New copper metallodrug CuL as antitumoral candidate."J. Velazquez Gutierrez, A. Gomez Quiroga, I Sánchez Pérez 2nd SRUK/CERU Cancer Research Networking Day. Comunicación Oral 18-09-2023 Madrid, Centro Nacional de Investigaciones Oncológicas (CNIO). Award Fundación Cris Contra El Cáncer.

Molecular Mechanisms of Aging and Cancer

PRINCIPAL INVESTIGATOR
Link, Wolfgang

ASSOCIATED INVESTIGATOR
Mayoral Varo, Víctor

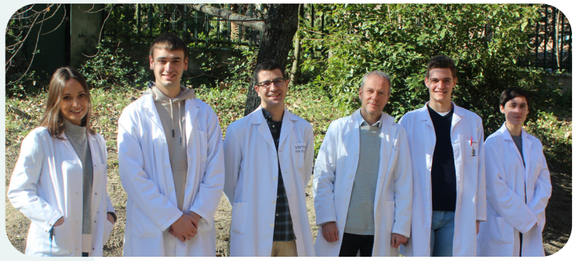
STAFF INVESTIGATOR
Jiménez Gómez, Lucía

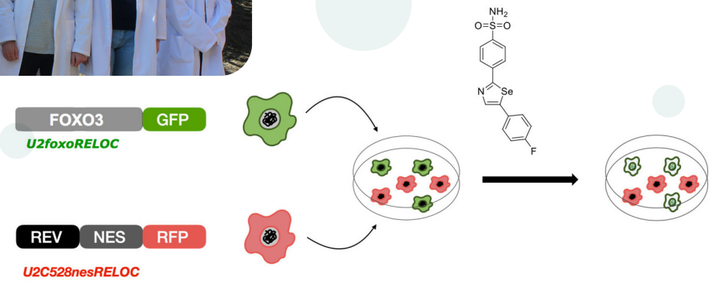
PREDOCTORAL INVESTIGATOR
Amenábar Blázquez, Carlos

MASTER THESIS STUDENT
Carmona Mayoral, Paula

UNDERGRADUATE STUDENT
Domínguez Esteban, Lucia

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





CRM1 is a nuclear export receptor that has been considered as a promising therapeutic target for the treatment of many types of cancers and viral infections. Here we report the development of a multiplexed platform to analyze the CRM1-dependence of any protein of interest as shown for the human proteins PDK1, p110α, STAT5A, FOXO1/3/4 and TRIB2. Compound screening revealed the striking inhibitory activity of an organoselenium compound on CRM1 and nuclear export of. endogenous CRM1 substrates.

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:

Santos, B; Grenho, I.; Martel, P; Ferreira, B.I. and Link W. (2023) FOXO family isoforms. *Cell Death Dis.* **2023**, *14*, 702. DOI: 10.1038/s41419-023-06177-1.

Velasco, G. and Link W. Pseudokinases, Tribbles Proteins and Cancer. *Cancers*. **2023**, *15*, 3547. DOI: 10.3390/cancers15143547.

Gacias, M; O'Neill, B; Relat, J.; Link, W.; Haro, D.; Marrero, P. and De Sousa-Coelho A.L. FOXO1 Represses PPARa-Mediated Induction of FGF21 Gene Expression. *Biochemical and Biophysical Research Communications.* **2023**. *644*, 122-129. DOIi: 10.1016/j.bbrc.2023.01.012

DOCTORAL THESES AND OTHER WORKS:

Lucia Domínguez Esteban

"Final degree's project: *Characterization of inhibitor compounds of CRM1 and validation in glioblastoma cell models*". Universidad Autónoma de Madrid. 2023. Supervisors: Wolfgang Alexander Link and Lucía Jiménez Gómez

FUNDING:

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

Biomarkers and Tumor Microenvironment

PRINCIPAL INVESTIGATOR
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Collado Valero, Manuel

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 **close 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 cancer.

PUBLICATIONS:

Collado, M.; Castillo, M.; Muñoz de Mier, GJ.; del Pinta. C.; Peña. C. The Diet as a Modulator of Tumor Microenvironment in Colorectal Cancer Patients. *Int J Mol Sci.* **2023**, 24, 7317. DOI: 10.3390/ijms24087317

DOCTORAL THESES AND OTHER WORKS:

Ángela Aparicio Valencia
"Final degree's project: "*Puesta a punto del estudio del papel de los fibroblastos en los mecanismos de radiorresistencia en cáncer colorrectal*". Universidad de Alcalá. Biosanitaria. 2023. Supervisor/s: Cristina Peña. Grade: Sobresaliente

FUNDING:

"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

"Plataforma biobancos y biomodelos . PT20/00045". ISCIII. 2021-2023

"Identificación de pacientes con cáncer de colon en estadio III con alto riesgo de recurrencia basado en un modelo radiómico derivado de biomarcadores del microambiente tumoral. PI20/00602". ISCIII. 2021-2023

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

"Analysis of music on cell behavior. PID2436". MICINN. 2016-2019

Molecular Mechanisms Involved In Hepatocelular Carcinome Development

PRINCIPAL INVESTIGATOR
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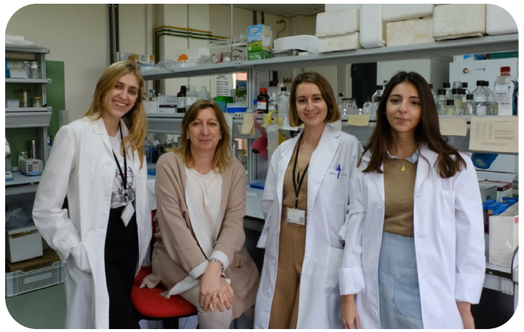
PREDOCTORAL INVESTIGATOR
López López Ana
Camblor Murube, Marina

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



RESEARCH LINES:

Hepatocellular carcinoma molecular mechanisms

Researchers involved: Camblor Murube, Marina.

Hepatocellular carcinoma (HCC) is the leading cause of liver transplantation and one of the most common cancers. Previous results from our laboratory point to AURKB, a protein that regulates chromosomal segregation and cytokinesis processes during mitosis, as a possible marker for the evolution of liver fibrosis and/or cirrhosis and HCC. Therefore, we are studying the role of AURKB in the development of fibrosis/cirrhosis and HCC, on a cohort of 348 patients with chronic hepatitis C that demonstrates how the presence of two AURKB SNPs is significantly associated with liver fibrosis progression and HCC outcome. One of these SNPs is codified for a threonine residue that contributes to the kinase activity of AURKB, essential in the phosphorylation of P53 and the CHMP4C protein. Thus, these SNPs could contribute to precancerous lesions development in the liver through defects in the cell cycle progression and the chromosomal segregation and cytokinesis.

Genetic studies performed in our laboratory by next-generation sequencing assays of cell populations in which the expression of these variants might explain the fibrosis, cirrhosis, and/or hepatocarcinoma development observed in a cohort of patients infected with the hepatitis C virus.

Microbiota modifications in oncological patients treated with biological therapy

Researchers involved: López López, Ana; Camblor Murube, Marina

Our laboratory is interested in studying the effects of immunotherapy on the intestinal microbiota population. 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.

These treatments increase the survival of cancer patients of different types but show a high incidence (around 85%) of gastrointestinal side effects, primary colitis, or intestinal perforations that compromise the continuity of therapy. In addition, one of the most serious processes, frequently associated with cancer, is malnutrition. 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. Microbiota is essential for proper body growth, with key functions such as metabolism, immunity regulation, and mediating systemic inflammation. Recent studies have shown that modifications in the intestinal microbiota could lead to chronic inflammation processes, which could be considered a predictive factor for side effects outcomes at the level of digestive function. Therefore, we have analyzed the composition and evolution of the microbiota in cancer patients using a new technology performed in our laboratory by nanopore massive sequencing (MinION). These studies are focused on anticipating both the appearance of nutritional disorders that compromise adherence to treatment and identifying the appearance of risk factors for the development of intestinal inflammation

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 can contribute to designing innovative treatments for chronic liver disease, including HCC.

PUBLICATIONS:

García-Crespo, C; Francisco-Recuero, I; Gallego, I; Camblor-Murube, M; Soria, ME; López-López, A; de Ávila, AI; Madejón, A; García-Samaniego, J; Domingo, E*; Sánchez-Pacheco, A*; Perales C*. *Corresponding authors. "Hepatitis C virus fitness can influence the extent of infection-mediated epigenetic modifications in the host cells".
Front Cell Infect Microbiol. **2023** *Mar13*, 13:1057082. DOI:10.3389/fcimb.2023.1057082.

DOCTORAL THESES AND OTHER WORKS:

Ana López López.
"Ph.D. tesis: Mención Doctorado Industrial. *Patrones de microRNAs y alteraciones de la microbiota intestinal en pacientes oncológicos tratados con inmunoterapia*". Universidad Autónoma de Madrid. Biociencias Moleculares. 2023. Supervisor: Aurora Sánchez Pacheco. Grade: Sobresaliente Cum Laude.

FUNDING:

"Formulas nutricionales para el control del déficit de ácido docosahexaenoico DHA y ácido araquidónico 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.

PATENTS:

"A stem-loop primer and a method for short length RNA detection". Sánchez-Pacheco A.; López-Lopez A.; Camblor M. Murube. EP22383065.4.2022.

AWARDS:

"3er premio al mejor proyecto de emprendimiento. Aurora Sánchez Pacheco. Proyecto Cancer Catch. Programa de Emprendimiento para investigadores de la Salud de UAM emprende y Genesis Biomed 2023. DIHBio CAM". 2023

"XXIX CONGRESO Neonatología y Medicina Perinatal.. Variaciones genéticas en FADS 1 Y 2 correlacionan con diferentes concentraciones de PUFA en neonatos prematuros. Oral presentation: Camblor Murube M". Santiago de Compostela, 2023

"XXII CONGRESO Seinap.. Variaciones genéticas en FADS 1 Y 2 correlacionan con diferentes concentraciones de PUFA en neonatos prematuros. Oral presentation: Camblor Murube M ". Madrid, 2023

Melanoma Plasticity In Metastasis And Immunotherapy Resistance

PRINCIPAL INVESTIGATOR
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TECHNICAL SUPPORT PERSONNEL
López Rodrigo, María Isabel

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 BioRxiv (Daugherty-Lopès et al., BioRxiv doi: https://doi.org/10.1101/2024.08.26.609785). We also contributed to the preparation of a white review paper summarizing the conclusions of the Melanoma Research Foundation Brain Metastases Summit 3.0 (Karz et al., 2022. Pigment Cell Melanoma Res. DOI: 10.1111/pcmr.13059).

PUBLICATIONS:

Pagadala, M.; Sears, T.J.; Wu, V.H.; Pérez-Guijarro, E.; Kim, H.; Castro, A.; Talwar, J.V.; Gonzalez-Colin, C.; Cao, S.; Schmiedel, B.J.; Goudarzi, S.; Kirani, D.; Au, J.; Zhang, T.; Landi, T.; Salem RM.; Morris GP.; Harismendy O.; Patel SP.; Alexandrov LB.; Mesirov JP.; Zanetti M.; Day CP.; Fan CC.; Thompson, W.K.; Merlino, G.; Gutkind, J.S.; Vijayanand, P.; Carter, H. Germline modifiers of the tumor immune microenvironment implicate drivers of cancer risk and immunotherapy response. *Nat. Commun.* **2023**, *14(1)*, 2744. DOI: 10.1038/s41467-023-38271-5.

Gruen, C.; Yang, H.H.; Sassano, A.; Wu, E.; Gopalan, V.; Marie, K.L.; Castro, A.; Mehrabadi, F.R.; Wu, C.H.; Church, I.; Needle, G.A.; Smith, C.; Chin, S.; Ebersole, J.; Marcelus, C.; Fon, A.; Liu, H.; Malikic, S.; Sahinalp, C.; Carter, H.; Hannenhalli, S.; Day, C.P.; Lee, M.P.; Merlino, G.; Pérez-Guijarro, E. Melanoma clonal subline analysis uncovers heterogeneity-driven immunotherapy resistance mechanisms. *bioRxiv [Preprint].* **2023** Apr 5:2023.04.03.535074. DOI: 10.1101/2023.04.03.535074.

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

AWARDS:

"Poster award to E. Perez-Guijarro at CNIO-CaixaResearch Frontiers Meeting 2023- Metastasis." 2023

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

PRINCIPAL INVESTIGATOR
Moreno Bueno, Gema

STAFF INVESTIGATOR
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Lázaro Encinas, Sara
Klett Mingo, José Ignacio

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Ballesteros Sánchez, Sandra

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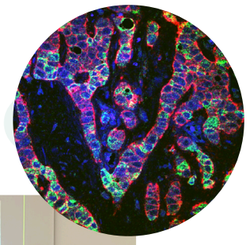
González Paramos, Cristina



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 A) 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 new approaches to recapitulate genomic and intratumoral genetic heterogeneity.
- 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.

PUBLICATIONS:

Alonso-Nocelo, M.; Ruiz-Canas, L.; Sancho, P.; Gorgulu, K.; Alcala, S.; Pedrero, C.; Vallespinos, M.; Lopez-Gil, J. C.; Ochando, M.; Garcia-Garcia, E.; et al. Macrophages direct cancer cells through a LOXL2-mediated metastatic cascade in pancreatic ductal adenocarcinoma. *Gut.* **2023**, *72 (2)*, 345-359. DOI: 10.1136/gutjnl-2021-325564.

Carretero-Barrio, I.; Caniego-Casas, T.; Rosas, M.; Sánchez, M.; Martínez-Jáñez, N.; Chiva, M.; Sarrió, D.; Moreno-Bueno, G.; Palacios, J.; Pérez-Mies, B. Evaluation of ERBB2 mRNA Expression in HER2-Equivocal (2+) Immunohistochemistry Cases. *CANCERS.* **2023**, *15 (6)*. DOI: 10.3390/cancers15061688.

Casas-Arozamena, C.; Moiola, C. P.; Vilar, A.; Bouso, M.; Cueva, J.; Cabrera, S.; Sampayo, V.; Arias, E.; Abalo, A.; Garcia, A.; et al. Noninvasive detection of microsatellite instability in patients with endometrial cancer. *International journal of cancer.* **2023**, *152 (10)*, 2206-2217. DOI: 10.1002/ijc.34435.

Colomo, S.; Ros-Pardo, D.; Oltra, S.; Gomez-Puertas, P.; Sarrio, D.; Moreno-Bueno, G. Structural and functional insights into GSDMB isoforms complex roles in pathogenesis. *CELL CYCLE.* **2023**, *22 (20)*, 2346-2359. DOI: 10.1080/15384101.2023.2287933.

(5) Lores, S.; Gámez-Chiachio, M.; Cascallar, M.; Ramos-Nebot, C.; Hurtado, P.; Alijas, S.; López, R.; Piñeiro, R.; Moreno-Bueno, G.; de la Fuente, M. Effectiveness of a novel gene nanotherapy based on putrescine for cancer treatment. *BIOMATERIALS SCIENCE.* **2023**, *11 (12)*, 4210-4225. DOI: 10.1039/d2bm01456d.

(6) Mendoza, G.; González-Pastor, R.; Sánchez, J.; Arce-Cerezo, A.; Quintanilla, M.; Moreno-Bueno, G.; Pujol, A.; Belmar-López, C.; de Martino, A.; Riu, E.; et al. The E1a Adenoviral Gene Upregulates the Yamanaka Factors to Induce Partial Cellular Reprogramming. *CELLS.* **2023**, 12 (9). DOI: 10.3390/cells12091338.

(7) Oltra, S. S.; Colomo, S.; Sin, L.; Perez-Lopez, M.; Lazaro, S.; Molina-Crespo, A.; Choi, K.-H.; Ros-Pardo, D.; Martinez, L.; Morales, S.; et al. Distinct GSDMB protein isoforms and protease cleavage processes differentially control pyroptotic cell death and mitochondrial damage in cancer cells. Cell death and differentiation. **2023**, *30 (5)*, 1366-1381. DOI: 10.1038/s41418-023-01143-y.

Tundidor, I.; Seijo-Vila, M.; Blasco-Benito, S.; Rubert-Hernández, M.; Adámez, S.; Andradas, C.; Manzano, S.; Alvarez-López, I.; Sarasqueta, C.; Villa-Morales, M.; et al. Identification of fatty acid amide hydrolase as a metastasis suppressor in breast cancer. *NATURE COMMUNICATIONS*. **2023**, *14 (1)*. DOI: 10.1038/s41467-023-38750-9.

DOCTORAL THESES AND OTHER WORKS:

Manuel Gámez Chiachio
"Ph.D. thesis: *The dual implication of GSDMB in cancer biology: from HER2-targeted therapy resistance to cell death induction*" Universidad Autónoma de Madrid. Medicina. 2023. Supervisor/s: Gema Moreno Bueno, José David Sarrió López. Grade: Sobresaliente Cum Laude

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". Agencia Estatal de Investigación (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.

"Identification of a Gasdermin B-related pyroptotic signature (GSDMB) as a predictor of response in HER2 breast tumours (PIROPTEST)" Fundación Contigo Contra el Cáncer de la Mujer. 2022-2023.

"Molecular characterization of Gasdermin B cytotoxic activity and its potential application for the treatment of cancer (GB-citoTOX). PID2019-104644RB-I00" Ministerio de Ciencia e Innovación. Ministerio de Universidades. 2020-2023.

"Targeting Gasdermin-B overexpression as a new therapeutic approach in Her2+ cancers. PROYE19036MORE" FCAECC. 2019-2023.

"Targeting the most aggressive type of endometrial carcinoma. GCTRA-18014MATI". Fundación Científica Asociación Española Contra el Cáncer (FCAECC). 2018-2024.

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

PATENTS:

"Fragments of the N-Terminal domain of GSDMB for the treatment of cancer". María De La Fuente Freire, Saínza Lores Touriño, José David Sarrió López, Manuel Gamez Chiachio, Gema Moreno Bueno. EP23382104.0" 2023

AWARDS:

"*Premio Con Nombre de Mujer por la participación destacada en CONÓCELAS 2022. Premios Fundación Merck Salud- ASEICA, al Impulso de las Vocaciones Científicas*" 2023

Cellular Senescence in Cancer and Other Pathologies

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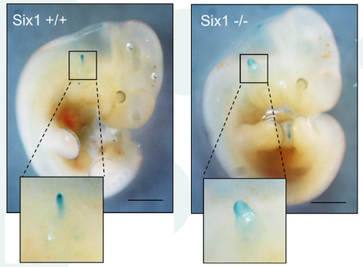
PRE-DOCTORAL INVESTIGATOR
Casa Rodríguez, Nuria

UNDERGRADUATE STUDENT
Rodríguez Diaz, Enriqueta

KEYWORDS
Cell senescence, Cancer, Plasticity, Development.



Aberrant senescence in the developing inner ear of *Six1*-deficient E11.5 mouse embryos, detected with SA-BetaGal staining, in blue (*De Lope, Development 2023*).

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 to this role against stress, senescence also participates 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 cancer and development

Previous work from our group showed that the homeoprotein SIX1 is an essential repressor of senescence in different cellular settings (Adrados, Oncogene, 2016, PMID 26500063). SIX1 is a member of the SIX/EYA developmental regulatory pathway, and alterations in this pathway are the genetic basis for the rare human BOR (Branchio-Oto-Renal) developmental syndrome (OMIM 113650, ORPHA 107). On the other hand, SIX1 is an oncogene, frequently overexpressed in different types of tumors, where it is usually associated to poor prognosis, stem phenotypes and invasiveness. Based on the well-established role of senescence in both embryogenesis and tumorigenesis, we have studied the relevance of the link SIX1-senescence in the context of cancer (De Lope, Sci Rep, 2019, PMID 30723235) and embryonic development (De Lope, Development, 2023, PMID 37017267). During this period, we characterized the role of senescence in developmental defects associated to defective SIX/EYA function. To do this, we used Six1-null embryos, a mouse model of the BOR syndrome, focusing on the developing inner ear, an organ with physiological senescence that is severely affected in Six1-deficient mice and BOR patients. Using a combination of immunohistochemical and transcriptional studies, we showed aberrant levels and distribution of senescence markers in Six1-deficient inner ears, concomitant with defective morphogenesis. Gene expression profiling and ex vivo senescence manipulation assays supported a link between aberrant senescence and altered morphogenesis in this model, associated with deregulation of the TGFβ/BMP pathway (De Lope, Development, 2023, PMID 37017267). These results support the notion that mis-regulation of embryo senescence may lead to genetic developmental disorders, expanding the connection between senescence and disease.

PUBLICATIONS:

de Lope, C.; Garcia-Lucena, R.; Magarinos, M.; Leon, Y.; Casa-Rodriguez, N.; Contreras, N.; Escudero-Iriarte, C.; Varela-Nieto, I.; Maire, P.; Palmero, I., Dysfunction of programmed embryo senescence is linked to genetic developmental defects. *Development.* **2023**, *150* (9).

DOCTORAL THESES AND OTHER WORKS:

Enriqueta Rodríguez Diaz
"*Final degreé s project: Senescence and cancer: SASP-mediated paracrine communication in the tumor microenvironment*". Universidad Autónoma de Madrid. 2023. Supervisor: Ignacio Palmero. Grade: Sobresaliente

FUNDING:

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

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

department
of **Metabolic & Immune Diseases**

Cell Compartmentalization, Homeostasis and Inflammation

PRINCIPAL INVESTIGATOR
Sánchez-Álvarez, Miguel

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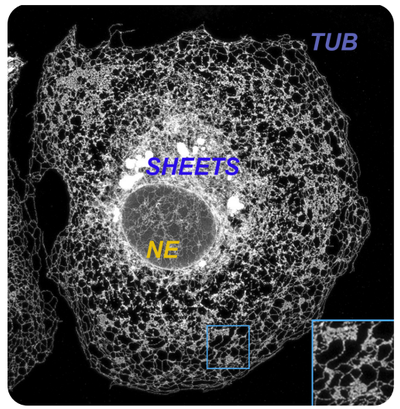
KEYWORDS
Organelles, Secretory Apparatus, Cell stress, Systems biology, Cell defense.



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 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.

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:

Lolo, F.-N.; Walani, N.; Seemann, E.; Zalvidea, D.; Pavón, D. M.; Cojoc, G.; Zamai, M.; Viaris de Lesegno, C.; Martínez de Benito, F.; Sánchez-Álvarez, M.; Uriarte, J. J.; Echarri, A.; Jiménez-Carretero, D.; Escolano, J.-C.; Sánchez, S. A.; Caiolfa, V. R.; Navajas, D.; Trepat, X.; Guck, J.; Lamaze, C.; Roca-Cusachs, P.; Kessels, M. M.; Qualmann, B.; Arroyo, M.; del Pozo, M. A. Caveolin-1 dolines form a distinct and rapid caveolae-independent mechanoadaptation system. *Nat. Cell. Biol.* **2023**, *25 (1)*, 120–133. DOI: 10.1038/s41556-022-01034-3.

Aizarna-Lopetegui, U.; García-Astrain, C.; Renero-Lecuna, C.; González-Callejo, P.; Villaluenga, I.; del Pozo, M. A.; Sánchez-Álvarez, M.; Henriksen-Lacey, M.; Jimenez de Aberasturi, D. Remodeling Arteries: Studying the Mechanical Properties of 3D-Bioprinted Hybrid Photoresponsive Materials. *J. Mater. Chem.* B **2023**, *11* (39), 9431–9442. DOI:10.1039/D3TB01480K.

Safi, R.; Sánchez-Álvarez, M.; Bosch, M.; Demangel, C.; Parton, R. G.; Pol, A. Defensive-Lipid Droplets: Cellular Organelles Designed for Antimicrobial Immunity. *Immunol. Rev.* **2023**, *317* (1), 113–136. DOI: 10.1111/imr.13199.

López-Méndez, T. B.; Sánchez-Álvarez, M.; Trionfetti, F.; Pedraz, J. L.; Tripodi, M.; Cordani, M.; Strippoli, R.; González-Valdivieso, J. Nanomedicine for Autophagy Modulation in Cancer Therapy: A Clinical Perspective. *Cell. Biosci.* **2023**, *13* DOI: 10.1186/s13578-023-00986-9.

DOCTORAL THESES AND OTHER WORKS:

María Heredia García
"Final degree's project: *Novel mechanisms regulating extracelular matrix remodeling: physiopathological relevance*". Universidad Autónoma de Madrid. Biochemistry. 2023. Supervisor/s: Miguel Sánchez Álvarez, Miguel Ángel del Pozo. Grade: 9.4

FUNDING:

"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 mechanoadaptation and innate immunity. Ramón y Cajal contract RYC2020-029690-I". MICINN. 2022-2027

Transcriptional Control of Metabolic Homeostasis

PRINCIPAL INVESTIGATOR
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Staff Investigator
Mirasierra Cuevas, Mercedes

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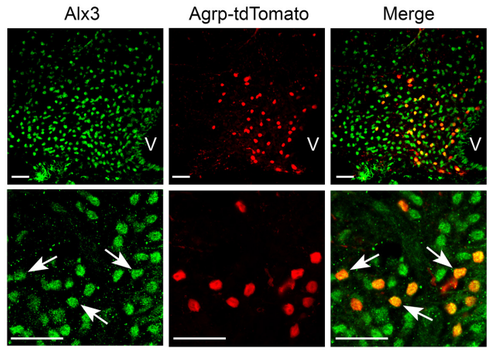
KEYWORDS
Metabolic homeostasis, Diabetes, Pancreatic islets, Hypothalamus, Energy expenditure.



Expression of Alx3 in the hypothalamic arcuate nucleus.

RESEARCH LINES:

Overview

Our group is interested 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.

Hypothalamic regulation of energy metabolism

During this period, we continued with our studies on the central role of circadian rhythms as key components for the regulation of cyclic food intake and energy expenditure. It has been well established for decades that the suprachiasmatic nucleus in the hypothalamus acts as a master circadian pacemaker synchronizing the diurnal oscillations of molecular clocks in the brain and in peripheral tissues. In previous work we found that Aphakia mice used as a model characterized by congenital lack of innervation of the suprachiasmatic nucleus by retinal axons due to hypomorphic expression of the transcription factor gene Pitx3 ($Pitx3^{ak}$), exhibit out-of-phase daily cycles of locomotor activity, feeding patterns, energy expenditure, and corticosterone secretion that are resistant to metabolic entrainment by time-restricted feeding. Our more recent studies carried out during this period demonstrate that lack of food anticipatory activity, a goal-directed reward-seeking behavior observed in these mice, correlates with decreased dopaminergic innervation of the suprachiasmatic nucleus and the nucleus accumbens of the striatum by neurons located in the ventral tegmental area. Thus revealing a close association between motivational and metabolic components of cyclic feeding behavior depending on oscillatory nutritional information. In addition, we have further pursued the study of the mechanisms by which transcription factor Alx3, that has been the focus of our interest for many years, maintains metabolic homeostasis at the systemic level. We have discovered that, apart from its important role on modulating insulin secretion in pancreatic islets, Alx3 is expressed in the arcuate nucleus of the hypothalamus and plays important roles in the regulation of feeding and in metabolic partitioning in peripheral organs.

Dysfunctional metabolic regulation in peripheral tissues

We have also participated in a collaborative study on the regulation of energy metabolism by the newly discovered succinate/SUCNR1 axis in adipocytes. In this study, succinate as been shown to play an essential role in the production and secretion of leptin, a hormone synthetized in adipocytes and secreted upon eating that regulates appetite by generating a sense of satiety. In addition, stimulation of SUCNR1 by succinate controls the cyclic oscillations of leptin secretion via regulation of the molecular clock in these cells.

PUBLICATIONS:

Villanueva-Carmona, T.; Cedó, L.; Madeira, A.; Ceperuelo-Mallafré, V.; Keirán, N.; Núñez-Roa, C.; Mirasierra, M.; Pimenta-Lopes, C.; Sabadell-Basallote, J.; Rodríguez, M.; Bosch, R.; Caubet, L.; Maymó-Masip, E.; Escolá-Gil, J.C. Fernández-Real, J.M.; Vilarrasa, N.; Ventura, F.; Vallejo, M.; Vendrell, J.; Fernández Veledo, S. SUCNR1 signaling in adipocytes controls energy metabolism by modulating circadian clock and leptin expression. *Cell Metab*. **2023**, 35, 1-19. DOI: 10.1016/j.cmet.2023.03.004

FUNDING:

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

Beta Cell Mass and Pancreatic Islet Development

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PRE-DOCTORAL INVESTIGATOR
Matas Aguado, Diego

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Pallarola Martínez, Paula

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Gordo Vega, Alicia

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:

Bartolomé, A. The Pancreatic Beta Cell: Editorial. *Biomolecules.* **2023**, 13, 495. DOI: 10.3390/biom13030495
Kang, J; Postigo-Fernandez, J; Kim, K; Zhu, C; Yu, J; Meroni, M; Mayfeld, B; Bartolomé, A; et al. Notch-mediated hepatocyte MCP-1 secretion causes liver fibrosis. *JCI Insight.* **2023**. 8. e165369. DOI: 10.1172/jci.insight.157694

DOCTORAL THESES AND OTHER WORKS:

Paula Pallarola Martínez
"Master's thesis: *Unraveling the role of NOTCH2 in human pancreas development*". Universidad Autónoma de Madrid. Master's Program in Biotechnology. 2023. Supervisor: Alberto Bartolomé. Grade: 9.4

Alicia Gordo Vega
"Final degree's project: *Caracterización De PCBD1 Como Genrelevante en el Desarrollo del Páncreas Endocrino Mediante Modelado a Partir de Células Madre Humanas*". Universidad Autónoma de Madrid. Bioquímica. 2023. Supervisor: Alberto Bartolomé. Grade: 8.9

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.

Physiopathology and Molecular Mechanisms of Obesity and Comorbidities

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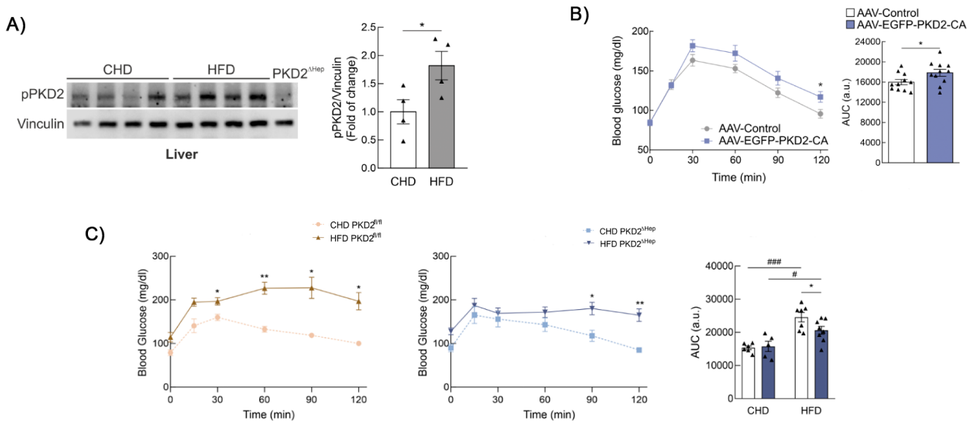
KEYWORDS

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



A) Western Blot image of phospho-PKD2 protein levels in liver extracts from chow diet (CHD)- and high fat diet (HFD)-fed PKD2$^{fl/fl}$ male mice.

B) Pyruvate tolerance test was performed in 16-h fasted mice injected sodium pyruvate. Bar graphs depict the area under the curve (AUC).

C) Pyruvate tolerance test was performed in 16-h fasted mice injected sodium pyruvate. Bar graphs depict the area under the curve (AUC).

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, hepatocellu-

lar carcinoma (HCC). Our group investigates the molecular basis 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 secretion of incretins 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 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 produced 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; Hitos, Ana B.; González Rodríguez, Águeda; Martínez Valverde, Ángela.

Liver progenitor cells (LPCs) have an emerging role in the regenerative responses of the liver due to their plasticity and differentiation capacity to hepatocytes or cholangiocytes. However, the role of LPC fate in liver regeneration during chronic liver diseases is less known. This research line is focused in investigate the susceptibility of LPCs to the inflammatory environment of MASLD and PSC and the role of the protein tyrosine phosphatase 1B (PTP1B) and the transcription factor GATA4 in modulating LPC fate in these pathological contexts. Transcriptomic analysis revealed changes in several molecular pathways in PTP1B-deficient LPCs, including upregulation of oxidative metabolism and hepatocyte-related genes and downregulation of apoptosis-related genes. PTP1B deficiency in LPCs enhanced survival signaling, induced NRF2 nuclear translocation and its antioxidant targets, improved mitochondrial bioenergetics and reduced apoptosis upon treatment with macrophage-derived lipo-inflammatory conditioned medium (CM). On the other hand, stimulation of LPCs with a PSC-like CM from PTP1B-/- macrophages increased survival signaling and proliferation. We also found that GATA4 is expressed in LPCs and we are investigating its role in LPC fate during fibrosis and hypoxia.

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

Researchers involved: García Martínez, Irma; Alen, Rosa; Izquierdo Pastor, Manuel; Ros, Manuel; Martínez Valverde, Ángela.

Cell-to-cell communication by sEV is an emerging issue in liver diseases including MASLD. In this research line we aim to characterize the exosome fraction of the sEV secreted by the hepatocytes under the lipotoxic conditions of MASLD and the impact of lipotoxic sEV in macrophages/Kupffer cells inflammation, as well as in insulin signaling in hepatocytes. A step further, since MASLD and T2DM are closely interconnected and the extent and relevance of sEV-mediated communication between hepatocytes and insulin-secreting β-cells remain largely unexplored, in this research line we also aim to provide new knowledge on the role of hepatocyte-released sEV during the progression of MASLD in the liver-pancreas interactome in order to identify new therapeutic/diagnostic targets for both pathologies. Briefly, we demonstrated that sEV released by the hepatocytes under MASLD conditions cause liver inflammation and insulin resistance in hepatocytes via a paracrine hepatocyte-macrophage-hepatocyte crosstalk. We identified sEV as transporters of saturated fatty acids and potent lipotoxic inducers of liver inflammation. Toll-like receptor 4 (TLR4) deficiency or its pharmaco-

logical inhibition ameliorated liver inflammation induced by hepatocyte-derived lipotoxic sEV. Evidence of this macrophage-hepatocyte interactome was also found in patients with MASLD, pointing to the relevance of sEV in SFA-mediated lipotoxicity in MASLD.

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 San Lorenzo, Ángela; Martínez Valverde, Ángela.

Bariatric surgery is effective for the treatment of obesity and T2D remission. Pharmacological approaches which exert similar metabolic adaptations are an active area of research. This research line investigates 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 white adipose tissue (WAT), pancreas, and liver. By using different genetically modified mouse models, we have uncovered that this complex interactome is initiated by a rapid release of free fatty acids by epididymal WAT leading to subsequent elevations in insulin, adiponectin and FGF21, resulting in beiging of WAT depots, brown adipose tissue (BAT) activation and increased energy expenditure. OXM elevation and similar metabolic profile were found in plasma from obese patients after malabsorptive bariatric surgery. These results, together with ongoing in depth research on WAT lipolysis as a trigger of the inter-organ cross talk mediated by G49 that ultimately leads to increase energy expenditure and reduce body weight, suggest that treatment of obesity with G49 represents a potential pharmacological alternative to bariatric surgery. In collaboration with Pep2Tango Therapeutics we are currently investigating the efficacy of next generation incretin receptor multiagonists in reducing obesity 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 the liver. We have unraveled molecular signatures in the hypothalamus-liver interactome identifying hypothalamic JNK as driver of the peripheral effects of OLA in the liver, as well as sex-depending effects. Mechanistically, we found that OLA i.p. treatment induces mild oxidative stress and inflammation in the hypothalamus in a JNK1-independent and dependent manner, respectively, without features of cell death. Hypothalamic JNK activation up-regulated lipogenic gene expression in the liver though the vagus nerve. This effect concurred with an unexpected metabolic rewiring in the liver in which ATP depletion resulted in increased AMPK/ACC phosphorylation. This starvation-like signature prevented steatosis. By contrast, intrahepatic lipid accumulation was observed in WT mice treated orally with OLA; this effect being absent in PTP1B-KO mice. We also demonstrated an additional benefit of PTP1B inhibition against hypothalamic JNK activation, oxidative stress and inflammation induced by chronic OLA i.p. treatment, thereby preventing hepatic lipogenesis.

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

Researchers involved: Rey, Esther; Fernández, Carlos Ernesto; Díez, Paula; 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. Also, we have demonstrated that BMP8A expression is enhanced in liver fibrosis states. These observations derived from different preclinical models of fibrosis, in which hepatic and serum levels of BMP8A were increased. Similarly, in a human cohort of MASH patients, there was an increase in circulating BMP8A levels in advanced fibrosis patients (F3-F4). In fact, an algorithm based on serum BMP8A levels was developed, called BFS (BMP8A fibrosis score), which efficiently predicts advanced fibrosis in MASLD patients.

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

Researchers involved: del Peso, Natalia; del Fresno, Elena; Ben Jeddou, Ikram; 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 both different cellular systems and a mouse model of MASLD, we have demonstrated that AIK selectively reduces LPS and palmitate-induced IL1β synthesis in macrophages, blocking their proinflammatory activation. Moreover, AIK protects against palmitic acid (PA)-induced lipotoxicity in hepatocytes by inhibiting JNK signalling induced by this fatty acid, and, selectively, IL1β synthesis. In addition, the compound transiently prevents the activation of the transcription factor transcription factor NFκB and p38 MAPK signalling pathways induced by PA in hepatocytes, reflected in a short-term reduction of TNFα expression. In addition, the results of the preclinical study revealed that AIK ameliorates MASLD progression and reduces intrahepatic lipid accumulation in a MASLD mouse model (mice fed with high fat diet).

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

Researchers involved: Hernández, Miguel Ángel; Fernández, Carlos Ernesto; González Rodríguez, Águeda.

Chronic obstructive pulmonary disease (COPD) and obstructive sleep apnea (OSA) are chronic respiratory diseases with a high prevalence worldwide and high morbidity and mortality, largely due to the presence of associated metabolic and cardiovascular disorders. These research line explores possible factors responsible for the association between these chronic respiratory diseases and the cardiometabolic associated-disorders. In this regard, we have described that metabolic comorbidities are more frequent in respiratory diseases with nocturnal intermittent hypoxia (IH) such as OSA, while cardiovascular comorbidities were more frequent in COPD, which is characterised with continuous hypoxia. Currently, the impact of IH on MASLD progression is being evaluated.

PUBLICATIONS:

Aguilar-Recarte, D.; Barroso, E.; Zhang, M.; Rada, P.; Pizarro-Delgado, J.; Peña, L.; Palomer, X.; Valverde, Á.M.; Wahli, W.; Vázquez-Carrera, M. A positive feedback loop between AMPK and GDF15 promotes metformin antidiabetic effects. *Pharmacol* Res. **2023**,*187*, 106578. DOI: 10.1016/j.phrs.2022.

Vranic, M.; Ahmed, F.; Hetty, S.; Sarsenbayeva, A.; Ferreira, V.; Fanni; G.; Valverde, Á.M.; Eriksson, J.W.; Pereira. M.J. Effects of the second-generation antipsychotic drugs aripiprazole and olanzapine on human adipocyte differentiation. *Mol Cell Endocrinol.* **2023**, *561*, 111828. DOI: 10.1016/j.mce.2022.111828.

Frutos, M.F.; Pardo-Marqués, V.; Torrecilla-Parra, M.; Rada, P.; Pérez-García, A.; Martín-Martín, Y.; de la Peña, G.; Gómez, A.; Toledano-Zaragoza, A.; Gómez-Coronado, D.; Casarejos, M.J.; Solís, J.M.; Rotllan, N.; Pastor, Ó.; Ledesma, M.D.; Valverde, Á.M.; Busto, R.; Ramírez, C.M. MiR-7 controls cholesterol biosynthesis through posttranscriptional regulation of DHCR24 expression. *Biochim Biophys Acta Gene Regul Mech.* **2023**, *1866(2)*, 194938. DOI: 10.1016/j.bbagrm.2023.194938.

Marañón, P.; Isaza, S.C.; Fernández-García, C.E.; Rey, E.; Gallego-Durán, R.; Montero-Vallejo, R.; de Cía, J.R.; Ampuero, J.; Valverde, Á.M.; Romero-Gómez, M.; García-Monzón, C.; González-Rodríguez, Á. Circulating bone morphogenetic protein 8A is a novel biomarker to predict advanced liver fibrosis. *Biomark Res.* **2023**, *11(1)*, 46. DOI: 10.1186/s40364-023-00489-2.

Carrasco, A.G;, Izquierdo-Lahuerta, A.; Valverde, Á.M.; Ni, L.; Flores-Salguero, E.; Coward, R.J.; Medina-Gómez, G.The protective role of peroxisome proliferator-activated receptor gamma in lipotoxic podocytes. *Biochim Biophys Acta Mol Cell Biol Lipids.* **2023**, *1868(7)*, 159329.DOI: 10.1016/j.bbalip.2023.159329.

Ferreira, V.; Folgueira, C.; García-Altares, M.; Guillén, M.; Ruíz-Rosario, M.; DiNunzio, G.; Garcia-Martinez, I.; Alen, R.; Bookmeyer, C.; Jones, J. G.; Cigudosa, J. C.; López-Larrubia, P.; Correig-Blanchar, X.; Davis, R. J.; Sabio, G.; Rada, P.; Valverde, Á. M. Hypothalamic JNK1-Hepatic Fatty Acid Synthase Axis Mediates a Metabolic Rewiring That Prevents Hepatic Steatosis in Male Mice Treated with Olanzapine via Intraperitoneal: Additional Effects of PTP1B Inhibition. *Redox Biol.* **2023**, 63, 102741. DOI: 10.1016/j.redox.2023.102741.

Laiglesia, L. M.; Escoté, X.; Sáinz, N.; Felix-Soriano, E.; Santamaría, E.; Collantes, M.; Fernández-Galilea, M.; Colón-Mesa, I.; Martínez-Fernández, L.; Quesada-López, T.; Quesada-Vázquez, S.; Rodríguez-Ortigosa, C.; Arbones-Mainar, J. M.; Valverde, Á. M.; Martínez, J. A.; Dalli, J.; Herrero, L.; Lorente-Cebrián, S.; Villarroya, F.; Moreno-Aliaga, M. J. Maresin 1 Activates Brown Adipose Tissue and Promotes Browning of White Adipose Tissue in Mice. Mol. *Metab*. **2023**, 74, 101749. DOI: 10.1016/j.molmet.2023.101749.

Marsal-Beltran, A.; Rodríguez-Castellano, A.; Astiarraga, B.; Calvo, E.; Rada, P.; Madeira, A.; Rodríguez-Peña, M. M.; Llauradó, G.; Núñez-Roa, C.; Gómez-Santos, B.; Maymó-Masip, E.; Bosch, R.; Frutos, M. D.; Moreno-Navarrete, J. M.; Ramos-Molina, B.; Aspichueta, P.; Joven, J.; Fernández-Real, J. M.; Quer, J. C.; Valverde, Á. M.; Pardo, A.; Vendrell, J.; Ceperuelo-Mallafré, V.; Fernández-Veledo, S. Protective Effects of the Succinate/SUCNR1 Axis on Damaged Hepatocytes in NAFLD. *Metabolism.* **2023**, 145, 155630. DOI: 10.1016/j.metabol.2023.155630.

Garcia-Martinez, I.; Alen, R.; Pereira, L.; Povo-Retana, A.; Astudillo, A. M.; Hitos, A. B.; Gomez-Hurtado, I.; Lopez Collazo, E.; Boscá, L.; Francés, R.; Lizasoain, I.; Moro, M. Á.; Balsinde, J.; Izquierdo, M.; Valverde, Á. M. Saturated Fatty Acid-Enriched Small Extracellular Vesicles Mediate a Crosstalk Inducing Liver Inflammation and Hepatocyte Insulin Resistance. *JHEP Rep.* **2023**, *5(8)*, 100756. DOI: 10.1016/j.jhepr.2023.100756.

Zhang, M.; Barroso, E.; Ruart, M.; Peña, L.; Peyman, M.; Aguilar-Recarte, D.; Montori-Grau, M.; Rada, P.; Cugat, C.; Montironi, C.; Zarei, M.; Jurado-Aguilar, J.; Camins, A.; Balsinde, J.; Valverde, Á.M.; Wahli, W.; Palomer, X.; Vázquez-Carrera, M. Elafibranor upregulates the EMT-inducer S100A4 via PPARβ/δ. *Biomed Pharmacother.* **2023**, *167*, 115623. DOI: 10.1016/j.biopha.2023.115623.

Peyman, M.; Barroso, E.; Turcu, A.L.; Estrany, F. Jr.; Smith, D.; Jurado-Aguilar, J.; Rada, P.; Morisseau, C.; Hammock, B.D.; Valverde, Á.M.; Palomer, X.; Galdeano, C.; Vázquez, S.; Vázquez-Carrera, M. Soluble epoxide hydrolase-targeting PROTAC activates AMPK and inhibits endoplasmic reticulum stress. *Biomed Pharmacother.* **2023**, *168:*115667. DOI: 10.1016/j.biopha.2023.115667.

Solís-Muñoz, P.; de la Flor-Robledo, M.; García-Ruíz, I.; Fernández-García, C. E.; González-Rodríguez, Á.; Shah, N.; Bataller, R.; Heneghan, M.; García-Monzón, C.; Solís-Herruzo, J. A. Mitochondrial Respiratory Chain Activity Is Associated with Severity, Corticosteroid Response and Prognosis of Alcoholic Hepatitis. *Aliment. Pharmacol. Ther.* **2023**, *57 (10)*, 1131-1142. DOI: 10.1111/apt.17434.

Landete, P.; Fernández-García, C. E.; Muñoz, J. M.; Friera, A.; Ancochea, J.; González-Rodríguez, Á.; García-Monzón, C. Sleep Apnea-COPD Overlap Syndrome Is Associated with Larger Left Carotid Atherosclerotic *Plaques. Front. Cardiovasc. Med.* **2023**, *10*, 1104377. DOI: 10.3389/fcvm.2023.1104377.

Infante-Menéndez, J.; López-Pastor, A. R.; González-Illanes, T.; González-López, P.; Huertas-Lárez, R.; Rey, E.; González-Rodríguez, Á.; García-Monzón, C.; Patil, N. P.; Vega de Céniga, M.; Baker, A. B.; Gómez-Hernández, A.; Escribano, O. Increased let-7d-5p in Non-Alcoholic Fatty Liver Promotes Insulin Resistance and Is a Potential Blood Biomarker for Diagnosis. *Liver Int.* **2023**, *43 (8)*, 1714-1728. DOI: 10.1111/liv.15581.

DOCTORAL THESES AND OTHER WORKS:

Rosa Alén
"*Ph.D. thesis: Hepatocyte-derived small extracellular vesicles in the intrahepatic and liver pancreas interactome in the context of non-alcoholic fatty liver disease*". Universidad Autónoma de Madrid. Medicina. 2023. Supervisors: Irma García Martínez, Ángela Martínez Valverde. Grade: Sobresaliente Cum Laude.

Victor Manuel Ferreira
"Ph.D. thesis: *Modulation of hypothalamic AMPK and JNK1 by olanzapine controls energy balance and hepatic lipogenesis in mice:additional benefits of PTP1B inhibition*". Universidad Autónoma de Madrid. Medicina. 2023. Supervisors: Patricia Rada LLano, Ángela Martínez Valverde. Grade: Sobresaliente Cum Laude.

Patricia Marañón Barnusell
"Ph.D. thesis: *Implicación de las proteínas morfogenéticas óseas en la fisiopatología hepática*". Universidad Complutense de Madrid. Farmacia. 2023. Supervisors: Águeda González Rodríguez. Grade: Sobresaliente Cum Laude.

Elvira del Pozo Maroto
"Ph.D. thesis: *Caracterización de la prevalencia y morbi-mortalidad de la enfermedad hepática grasa no alcohólica: Estudio prospectivo longitudinal de una cohorte de pacientes con colelitiasis*". Universidad Complutense de Madrid. Medicina. 2023. Supervisors: Águeda González Rodríguez, Carmelo García Monzón. Grade: Sobresaliente Cum Laude.

Paula Díez Roda
"Master´s thesis: *La proteína morfogenética ósea 2 (BMP2) es una nueva diana molecular relacionada con la enfermedad de hígado graso no alcohólico (NAFLD) y el daño vascular asociado*". Universidad Complutense de Madrid. 2023. Supervisor/s: Águeda González Rodríguez, Oscar Escribano Llanes. Grade: 9

Miguel Hernández García
"Final degree's project: *Identificación y caracterización de nuevas dianas moleculares implicadas en la progresión de la enfermedad del hígado graso no alcohólico*". Universidad Autónoma de Madrid. 2023. Supervisor/s: Águeda González Rodríguez. Grade: 8,7

Natalia del Peso Casado
"Final degree's project: *Evaluación del inhibidor alostérico de JNK1 en la progresión de la enfermedad del hígado graso no alcohólico*". Universidad Politécnica de Madrid. 2023. Supervisor/s: Águeda González Rodríguez. Grade: 9

FUNDING:

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

"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

"Extracelular vesicles: new insights into their role in liver-pancreas interactome in T2D". European Foundation for the Study of Diabetes (EFSD). 2021-2023

"Identification of metabolic biomarkers for chronic diseases and treatments". MICIU. 2021-2023

"Unravelling lipid droplets dynamics in liver progenitor cells in non-alcoholic fatty liver disease". CIBER (ISCIII). 2021-2023

"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.

"Evaluation of the effects of the allosteric JNK1 inhibitor on the progression of non-alcoholic fatty liver disease". Allinky Biopharma. From 2019.

AWARDS:

"Premio ASISA del Concurso Científico de la Real Academia Nacional de Farmacia" 2023

Cardiovascular Physiopathology

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

RESEARCH LINES:

Line of work: Cardiovascular function
Our work aimed to provide a novel understanding of the interaction between cardiac immune myeloid cells and cardiomyocytes, to ensure proper cardiac function, as well as the alterations that lead to cardiac dysfunction, hypertrophy, cardiac fibrosis, and ultimately heart failure (HF). The focus was on establishing the role of the nuclear receptors VDR and AhR as key players in this protective interaction and how they can be modulated by molecules released by macrophages, and trophic factors that alter the transcription pattern in both cell types. We discovered that macrophages contribute to providing immunometabolic support to cardiomyocytes, which ensures a correct physiological activity. In addition to this, cardiomyocytes also have a high responsiveness to these nuclear receptors. Therefore, the activation of this homeostatic system seems to contribute to preventing cardiac dysfunction and, in some cases, irreversible HF. In this scenario, the regulation of the activity of the nuclear receptors VDR and AhR has been the main focus of our work, including the role of VDR and AhR in the outcome of human HF after myocardial infarction, aortic stenosis and/or other low-grade proinflammatory comorbidities.

Line of work: COVID-19 and pro-resolving mediators
SARS-CoV-2 and other infections can cause pulmonary complications such as acute respiratory distress syndrome (ARDS) or bilateral pneumonia, due to the excessive generation of proinflammatory cytokines in a process known as “cytokine storm,” which is induced due to faulty resolution of the inflammatory reaction. Since the main molecules involved in this step are specialized pro-resolving lipid mediators, we studied the role of lipoxin A4, the most abundant pro-resolving mediator, in the context of COVID-19. Our data show that the native form of lipoxin A4/B4, 15-epi-lipoxin A4/B4 epimers, and the synthetic lipoxin A4 analog BML-111 play a key role in the resolution of ARDS. Among the mechanisms involved, these molecules inhibit neutrophil infiltration, induce an “alternative activation” of macrophage polarization, and reduce ROS production. At the molecular level, these lipids modulate survival and proliferation pathways such as ERK, AKT, or AMPK/ACC. Furthermore, they activate NRF2, considered a “master” regulator of the antioxidant response, inducing its translocation to the nucleus and promoting the expression of antioxidant genes.

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 KChIP2, sigma-1 receptor and Lgi3-4.

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:

Paz-García, M.; Povo-Retana, A.; Jaén,, R.I.; Prieto P.; Peraza, D.A.; Zaragoza, C.; Hernandez-Jimenez ,M.; Pineiro, D.; Regadera, J.; García-Bermejo, M.L.; Rodríguez-Serrano, E.M.; Sánchez-García, S.; Moro, M.A.; Lizasoaín, I.; Delgado, C.; Valenzuela, C.; Boscá, L. Beneficial effect of TLR4 blockade by a specific aptamer antagonist after acute myocardial infarction. *Biomed. Pharmacother.* **2023**,*158*, e114214. DOI: 10.1016/j.biopha.2023.114214.

Hamdy, N.M.; Bosca, L.; Singh, S.M.; Reddy Bonam S.; Kiss, I.; Kumar, D.P.; Banerjee, A. Women in gastrointestinal cancers. *Front. Oncol.* **2023**,*13*, e1192814. DOI:10.3389/fonc.2023.1192814.

Garcia-Martinez, I.; Alen, R.; Pereira, L.; Povo-Retana, A.; Astudillo, A.M.; Hitos, A.B.; Gomez-Hurtado, I.; Lopez-Collazo, E.; Boscá, L.; Francés, R.; Lizasoain, I.; Moro, M.A.; Balsinde, J.; Izquierdo, M.; Valverde, A.M. Saturated fatty acid-enriched small extracellular vesicles mediate a crosstalk inducing liver inflammation and hepatocyte insulin resistance. *JHEP Reports.* **2023***; 5,* e100756. DOI: 10.1016/j.jhepr.2023.100756.

Povo-Retana, A.; Landauro-Vera, R.; Fariñas, M.; Sánchez-García, S.; Alvarez-Lucena, C.; Marin, S.; Cascante, M.; Boscá L. Defining the metabolic signatures associated with human macrophage polarisation. *Biochem. Soc. Trans.* **2023**, *51*, 1429-1436. DOI:10.1042/BST20220504.

Povo-Retana, A.; Fariñas, M.; Landauro-Vera, R.; Mojena, M.; Alvarez-Lucena, C.; Fernández-Moreno, M.A.; Castrillo, A.; de la Rosa Medina, J.V.; Sánchez-García, S.; Foguet, C.; Mas, F.; Marin, S.; Cascante, M.; Boscá L. Immunometabolic actions of trabectedin and lurbinectedin on human macrophages: relevance for their anti-tumor activity. *Front. Immunol.* **2023**, *14*, e1211068. DOI: 10.3389/fimmu.2023.1211068.

Sánchez-García, S.; Jaén, R.I.; Fernández-Velasco, M.; Delgado, C.; Boscá, L.; Prieto P. Lipoxin-mediated signaling: ALX/FPR2 interaction and beyond. *Pharmacol. Res.* **2023**, *197*, e106982. DOI: 10.1016/j.phrs.2023.106982.

Cerrato, G.; Alvarez-Lucena, C.; Sauvat, A.; Hu, Y.; Forveille, S.; Chen, G.; Durand, S.; Aprahamian, F.; Leduc, M.; Motiño, O.; Boscá, L.; Xu, Q.; Kepp, O.; Kroemer, G. 3;4-dimethoxychalcone induces autophagy and reduces neointimal hyperplasia and aortic lesions in mouse models of atherosclerosis. *Cell. Death. Dis.* **2023**, *14*, e758. DOI: 10.1038/s41419-023-06305-x.

Peraza, D.A.; Povo-Retana, A.; Mojena, M.; García-Redondo, A.B.; Avilés, P.; Boscá, L.; Valenzuela, C. Trabectedin modulates macrophage polarization in the tumor-microenvironment. Role of Kv1.3 and Kv1.5 channels. *Biomed. Pharmacother.* **2023**, *161*, e114548. DOI: 10.1016/j.biopha.2023.114548.

Díaz del Campo, L.; García-Redondo, A.B.; Duro-Sánchez, S.; Zaragoza, C.; Palmas, F.; Peraza, D.A.; de Benito-Bueno, A.; Socuéllamos, P.G.; Rodrigues-Diez, R.; Valenzuela, C.; Dalli, J.; Salaices, M.; Briones, A.M. Treatment with resolvin D2 attenuates cardiovascular damage in angiotensin II-induced hypertension. *Hypertension.* **2023**, *80*, 84-96,DOI: 10.1161/HYPERTENSIONAHA.122.19448.

Vera-Zambrano, A.; Baena-Nuevo, M.; Rinné, S.; Villegas-Esguevillas, M.; Barreira, B.; Telli, G.; de Benito-Bueno, A.; Blázquez, J.A.; Climent, B.; Pérez-Vizcaino, F.; Valenzuela, C.; Decher, N.; Gonzalez, T.; Cogolludo, A. Sigma-1 receptor modulation fine-tunes Kv1.5 channels and impacts pulmonary vascular function. *Pharmacol. Res.* **2023**, *189*, 106684. DOI: 10.1016/j.biopha.2023.114214.

DOCTORAL THESES AND OTHER WORKS:

Abdoul Nasser Mahamadou Bafoutche
"Master´s thesis: *Effect of 25-OH-D in human primary cell culture of macrophages and T lymphocyte subsets in the modulation of the inflammatory response*". Universidad Complutense de Madrid. Medicina. 2023. Supervisor/s: Adrián Povo / Lisardo Boscà. Grade: Notable.

María Durán Sáez
"Master´s thesis: *Papel protector de las lipoxinas en la viabilidad y funcionalidad de células pulmonares. Implicaciones en COVID-19*". Universidad Complutense de Madrid. Medicina. 2023. Supervisor/s: Patricia Prieto / Lisardo Boscà. Grade: Sobresaliente.

María Redondo Moya
"Master´s thesis: *Análisis electrofisiológico de la modulación diferencial ejercida por las proteínas Lgi3 y Lgi4 sobre los canales de potasio Kv4.2 y Kv4.3*". Universidad de Alcalá. 2023. Supervisor: Carmen Valenzuela Miranda. Grade: Sobresaliente (9,3).

Paula García Socuéllamos
"Doctoral Thesis: *Unravelling Lgi3-4 role in cardiac electrophysiology*". Universidad Autónoma de Madrid. 2023. Supervisor: Carmen Valenzuela Miranda. Grade: Sobresaliente cum laude.

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

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

"Estudio de las proteínas integrantes de los canalosomas Kv1.5 y Kv4.3 como dianas para la fibrilación auricular. PID2019-104366RB-C21". AEI. 2020-2023.

"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.

"Mechanisms underlying the cardioprotective effects of nuclear vitamin D receptor activation in heart failure". UCRAN2005. Programa CSIC de cooperación científica con Ucrania. 2022-2025.

Mitochondrial Function in Health and Disease

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KEYWORDS
Mitochondria, oxidative metabolism, oxidative stress, PGC-1α, 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.; Rincón, L; 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.; del Rio, M; García, R.; Doblado, L.; Labalde, M.; Ferrero, E.; Monsalve, M.
The third line of research focuses on understanding how the systemic metabolism impacts on CRC development.

Thyroid Cancer (CRC)
Researchers involved: Moreno, O.; García, R.; Labalde, M.; Ferrero, E.; Monsalve, M.
The fourth line of research aims to make use of the evaluation of mitochondrial markers to support the non-invasive risk assessment of thyroid cancer.

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

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

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:

Bernal-Tirapo, J.; Bayo Jiménez, M.T.; Yuste-García, P.; Cordova, I.; Peñas, A.; García-Borda, F.J.; Quintela, C.; Prieto I.; Sánchez-Ramos, C.; Ferrero-Herrero, E.; Monsalve, M. Evaluation of Mitochondrial Function in Blood Samples Shows Distinct Patterns in Subjects with Thyroid Carcinoma from Those with Hyperplasia.
Int J Mol Sci. **2023**, *24*, 6453. DOI: 10.3390/ijms24076453.

Ferrero Herrero, E.; Labalde Martínez, M.; Guadarrama González, F.J.; García Villar, Ó.; Nevado García, C.; Alonso Gómez, S.; Fernández Miguel, T.; Bernal Tirapo, J.; García Villalon, Á.L.; Monsalve Pérez, M. Oncología quirúrgica personalizada. Personalized surgical oncology. The road to excellence. *Archivos de Cirugía. ISSN: 3020-2655.* **2023**, *1*, 7.1-2023(1-2023). DOI: 10.14679/2327.

Samhan-Arias AK, Poejo J, Marques-da-Silva D, Martínez-Costa OH, Gutierrez-Merino C. Are There Lipid Membrane-Domain Subtypes in Neurons with Different Roles in Calcium Signaling? Molecules. **2023**;28(23):7909. doi: 10.3390/molecules28237909

Salazar J, Samhan-Arias AK, Gutierrez-Merino C. Hexa-Histidine, a Peptide with Versatile Applications in the Study of Amyloid-β(1-42) Molecular Mechanisms of Action. Molecules. **2023**;28(20):7138. doi: 10.3390/molecules28207138

DOCTORAL THESES AND OTHER WORKS:

Gaurang Kumar Patel
"Ph.D. thesis: *Role of Antipsychotics-Induced Mitochondrial Dysfunction in Increased Cardiovascular Risk*". Universidad Autónoma de Madrid (UAM). Medicine. 2023. Supervisor/s: María Monsalve, Santiago Lamas.

Lucía Gómez Rincón
"Master's thesis: *Loss of mitocondrial plasaticity associated to the chronic administration of SGAs*". Universidad Complutense de Madrid (UCM). Biology. 2023. Supervisor/s: María Monsalve, Laura Benítez Rico.

Mariano Patrizio del Rio Reinoso
"Final degree's projec: *Identification and evaluation of metabolic biomarkers for riks stadification of subjects with colorectal cancer*". Universidad Autónoma de Madrid (UAM). Biology. 2023. Supervisor/s: María Monsalve, Jose Luis Bella Sombria.

Alí Ben Salah
"Master's thesis: *Flavin Redox Bifurcation as a Mechanism for Controlling Electron Flow between the Cytochrome b5 reductase and Cytochrome* b_5". Universidad Autónoma de Madrid (UAM). Master in Biotechnology. Pending of evaluation. Supervisor/s: Alejandro K. Samhan Arias, Oscar H. Martinez-Costa.

FUNDING:

"Identificación de biomarcadores metabólicos para enfermedades crónicas y sus tratamientos. EIN2020-112263". MICIU. 2021-2023.

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

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Hypoxia and Angiogenesis

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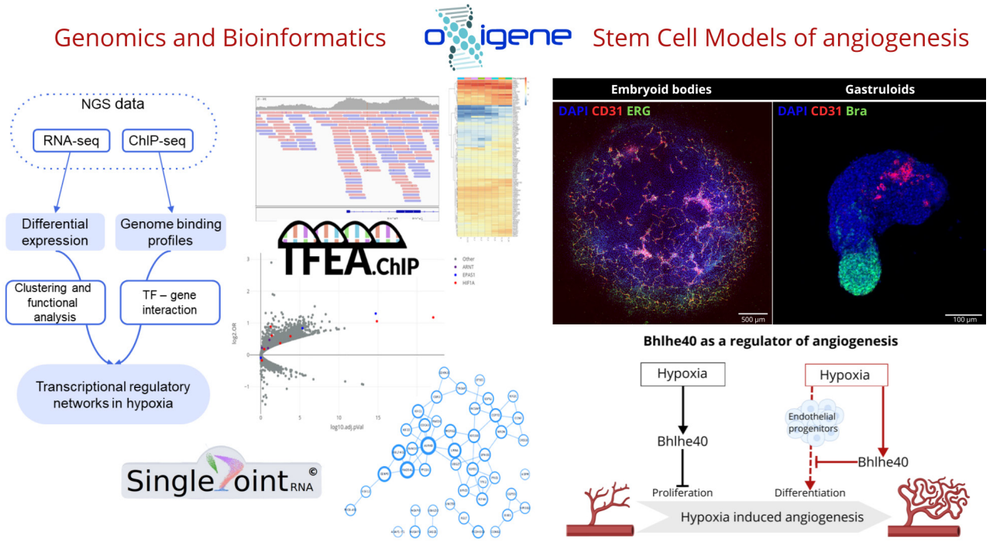
KEYWORDS
Hypoxia, Genomics, Bioinformatics, Angiogenesis.



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

The disruption of HIF prevents both the upregulation and downregulation of genes triggered by hypoxia. However, a comprehensive examination of HIF-binding sites on a genome-wide scale has revealed that HIF directly governs only gene induction. To gain deeper insights into this response, we con-

ducted a meta-analysis of published hypoxic transcriptomic profiles, resulting in the identification of a robust and reliable hypoxic signature. This analysis has also unveiled Bhlhe40 as the gene most consistently induced by hypoxia. Bhlhe40 is a transcriptional repressor known for its role in regulating cell proliferation, differentiation, and lipid metabolism. Our current focus is on elucidating the specific function of Bhlhe40 in the transcriptional response to hypoxia.

As part of our transcriptomic analysis pipelines, we have developed singlepointRNA, a software tool designed to simplify single-cell RNA sequencing (scRNA-seq) analysis.

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

Obstructive sleep apnea (OSA) is a highly prevalent condition characterized by the intermittent obstruction of the upper respiratory tract during sleep, leading to cyclic hypoxia. OSA patients face an increased risk of cancer and cardiovascular diseases, but the mechanisms connecting these conditions remain poorly understood. We hypothesize that the intermittent hypoxia seen in OSA activates the HIF/Bhlhe40 axis, ultimately disrupting adipose tissue homeostasis, which, in turn, contributes to the development of metabolic syndrome and its association with cancer and cardiovascular diseases.

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.

DOCTORAL THESES AND OTHER WORKS:

Laura Puente Santamaría

"Ph.D. thesis: *A computational approach to transcriptional regulation in response to hypoxia*". Universidad Autónoma de Madrid. Medicina. 2023. Supervisor/s: Luis del Peso Ovalle y Ricardo Ramos. Grade: Sobresaliente Cum Laude

Marta Abanades Salmerón

"Final degree's project: *Role of the HIF/BHLHE40 axis in brown adipocyte differentiation*". Universidad Autónoma de Madrid. Facultad de Medicina. 2023. Supervisor/s: Luis del Peso Ovalle. Grade: Sobresaliente

Marta Sanz Gómez

"Final degree's project: "*Papel de BHLHE40 en la regulación de la diferenciación y proliferación en la angiogénesis inducida por hipoxia*". Universidad Autónoma de Madrid. Facultad de Medicina. 2023. Supervisor/s: Benil de Jiménez Cuenca y Bárbara Acosta Iborra. Grade: Sobresaliente

FUNDING:

"Contribution of BHLHE40 to the transcriptional response to hypoxia and its implication in metabolic and respiratory diseases. PID2020-118821RB-I00". MICINN. 2021-2024

"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.

Oxygen Homeostasis in the Cardiovascular System

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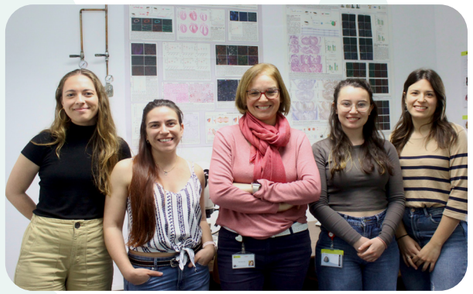
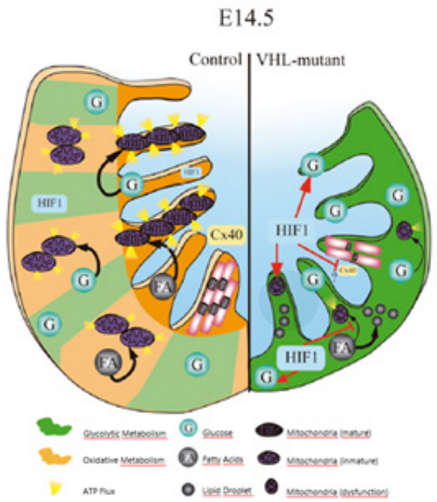
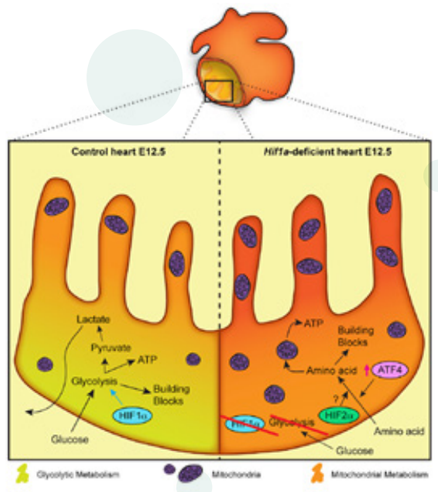
KEYWORDS
Hypoxia, Heart, Oxygen, Cardiovascular System, HIF, VHL







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 **cardiovascular system** and is involved in the appearance and evolution of numerous CVD.

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

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

One of our research lines is dedicated to investigating the influence of HIF signaling during heart development and maturation. We have discovered the fundamental role of VHL/HIF1 axis signaling in the establishment of metabolic territories in the embryonic heart 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.

Proposed Working Models of Embryonic Cardiac Metabolism in VHL-KO and HIF1-KO mice

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.

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).

"Redox Regulation of Cardiomyocyte Renewal.17CVD04". Foundation Le-Ducq. 2018-2023 (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.

PATENTS:

"License approval for the use of hybridomas to obtain monoclonal human HIF1 alpha antibodies". Puig Silvia, Roncador Giovanna. CNIO23-112 23-08 10".

Immunity, Immunopathology and Emergent Therapies

PRINCIPAL INVESTIGATOR
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KEYWORDS
Leukemia, Lymphoma, Small chemicals, Chemoresistance, immunotherapy, TNFR, Proteomics, Macrophage, Liver X Receptors, LXR, Inflammation, Transcriptional Regulation, Innate Immunity.

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.

Indole-3-Carbinol synergized with inhibitors of the B cell receptor pathway in B cell lymphoma y leukemia

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

I3C is a nutraceutical present in vegetables of the Brassica genus that are very common in the diet. I3C has been already tested in phase 2 clinical trials for precancerous papilloma and it has negligible toxic effects in patients and mice. Our results show that this compound, at bioavailable concentrations, synergizes with new generation drugs targeting Bruton´s tyrosine kinase (BTK) resulting in B cell receptor (BCR) signaling inhibition. The combination treatment with I3C and these compounds is still highly effective in conditions mimicking the B cell neoplasia proliferation centers in the lymph nodes and spleen, including culturing under hypoxia and co-culturing with mesenquimal stromal cells. Analysis of the effect of the I3C and BCR inhibitor combination treatment indicates that the combination treatment strongly downmodulates the expression of key survival proteins and induces apoptosis. We continue working in the identification of the mechanism of action of I3C.

CD137 (4-1BB) requires physically associated cIAPs for signal transduction and antitumor effects

Researchers involved: Zapata, Juan Manuel

CD137 (4-1BB) is a member of the TNFR family that mediates potent T cell costimulatory signals upon ligation by CD137L or agonist monoclonal antibodies (mAbs). CD137 agonists attain immunotherapeutic antitumor effects in cancer mouse models, and multiple agents of this kind are undergoing clinical trials. In collaboration with the Group of Dr. Ignacio Melero (Clinica Universitaria de Navarra, CIMA) we have shown that cIAP1 and cIAP2 are physically associated with the CD137 signaling complex. Moreover, cIAPs are required for CD137 signaling toward the NF-κB and MAPK pathways and for costimulation of human and mouse T lymphocytes. SMAC mimetics that trigger cIAP degradation and expression of cIAP dominant-negative variants abrogated the anti-tumoral effects of CD137, thus demonstrating that the antitumor effects of agonist anti-CD137 mAbs are critically dependent on the integrity of cIAPs.

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 LXRα in macrophages, through in vitro and in vivo studies with mouse models of LXRα deficiency, and knockin transgenics of conditional absence or reporter mice. Our recent results suggest that LXRα exhibits distinct activities in macrophages. On one hand, in healthy tissues it exerts homeostatic functions in certain subtypes of tissue-resident macrophages, and on the other hand, in situations of inflammation or infection it promotes antimicrobial and inflammatory polarization actions in recruited, monocyte-derived, macrophages. Specifically, we approached these studies from 2 main angles. First, we study the role of LXRα in the differentiation and transcriptional activity of tissue-resident macrophages. Using genomic strategies we search for the genome-wide localization of LXRα, and we will analyze by proteomic approaches the molecular interactions of LXRα in different situations, using tissue-derived macrophages and with macrophages in culture. The second major direction of our research will aim to analyze the function of LXRα in macrophages in pathological situations through inflammation and infection models. We envision that our contributions may translate into future strategies for therapeutic intervention in diseases by manipulating macrophage activity.

PUBLICATIONS:

Glez-Vaz, J; Azpilikueta, A; Ochoa, MC; Olivera, I; Gomis, G; Cirella, A; Luri-Rey, C; Álvarez, M; Pérez-Gracia, JL; Ciordia, S; Eguren-Santamaria, I; Alexandru, R; Berraondo, P; de Andrea, C; Teijeira, Á; Corrales, F; Zapata, JM; Melero, I. CD137 (4-1BB) requires physically associated cIAPs for signal transduction and antitumor effects. *Sci. Adv.* **2023**. *9* (33):eadf6692. DOI:10.1126/sciadv.adf6692

Gutierrez, AH; Mazariegos, MS; Alemany, S; Nevzorova, YA; Cubero, FJ; Sanz-García, C. Tumor progression locus 2 (TPL2): A Cot-plicated progression from inflammation to chronic liver disease. Biochim. Biophys. Acta Mol. *Basis Dis.* **2023**. 1869 (*4*):166660. DOI:10.1016/j.bbadis.2023.166660

Contreras-Jurado, C; Montero-Pedrazuela, A; Pérez, RF; Alemany, S; Fraga, MF; Aranda, A. The thyroid hormone enhances mouse embryonic fibroblasts reprogramming to pluripotent stem cells: role of the nuclear receptor corepressor 1. *Front. Endocrinol* (Lausanne). **2023**. *14*:1235614. DOI:10.3389/fendo.2023.1235614.

de la Aleja, AG; Herrero, C; Torres-Torresano, M; Schiaffino, MT; Del Castillo, A; Alonso, B; Vega, MA; Puig-Kröger, A; Castrillo, A; Corbí, ÁL. Inhibition of LXR controls the polarization of human inflammatory macrophages through upregulation of MAFB. **2023**. *Cell. Mol. Life Sci. 80 (4):96* DOI:10.1007/s00018-023-04745-4.

Povo-Retana, A; Fariñas, M; Landauro-Vera, R; Mojena, M; Alvarez-Lucena, C; Fernández-Moreno, MA; Castrillo, A; de la Rosa Medina, JV; Sánchez-García, S; Foguet, C; Mas, F; Marin, S; Cascante, M; Boscá, L. Immunometabolic actions of trabectedin and lurbinectedin on human macrophages: relevance for their anti-tumor activity. **2023**. *Front. Immunol.14:1211068*. DOI:10.3389/fimmu.2023.1211068.

Fernández-Pérez, L; Guerra, B; Recio, C; Cabrera-Galván, JJ; García, I; De La Rosa, JV; Castrillo, A; Iglesias-Gato, D; Díaz, M. Transcriptomic and lipid profiling analysis reveals a functional interplay between testosterone and growth hormone in hypothyroid liver. Front. Endocrinol. **2023**, 14:1266150. DOI:10.3389/fendo.2023.1266150.

DOCTORAL THESES AND OTHER WORKS:

Haisea Aguado Iglesias
"Master thesis: *Cell autonomous role of constitutive TRAF1 expression in T cells: Role in TNFR1 and TNFR2 signallings*". Universidad Autónoma de Madrid. Facultad de Ciencias Biológicas. 2023. Supervisor: Juan Manuel Zapata Hernández. Grade: 9,8

FUNDING:

"Regulación transcripcional del metabolismo del hierro, la hemofagocitosis y la eritropoyesis por receptores nucleares LXR en macrófagos residentes en tejidos. PID2019-104284RB-I00". Agencia Estatal de Investigación (AEI), Ministerio de Ciencia, Investigación y Universidades. 2020-2023.

"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.

"TNF-Receptor Associated Factor (TRAF)-3 and Tripartite Motif (TRIM)-37: Elucidating new roles in the control of innate and adaptive humoral immune responses against pathogen. PID2019-110405RB-I00". Agencia Estatal de Investigación (AEI), Ministerio de Ciencia, Investigación y Universidades. 2020-2023.

"New Functions of TRAF1 in T Lymphocyte immune Responses, Homeostasis and Aging. PID2022-136909OB-I00.". Agencia Estatal de Investigación (AEI), Ministerio de Ciencia, Investigación y Universidades. 2023-2026.

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

AWARDS:

El proyecto de spin-off "Nabiza Pharmaceutics", una empresa dedicada al descubrimiento de fármacos de plantas (Brasicaceas), presentado por Juan Manuel Zapata Hernández y Gema Pérez Chacón, fue galardonado con el segundo premio en el Pich day del Programa de Emprendimiento para investigadores de la Salud de UAM emprende y Genesis Biomed 2023 https://eventos.uam.es/99778/detail.

Atherosclerosis Associated Chronic Inflammation in the Progression and Response of Cancer to Immunotherapies: MDSC Modulation by Platelets.

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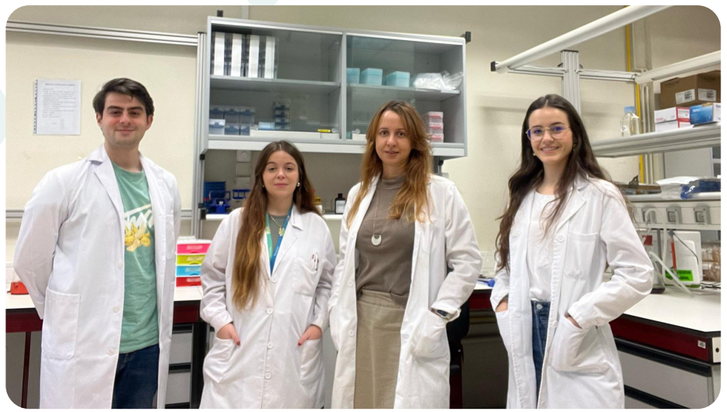
KEYWORDS
Platelets, Immunotherapy, Cancer Immunology, Myeloid Suppressor Cells, Microenvironment.



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

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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.

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-20217524". (2022-2024)

"MATERIAL TRANSFER AGREEMENT between CONSEJO SUPERIOR DE INVESTIGACIONES CIENTÍFICAS, M.P. and Gossamer Bio Services." (2022-2025)

MicroRNAs in immune tolerance, autoimmunity and cancer

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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:

Manuel dos Santos Mattias
"Master´s thesis: *MiR-148a regulation of CD8+ T cell activation in vitro*". University of Strasbourg. Biology. 2023. Supervisor: Alicia González Martín. Grade: Sobresaliente (9.1).

Leticia Villadangos Reyes
"Final degree's project: *Regulation of B cell activation by microRNAs*". Universidad Autónoma de Madrid. Medicina. 2023. 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.

"Ramon y Cajal Merit Award, RyC-2016-21155". Ministry of Science and Innovation. 2018-2023.

PATENTS:

"B cell receptor engineering in B cell lines and primary B cells". Voss J*, Huang D*, González-Martín A*, Andrabi R*, Burton D*. (* Equal contribution). International Patent TSRI 1816.1 PCT / TSR2234P - SL-W:1361.245WO1. 07/02/2019, The Scripps Research Institute. Licensed by Tabby Therapeutics, Israel, 2023.

Nanoimmunology of T Lymphocyte Activation and Apoptosis

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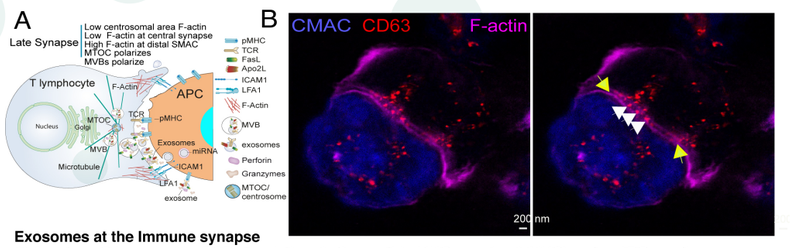
KEYWORDS
T lymphocytes, Immunological synapse, Exosomes, Multivesicular bodies, Actin cytoskeleton, Protein kinase C delta, FMNL1.



Exosomes at the Immune synapse
A) A mature IS is evoked by TCR stimulation via the peptide-MHC complex (pMHC) on the APC and the interaction of accessory molecules (ICAM1/ LFA-1). F-actin is reduced at the cIS, the central region of the IS, F-actin accumulates at the distal SMAC (dSMAC) and F-actin around the centrosome (MTOC) depolymerizes. These processes control MTOC traffic towards the IS and the convergence of MVB towards the F-actin-depleted area in the cIS, facilitating MVB fusion at the cIS, and the subsequent exosome secretion in the synaptic cleft.
B) STED Image of Jurkat T cell (up) forming immune synapse with a Raji cell (down, blue). MVB from the Jurkat cell (CD63+ red vesicles) are located nearby to the immune synapse. The yellow arrows label the edges of the synap cleft, which is the narrow, lane-shaped space between the two cells enclosed by the two F-actin-rich (magenta) plasma membranes.Some red nanovesicles (exosomes, white arrows) are located at the synaptic cleft.

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 filamentous actin (F-actin) accumulation 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 actin and tubulin 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 lymphocytes.

Analysis of centrosomal area actin reorganization and centrosome polarization upon lymphocyte activation at the immunological synapse

Researchers involved: Ruiz-Navarro, J.; Fernández-Hermira S.; Sanz-Fernández, I.; Botas, M.; Calvo, V.; Izquierdo, M.

IS formation is associated with an initial increase in cortical filamentous actin (F-actin) at the IS, followed by a decrease in F-actin density at the central region of the IS, which contains the secretory domain. This is followed by the convergence of secretion vesicles towards the centrosome, and the polarization of the centrosome to the IS. These reversible, cortical actin cytoskeleton reorganization processes occur during lytic granule secretion in cytotoxic T lymphocytes (CTL) and natural killer (NK) cells, proteolytic granules secretion in B lymphocytes and during cytokine-containing vesicle secretion in T-helper (Th) lymphocytes. In addition, several findings obtained in T and B lymphocytes forming IS show that actin cytoskeleton reorganization also occurs at the centrosomal area. F-actin reduction at the centrosomal area appears to be associated with centrosome polarization. In this research line we deal with the analysis of centrosomal area F-actin reorganization, as well as the study of centrosome polarization towards the IS (Fig. 1). This research line is on the frontier of Nanotechnology applied to Life Sciences. Its multidisciplinary nature stands out, which encompasses cell biology techniques and imaging techniques, and Nanotechnology tools, which is cross-sectionally required for the characterization of vesicular traffic and the function of exosomes. These techniques include multiparametric and high-throughput analysis of nanoparticles of biological origin, and the capture and enhancement of super-resolution fluorescence imaging applied to intracellular traffic (Fig.1). Following these approaches, we have shown that, upon Th lymphocyte IS formation, centrosomal area F-actin decreased concomitantly with centrosome polarization to the IS, and a linear correlation between these two parameters exists. Further experiments are in progress to characterize the molecular events involved in centrosomal area cytoskeletal reorganization, and to analyze their role on polarized secretion at the immune synapse.

PUBLICATIONS:

Fernández-Hermira, S.; Sanz-Fernández, I.; Botas, M.; Calvo, V.; Izquierdo, M., Analysis of centrosomal area actin reorganization and centrosome polarization upon lymphocyte activation at the immunological synapse. *Methods in cell biology.* **2023**, *173*, 15-32. DOI: 10.1016/bs.mcb.2021.11.002

Garcia-Martinez, I.; Alen, R.; Pereira, L.; Povo-Retana, A.; Astudillo, A. M.; Hitos, A. B.; Gomez-Hurtado, I.; Lopez-Collazo, E.; Boscá, L.; Francés, R.; Lizasoain, I.; Moro, M.; Balsinde, J.; Izquierdo, M.; Valverde Á, M., Saturated fatty acid-enriched small extracellular vesicles mediate a crosstalk inducing liver inflammation and hepatocyte insulin resistance. *JHEP reports: innovation in hepatology.* **2023**, *5* (8), 100756. DOI: 10.1016/j.jhepr.2023.100756

Gómez-Morón, Á.; Requena, S.; Pertusa, C.; Lozano-Prieto, M.; Calzada-Fraile, D.; Scagnetti, C.; Sánchez-García, I.; Calero-García, A. A.; Izquierdo, M.; Martín-Cófreces, N. B., End-binding protein 1 regulates the metabolic fate of CD4(+) T lymphoblasts and Jurkat T cells and the organization of the mitochondrial network. *Frontiers in immunology.* **2023**, *14*, 1197289. DOI: 10.3389/fimmu.2023.1197289

Ibáñez-Navarro, M.; Fernández, A.; Escudero, A.; Esteso, G.; Campos-Silva, C.; Navarro-Aguadero, M. Á.; Leivas, A.; Caracuel, B. R.; Rodríguez-Antolín, C.; Ortiz, A.; Navarro-Zapata, A.; Mestre-Durán, C.; Izquierdo, M.; Balaguer-Pérez, M.; Ferreras, C.; Martínez-López, J.; Valés-Gómez, M.; Pérez-Martínez, A.; Fernández, L., NKG2D-CAR memory T cells target pediatric T-cell acute lymphoblastic leukemia in vitro and in vivo but fail to eliminate leukemia initiating cells. *Frontiers in immunology*. **2023**, *14*. DOI: 10.3389/fimmu.2023.1187665

DOCTORAL THESES AND OTHER WORKS:

Sofía Blazquez Cucharero

"Final degree's project: *Estudio del tráfico polarizado en la sinapsis inmunitaria: función de FMNL1¨*. Universidad Autónoma de Madrid. Facultad de Biología. 2023. Supervisor/s: Manuel Izquierdo, Javier Ruiz-Navarro. Grade: Sobresaliente 9,2.

Carlos del Hoyo Gómez

"Final degree's project: *Estudio de las bases moleculares de la secreción polarizada de exosomas por los linfocitos T*". Universidad Autónoma de Madrid. Facultad de Medicina. 2023. Supervisor/s: Manuel Izquierdo, Javier Ruiz-Navarro. Grade: Sobresaliente 9,6.

Pablo del Barrio García

"Final degree's project: *Estudio de la proteína fmnl1 en el proceso de secreción polarizada de la sinapsis inmune.*". Universidad Alcalá de Henares. Facultad de Ciencias. 2023. Supervisor/s: Manuel Izquierdo, Javier Ruiz-Navarro. Grade: Sobresaliente 9,42.

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.

department
of **Neurological Diseases & Aging**

Biomedical Imaging Analysis and Multi-Omics Integration

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Miranda de Larra, Álvaro
Muñoz Silva, Miguel
Rubio Muñoz, Alejandra

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; 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 my 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: Muñoz Silva, Miguel; Muñoz Rubio, Alejandra; 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: Miranda de Larra, Álvaro; Ballesteros Gomez, Ana Karina; 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:

Alieva, M.; Wezenaar, A.; Wehrens, E.; Ríos, A. Bridging live-cell imaging and next-generation cancer treatment. *Nat Rev Cancer.* **2023**, *23(11)*, 731-745. DOI: 10.1038/s41568-023-00610-5.

Dekkers, J.; Alieva, M.; Cleven, A.; Keramati, F.; Wezenaar,A.; Van Vliet, E.; Puschhof, J.; Brazda, P.; Johanna, I.; Meringa, A.; Rebel, H.; Buchholz, M.; Barrera, M.; Zeeman, A.; de Blank, S.; Fasci, D.; Geurts, M.; Cornel, A.; Driehuis, E.; Millen, R.; Straetemans, T.; Nicolasen, M.; Aarts-Riemens, T.; Ariese, H.; Johnson, H.; Van Ineveld, R.; Karaiskaki, F.; Kopper, O.; Bar-Ephraim, Y.; Kretzschmar, K.; Eggermont, A.; Nierkens, S.; Wehrens, E.; Stunnenberg, H.; Clevers, H.; Kuball, J.; Sebestyen, Z.; Rios, A. Uncovering the mode of action of engineered T cells in patient cancer organoids. *Nat Biotechnol.* **2023**, *41(1)*, 60-69. DOI: 10.1038/s41587-022-01397-w.

DOCTORAL THESES AND OTHER WORKS:

Ana Karina Ballesteros Gomez
"Master´s thesis: *User-friendly deep learning-based morphometric unmixing of multiplex 3D imaging data*". Universidad Politecnica de Madrid. 2023. Supervisor/s: Maria Alieva Krasheninnikova. Grade: 9,3.

FUNDING:

"Deep learning-based 3D Virtual Multiplexing to explore microenvironment drivers of brain tumor progression. LEO23-2-10305-BBM-BAS-144". Fundacion BBVA. 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

AWARDS:

"European Society for Blood and Marrow Transplantation Jon van Rood award for the best paper of 2022 that significantly contributed to the field of cellular transplantation". 2023

"Leonardo Grant for Researchers and Cultural Creators in Biomedicine". 2023

"Second prize for Best Scientific Image at the ASEICA 40th conference". 2023

Neuroprotective Strategies for Neurodegenerative Diseases

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Escoll Guerrero, María Isabel

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

RESEARCH LINES:

Overview

Aging is the main factor contributing to Parkinson's (PD) and Alzheimer's (AD) diseases. These chronic, incurable diseases can have debilitating effects for years. Many brain 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 NRF2's role in protecting against oxidative damage and neuroinflammation in models of neurodegenerative diseases. 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.

Role of oxidative stress in neuronal death and neuroinflammation in neurodegenerative diseases

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

We studied the role of oxidative stress in neuronal death and neuroinflammation in neurodegenerative diseases, particularly Alzheimer's disease (AD). Our results highlighted how oxidative stress contributed to neuronal damage and inflammation, exacerbating neurodegenerative conditions. In neurodegenerative diseases, excessive levels of reactive oxygen species (ROS) led to lipid peroxidation, protein oxidation, and DNA damage, ultimately resulting in neuronal death. We emphasized that the brain, due to its high oxygen consumption and lipid content, was particularly vulnerable to oxidative damage. In our studies, we also discussed neuroinflammation as a major consequence of oxidative stress. We found that the activation of microglia and astrocytes in response to oxidative stress led to the release of pro-inflammatory cytokines, further damaging neurons. This inflammatory response created a vicious cycle, where oxidative stress and inflammation reinforced each other, accelerating neurodegeneration. In the context of Alzheimer's disease, we linked oxidative stress to key pathological hallmarks such as amyloid-beta (Aβ) accumulation and tau hyperphosphorylation. We suggested that oxidative stress promoted Aβ aggregation, which in turn triggered further ROS production, contributing to neuronal dysfunction and synaptic loss. We also explored therapeutic strategies targeting oxidative stress and identified NRF2 as a promising target. The activation of NRF2 enhanced the expression of detoxifying enzymes, reducing oxidative damage and neuroinflammation. Several potential drug candidates were selected for their ability to modulate NRF2 pathways, showing promise for neuroprotection. Overall, we provided insight into the critical role of oxidative stress in neuronal death and neuroinflammation, highlighting its potential as a therapeutic target in neurodegenerative diseases like Alzheimer's.

Validation of NRF2 as a new therapeutic target in neurodegenerative diseases.

Researchers involved: Fernández-Ginés, R; Cuadrado, A; Lastra, D, Escoll, M; Rojo, AI.

We explored NRF2 as a new therapeutic target in neurodegenerative diseases by analyzing its role in cellular defense mechanisms. We investigated the NRF2 signaling pathway and found that it controlled the expression of numerous cytoprotective genes, includ-

ing those involved in detoxification, anti-inflammatory responses, and mitochondrial function. NRF2 activation mitigated oxidative damage by upregulating antioxidant enzymes such as heme oxygenase-1 (HO-1) and superoxide dismutase (SOD1). We also explored the dysfunction of NRF2 in neurodegenerative diseases and found that in conditions like Alzheimer's, Parkinson's, and Huntington's disease, NRF2 activity was impaired, leading to increased oxidative stress and neuroinflammation. Pathological factors, such as amyloid-beta toxicity and tau aggregation, disrupted NRF2 signaling, exacerbating neuronal damage. To validate NRF2 as a therapeutic target, we examined pharmacological strategies aimed at enhancing its activity. We identified searched the literature for reliable NRF2 activators and their effects in preclinical models of neurodegeneration. We found that compounds such as curcumin, sulforaphane, and dimethyl fumarate successfully activated NRF2, leading to neuroprotection and reduced inflammation. We further validated some of these findings through in vitro and in vivo studies, demonstrating that NRF2 activation improved neuronal survival and cognitive function. Additionally, we assessed the translational potential of NRF2-based therapies by analyzing results of clinical trials targeting this pathway. We observed that NRF2 modulators showed promising results in reducing oxidative stress markers and improving disease outcomes in neurodegenerative disorders. Overall, our findings support the development of NRF2-targeting drugs as a potential strategy for mitigating oxidative stress, neuroinflammation, and neuronal loss in conditions such as Alzheimer's and Parkinson's disease.

Use of the NRF2 transcriptional signature as a biomarker of prognosis, progression and therapeutic efficacy

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

We analyzed how NRF2-regulated genes reflected cellular responses to oxidative stress and inflammation, making them potential indicators of disease status and treatment response. Our analysis of transcriptomic data allowed us to identified a distinct NRF2-driven gene expression profile associated with neurodegeneration. We found that genes involved in antioxidant defense, detoxification, and proteostasis were consistently dysregulated in conditions such as Alzheimer's and Parkinson's disease. The reduced NRF2 activity correlated with increased oxidative damage and neuroinflammation, suggesting that its transcriptional signature could serve as a prognostic marker. To evaluate the potential of NRF2 as a biomarker for disease progression, we tracked its transcriptional activity across different disease stages. We found that NRF2 target gene expression declined as neurodegeneration advanced, indicating a progressive loss of cellular defense mechanisms. We also noted that individuals with more severe cognitive impairment exhibited lower NRF2-related gene expression, reinforcing its value as a progression marker. We further assessed the utility of the NRF2 signature in monitoring therapeutic efficacy. We analyzed drug treatments known to activate NRF2 and found that successful NRF2 modulation resulted in the upregulation of its downstream genes. We validated this effect using both in vitro and in vivo models, demonstrating that NRF2 activation correlated with reduced neuronal damage and improved cognitive outcomes. Additionally, we explored the potential integration of NRF2 transcriptional profiling into clinical practice. We proposed that NRF2 gene expression patterns could be used to stratify patients, predict treatment responses, and optimize therapeutic strategies in neurodegenerative diseases.

Identification of NRF2-activating compounds by inhibiting its interaction with β-TrCP.

*Researchers involved: Fernández-Ginés, R; Cuadrado, A; García-Yagüe, AJ; Rojo, AI; Debasa, M.*The β-TrCP protein is part of the SCF (Skp1-Cullin-F-box) E3 ubiquitin ligase complex, and targets NRF2 for proteasomal degradation, reducing its transcriptional activity and disrupting this interaction stabilized NRF2 and enhanced its ability to regulate cytoprotective genes. To identify potential NRF2-activating compounds, we screened chemical libraries for small molecules capable of selectively inhibiting NRF2-β-TrCP binding. We employed computational docking and molecular dynamics simulations to predict compounds that could interfere with key binding sites. We prioritized candidates based on their binding affinity, specificity, and ability to prevent NRF2 ubiquitination. Then, we experimentally validated the most promising compounds in cellular models and found selected inhibitors that effectively stabilized NRF2, leading to increased expression of its downstream targets such as heme oxygenase-1 (HO-1) and superoxide dismutase (SOD1). We also observed reduced markers of oxidative stress and inflammation in treated neuronal cells, confirming their neuroprotective potential. Furthermore, we evaluated the pharmacokinetic properties and safety profiles of these compounds, identifying candidates suitable for further preclinical development. We proposed that NRF2-β-TrCP inhibitors could serve as novel therapeutic agents for neurodegenerative diseases, enhancing NRF2 activity independently of KEAP1 regulation.

NRF2: biomarker and evaluation as a therapeutic target for Amyotrophic Lateral Sclerosis

Researchers involved: Rojo, AI; Jiménez-Villegas, J.

There is an urgent need for biomarkers and disease-modifying strategies for Amyotrophic Lateral Sclerosis (ALS). Although pharmacological activation of NRF2 has shown benefits in other neurodegenerative models, its role in ALS is not well-studied. We found that NRF2

dysfunction contributed to motor neuron degeneration by reducing cellular antioxidant defenses, leading to increased reactive oxygen species (ROS) and mitochondrial damage. NRF2 transcriptional activity in ALS patient samples is significantly downregulated leading to impaired antioxidant responses. We propose that NRF2 expression levels could serve as a biomarker for disease progression, as lower NRF2 activity correlated with more severe motor impairment and faster disease progression. Durinf this year we have progressed to: 1) Validate NRF2-selective drugs in preclinical ALS models using dimethyl fumarate (DMF) 2) Identify predictive biomarkers by analyzing NRF2 transcriptional signatures in ALS patients' white blood cells. 3) Strengthen collaborations with the biopharmaceutical industry.

PUBLICATIONS:

Bourdakou, MM; Fernández-Ginés, R; Cuadrado, A; Spyrou, GM; Drug repurposing on Alzheimer's disease through modulation of NRF2 neighborhood. *Redox Biol.* **2023**. DOI: 10.1016/j.redox.2023.102881

García-Yagüe, A.J; Cuadrado, A. Mechanisms of NURR1 Regulation: Consequences for Its Biological Activity and Involvement in Pathology. *Int J Mol Sci.* **2023**. DOI: 10.3390/ijms241512280

Bourdakou, MM; Fernández-Ginés, R; Cuadrado, A; Spyrou, GM. Drug repurposing on Alzheimer's disease through modulation of NRF2 neighborhood. *Redox Biol.* **2023**. DOI: 10.1016/j.redox.2023.102881

Palomino-Antolín, A; Decouty-Pérez, C; Farré-Alins, V; Narros-Fernández, P; Lopez-Rodriguez, AB; Álvarez-Rubal, M; Valencia, I; López-Muñoz, F; Ramos, E; Cuadrado, A; Casas, AI; Romero, A; Egea, J. Redox Regulation of Microglial Inflammatory Response: Fine Control of NLRP3 Inflammasome through Nrf2 and NOX4. *Antioxidants.* **2023**. DOI: 10.3390/antiox12091729

Crisman, E; Duarte, P; Dauden, E; Cuadrado, A; Rodríguez-Franco, MI; López, MG; León, R. KEAP1-NRF2 protein-protein interaction inhibitors: Design, pharmacological properties and therapeutic potential. *Med Res Rev.* **2023**. 43(1). 237-287. DOI: 10.1002/med.21925

Bourgonje, AR; Kloska, D; Grochot-Przęczek, A; Feelisch, M; Cuadrado, A; van Goor, H. Personalized redox medicine in inflammatory bowel diseases: an emerging role for HIF-1α and NRF2 as therapeutic targets. *Redox Biol.* **2023**. DOI: 10.1016/j.redox.2023.102603

Silva-Llanes, I,; Shin, CH; Jiménez-Villegas, J: Gorospe, M; Lastres-Becker, I. The Transcription Factor NRF2 Has Epigenetic Regulatory Functions Modulating HDACs, DNMTs, and miRNA Biogenesis. *Antioxidants*. **2023.** DOI: 10.3390/antiox12030641.

DOCTORAL THESES AND OTHER WORKS:

Diego Lastra Martínez
"Ph.D. thesis: *Análisis de la función del factor de transcripción NRF2 en la progresión tumoral*". Universidad Autónoma de Madrid. Medicina. 2023. Supervisor/s: María Isabel Escoll y Antonio Cuadrado. Grade: Sobresaliente Cum Laude

Manuel Debasa Mouce
"Master´s thesis: *6-MSITC (isocianato-wasabi) como modulador de NRF2 y protector frente a la taupatía*". Universidad Complutense de Madrid. Ciencias Biológicas. 2023. Supervisor/s: Ángel Juan García y Antonio Cuadrado. Grade: Sobresaliente

FUNDING:

"El factor de transcripción NRF2 en la patofisiología de la enfermedad de Alzheimer. PID2019-110061RB-I00". MICINN. 2020-2023

"Desarrollo de nuevos fármacos anti-inflamatorios basados en la activación del factor de transcripción NRF2. PDC2021-121421-I00". MICINN. 2021-2023

"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

AWARDS:

"Premio de innovación y transferencia de conocimiento de la Universidad Autónoma de Madrid". 2023

"Beca Dr. Luis Álvarez 2023 a la publicación de artículos científicos de alto impacto. IdiPAZ". 2023

Neuroprotective Peptides in Excitotoxicity and Stroke

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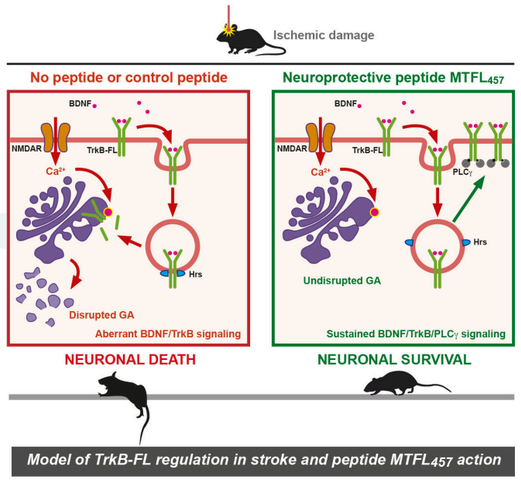
KEYWORDS
Cell-penetrating Peptides, Excitotoxicity, Neuroprotection, PSD-95, Stroke, TrkB.



Model of TrkB-FL regulation in stroke and peptide $MTFL_{457}$ action

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.

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, MTFL457, which efficiently prevents excitotoxicity-induced receptor processing and neuronal death by a PLCγ-dependent mechanism. In the stroke model, MTFL457 decreases the infarct size and improves the neurological outcome. Our recent 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, MTFL457 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 MTFL457 might preserve GA function and promote neuronal survival not only in stroke but also other neurodegenerative diseases.

We are also investigating MTFL457 potential to prevent cochlear synaptopathy (with I. Varela's group), induced by excitotoxicity or decreased neurotrophic support in the inner ear, or promote oligodendrocyte survival in models of multiple sclerosis (with C. Dreyfus).

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. TrkB-T1, which is also expressed in astrocytes, has TrkB-FL-independent functions, probably mediated by protein interactions established by a highly conserved intracellular sequence. Excitotoxicity alters TrkB-T1 levels and activity by mechanisms that include transcriptional upregulation, regulated intramembrane proteolysis (RIP), producing a receptor ectodomain acting as a BDNF-scavenger and intracellular fragments (ICDs) of unknown function and, probably, changes in protein interactions. As neuroprotective strategies, we are developing peptides to prevent TrkB-T1 cleavage by metalloproteinases, first and obligatory step for RIP, or interfere protein interactions established by this isoform intracellular sequences. In addition, TrkB-T1-containing CPPs are interesting to model damage induced by ICDs to neurons and astrocytes, investigate ICDs transcriptional activity and identify TrkB-T1-interacting proteins.

DOCTORAL THESES AND OTHER WORKS:

Elena Martín Buzo

"JAE Intro ICU fellowship: *Caracterización de cell-penetrating peptides (CPPs) dirigidos a prevenir el procesamiento por metaloproteinasas del receptor de neurotrofinas TrkB en excitotoxicidad e isquemia cerebral*". Conexión de Nanomedicina CSIC. Supervisor/s: Margarita Díaz-Guerra González

FUNDING:

"Estrategias neuroprotectoras y reparadoras con péptidos que promueven la vía de señalización BDNF/TrkB/CREB. PID2019-105784RB-100". MICINN. 2020-2023

"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

"Ayudas Extraordinarias para la preparación de proyectos a realizar en el marco del Plan estatal de I+D+i. 2023AEP097". AEI. 2023

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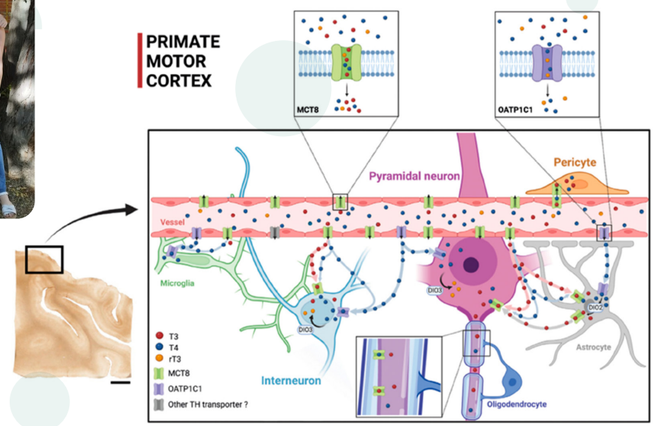
KEYWORDS
Thyroid hormones, Thyroid hormone transport, MCT8 deficiency, Brain, Blood-brain barrier, Thyromimetics



Model of thyroid hormones transport in human and primate motor cortex based on our contribution. Yu Wang et al 2023 IJMS

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 underlying an X-linked, inherited rare disease caused by mutations in the Monocarboxylate transporter 8 (MCT8), a specific thyroid hormone transporter. This condition is known as the Allan-Herndon-Dudley syndrome (AHDS) or MCT8 deficiency. It results in peripheral hyperthyroidism and profound neurological impairments, mainly attributed to brain hypothyroidism. However, the underlying pathophysiological mechanisms remain poorly understood.

To achieve our goals:

- We investigate the phenotype of several animal models, some of which have been developed in our laboratory. These models are animals with congenital hypothyroidism, animals deficient in MCT8, the main transporter of thyroid hormones in brain barriers and neural cells, and animals deficient in proteins involved in the metabolism and action of thyroid hormones.
- We use different experimental approaches, mainly in vivo studies. In addition, we perform preclinical studies with animal models of AHDS to test the action of different thyroid hormone analogs on thyroid hormone target neural cells under MCT8-deficient conditions.
- We analyze the histopathology of human autopsy brain tissue from patients with a genetic diagnosis of AHDS.

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; Rausell, E; Valcárcel-Hernández, V; Wang, T; Wang, Y.

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 working in this research line using different strategies or approaches:

1. To study histopathological alterations in the CNS of affected patients with different histological techniques and immunohisto-

chemistry, and magnetic resonance imaging techniques, as well as other non-invasive techniques to evaluate motor alterations

2. To investigate the spatiotemporal expression pattern of MCT8 in the human and non-human primate brain, both during development and in juvenile stages to characterize the subpopulations of neural cells that express MCT8 specifically. Knowing which cells and at what time may require MCT8 for proper development or function is fundamental for the development of therapies for the disease, especially gene therapies. These studies will also contribute to a better understanding of the role of MCT8 in the human brain.

Our studies investigating the expression of thyroid hormone transporters MCT8 and OATP1C1 in the human and non-human primate brain, highlight their crucial role in motor system regulation and brain homeostasis. Immunohistochemical analysis in human and primate motor cortices revealed that both transporters are present in long-projection pyramidal neurons, GABAergic interneurons, and astrocytes. MCT8 was identified at the neurovascular unit, whereas OATP1C1 appeared only in certain large vessels and, unexpectedly, in human-specific Corpora amylacea aggregates. These findings suggest that MCT8 and OATP1C1 modulate excitatory/inhibitory motor circuits, explaining the severe motor impairments seen in transporter deficiency syndromes. Further investigation in basal ganglia circuits showed MCT8/OATP1C1 expression in medium-sized spiny neurons of the striatum, local interneurons (including cholinergic cells), and projection neurons of the intrinsic and output nuclei. Their presence in the motor thalamus and nucleus basalis of Meynert suggests a key role in motor system modulation. Transporter dysfunction likely disrupts basal ganglia circuits, leading to profound movement disorders.

Years ago, we provided the first description available regarding the anatomical and cellular defects caused by MCT8 deficiency in the brain of a human fetus and a child. Recently we have further analyzed the brain pathophysiology of MCT8-deficient brains using brain samples from an MCT8-deficient patient and a validated mouse model of the syndrome: Mct8/Dio2KO mice. Using histological, ultraestructural, magnetic resonance imaging and in vivo blood brain barrier permeability studies we found that in MCT8-deficient humans and Mct8/Dio2KO mice exists neurovascular alterations, including blood-brain barrier (BBB) disruption, increased permeability, and impaired angiogenesis. These findings introduce vascular dysfunction as a novel AHDS pathophysiological mechanism and propose magnetic resonance angiography as a potential non-invasive tool for disease monitoring and therapeutic targeting.

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

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

MCT8 deficiency is 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.

The thyroid hormone analog TRIAC is currently widely used to normalize the peripheral hyperthyroidism of MCT8-deficient patients. We explore the potential of TRIAC to access the MCT8-deficient brain and exert thyromimetic actions. As in previous studies systemic treatment with TRIAC was not able to reach the brain in sufficient amounts to detect the analog and mediate the expression of T3-target genes in MCT8-deficiency, we investigated the effects of intracerebroventricular (ICV) administration of a high-dose TRIAC in juvenile Mct8/Dio2 KO mice. As expected, the treatment normalized peripheral hyperthyroidism without worsening brain hypothyroidism. However, while TRIAC content increased in several brain regions, its thyromimetic effects were limited to specific areas. These findings indicate that ICV TRIAC administration provides only modest benefits for brain function. We collaborated for these studies with Dr. Refetoff's group, one of the two research teams that discovered the etiology of AHDS.

We also further explore the potential of another thyroid hormone analog, Sobetirome, as we demonstrated in previous studies that it was able to reach the brain and drive the expression of Thyroid hormone-target genes, making Sobetirome a more promising candidate. We tested the effects of Sobetirome and its CNS-selective prodrug, Sob-AM2, in pregnant mice carrying Mct8/Dio2 KO fetuses. Sobetirome treatment led to spontaneous abortions, whereas Sob-AM2 successfully crossed both the placental and fetal brain barriers, exerting thyromimetic effects in fetal tissues by modulating the expression of thyroid hormone-dependent genes in the liver and brain. These findings suggest that early interventions with Sob-AM2 could address cerebral hypothyroidism from fetal stages, potentially preventing neurodevelopmental deficits in MCT8-deficient individuals.

In view of all these results, we continue exploring Sobetirome and Sob-AM2 as a promising therapy to improve the neurological condition of MCT8-deficient patients, performing preclinical studies in disease animal models.

PUBLICATIONS:

Grijota-Martínez, C.; Montero-Pedrazuela, A.; Hidalgo-Álvarez, J.; Bárez-López, S.; Guadaño-Ferraz, A. Intracerebroventricular High Doses of 3,3',5-Triiodothyroacetic Acid at Juvenile Stages Improve Peripheral Hyperthyroidism and Mediate Thyromimetic Effects in Limited Brain Regions in a Mouse Model of Monocarboxylate Transporter 8 Deficiency. *Thyroid.* **2023**, *33(4)*, 501-510. DOI: 10.1089/thy.2022.0562.

Wang, Y.; Wang, T.; Montero-Pedrazuela, A.; Guadaño-Ferraz, A.; Rausell, E. Thyroid Hormone Transporters MCT8 and OATP1C1 Are Expressed in Pyramidal Neurons and Interneurons in the Adult Motor Cortex of Human and Macaque Brain. *Int J Mol Sci.* **2023**, *24(4),* 3207. DOI: 10.3390/ijms24043207.

Greenwood, M.P.; Greenwood, M.; Bárez-López, S.; Hawkins, J.W.; Short, K.; Tatovic, D.; Murphy, D. Osmoadaptive GLP-1R signalling in hypothalamic neurones inhibits antidiuretic hormone synthesis and release. *Mol Metab.* **2023**, *70*, 101692. DOI: 10.1016/j.molmet.2023.101692.

Valcárcel-Hernández, V.; Guillén-Yunta, M.; Scanlan, T.S.; Bárez-López, S.; Guadaño-Ferraz, A. Maternal Administration of the CNS-Selective Sobetirome Prodrug Sob-AM2 Exerts Thyromimetic Effects in Murine MCT8-Deficient Fetuses. *Thyroid* **2023**, *33(5)*, 632-640. DOI: 10.1089/thy.2022.0612.

Wang, T.; Wang, Y.; Montero-Pedrazuela, A.; Prensa, L.; Guadaño-Ferraz, A; Rausell, E. Thyroid Hormone Transporters MCT8 and OATP1C1 Are Expressed in Projection Neurons and Interneurons of Basal Ganglia and Motor Thalamus in the Adult Human and Macaque Brain. *Int J Mol Sci*. **2023**, *24(11)*, 9643. DOI: 10.3390/ijms24119643.

Bárez-López, S.; Mecawi, A.S.; Bryan, N.; Pauža, A.G.; Duque, V.J.; Gillard, B.T.; Murphy, D.; Greenwood, M.P. Translational and Posttranslational Dynamics in a Model Peptidergic System. *Mol Cell Proteomics.* **2023**, *22(5)*, 100544. DOI: 10.1016/j.mcpro.2023.100544.

Neves, J.S.; Leite, A.R.; Conceição, G.; Gonçalves, A.; Borges-Canha, M.; Vale, C.; Von-Hafe, M.; Martins, D.; Miranda-Silva, D.; Leite, S.; Rocha-Oliveira, E.; Sousa-Mendes, C.; Chaves, J.; Lourenço, I.M.; Grijota-Martínez, C.; Bárez-López, S.; Miranda, I.M.; Almeida-Coelho, J.; Vasques-Nóvoa, F.; Carvalho, D.; Lourenço, A.; Falcão-Pires, I.; Leite-Moreira, A. Effects of Triiodothyronine Treatment in an Animal Model of Heart Failure with Preserved Ejection Fraction. *Thyroid.* **2023**, *33(8),* 983-996. DOI: 10.1089/thy.2022.0717.

Guillén-Yunta, M.; Valcárcel-Hernández, V.; García-Aldea, Á.; Soria, G.; García-Verdugo, J.M.; Montero-Pedrazuela, A.; Guadaño-Ferraz, A. Neurovascular Unit Disruption and Blood-Brain Barrier Leakage in MCT8 Deficiency. *Fluids Barriers CNS* **2023**, *20(1)*, 79. DOI: 10.1186/s12987-023-00481-w.

Bárez-López, S.; Gadd, G.J.; Pauža, A.G.; Murphy, D.; Greenwood, M.P. Isoflurane Rapidly Modifies Synaptic and Cytoskeletal Phosphoproteomes of the Supraoptic Nucleus of the Hypothalamus and the Cortex. *Neuroendocrinology.* **2023**, *113(10)*, 1008-1023. DOI: 10.1159/000531352.

Contreras-Jurado, C.; Montero-Pedrazuela, A.; Pérez, R.F.; Alemany, S.; Fraga, M.F.; Aranda. A.The thyroid hormone enhances mouse embryonic fibroblasts reprogramming to pluripotent stem cells: role of the nuclear receptor corepressor 1. *Front Endocrinol (Lausanne).* **2023,** *1;14,* 1235614. DOI: 10.3389/fendo.2023.1235614.

DOCTORAL THESES AND OTHER WORKS:

Yu Wang
"Ph.D. thesis: *Distribución de los transportadores de hormonas tiroideas MCT8 y OATP1C1 en la corteza motora de macaco y humano*". Universidad Autónoma de Madrid. Medicina. 2023. Supervisors: Estrella Rausell & Ana Guadaño. Grade: Sobresaliente Cum Laude

Víctor Valcárcel Hernández
"Ph.D. thesis: *Unraveling disease mechanisms, developing preclinical models and therapeutic approaches to target MCT8 deficiency*". Universidad Autónoma de Madrid. Medicina. 2023. Supervisors: Ana Guadaño & Soledad Bárez. Grade: Sobresaliente Cum Laude

Ting Wang
"Ph.D. thesis: *Distribución de los transportadores de hormonas tiroideas MCT8 y OATP1C1 en los ganglios basales de humano y macaco*". Universidad Autónoma de Madrid. Medicina. 2023. Supervisors: Estrella Rausell & Ana Guadaño. Grade: Sobresaliente Cum Laude

Andrea Rodríguez Cruz
"Master's thesis: *Caracterización funcional y estructural del sistema auditivo en ratones modelo del síndrome de Allan-Herndon-Dudley*".
Universidad Complutense de Madrid. Biología. 2023. Supervisor: Soledad Bárez. Grade: Notable.

Ainara Sanz Bógalo
"Final degree's project: *Astroglía y microglía a edades tempranas del desarrollo postnatal en la deficiencia de Mct8*". Universidad Autónoma de Madrid. Bioquímica. 2022-2023. Supervisors: Ana Guadaño & Marina Guillén. Grade: Sobresaliente

Irene Pazos Sanz
"Final degree's project: *Mutaciones en MCT8: afectación del eje hipotálamo-hipófisis-tiroides*"
Universidad Autónoma de Madrid. Bioquímica. 2022-2023. Supervisors: María del Carmen Grijota Martínez & Ana Guadaño Ferraz. Grade: Sobresaliente

Alexia Pérez Pestourie
"JAE Intro (JAEINT23_EX_0390) *Cómo mejorar los trastornos neurológicos asociados a una enfermedad rara*" Introduction to research Fellowship, from the CSIC development of studies Board. 2023-2024. Supervisor: Soledad Bárez.

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.

AWARDS:

"Best Flash Talk award "Addressing the neurological defects in MCT8 deficiency. At the IIBm Retreat La Cristalera, Miraflores de la Sierra".
14th of June 2023

Novel Targets in Neurodegeneration and Neuroprotection

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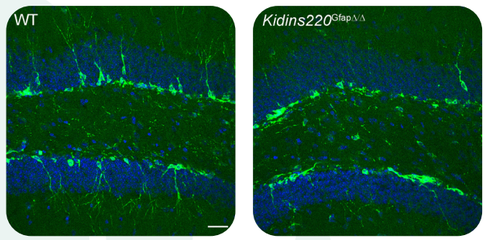
KEYWORDS
Stroke, Hydrocephalus, Neurogenesis, SINO Syndrome, Kidins220, Protein Kinase D



Neurogenic defects in Kidins220 deficient mice in the hippocampus. Staining for the neuroblast marker doublecortin (DCX, green) and DAPI (blue) at the subgranular zone (SGZ) of the dentate gyrus (DG) in the hippocampus of WT (left pannel) and Kidins220Gaps (right pannel) mice.
Kidins220 deficiency in adult neural stem cells (NSCs) markedly decreases type 2 cycling progenitors and the concomitant emergence of DCX+ neuroblasts and adult-born neurons, indicating that survival might be compromised in NSC-derived progeny when Kidins220 levels drop in the DG. Scale bar: 20 um.

RESEARCH LINES:

Overview

Our group investigates cellular and molecular mechanisms underlying dementia and neurodegeneration searching for neuroprotection strategies. **Dementia** is a chronic and progressive syndrome affecting about 50% of adults aged over 85. It is characterized by cognitive impairment and loss of memory that is accompanied by a decline in the ability to perform daily activities. Increased life expectancy and growth in elderly population makes this condition increasingly common. An estimated 50 million people in the world lived with dementia in the last year, and 10 million new cases are recorded each year. Causes of dementia are multiple, including chronic neurodegenerative diseases, such as Parkinson's disease, Huntington's disease (HD) and **Alzheimer's disease** (AD). Among these, AD is the most common form, accounting for 60–70% of cases. Contributing to this percentage is idiopathic Normal Pressure **Hydrocephalus (iNPH)**, the major form of chronic hydrocephalus in adults, a disease characterized by cerebrospinal fluid accumulation, ventricular enlargement and dementia. The second most common cause of dementia is vascular dementia, representing 20% of cases, with **ischemic stroke** as the major cause accounting for approximately 80% of all cases. In ischemic stroke, obstruction of cerebral blood vessels decreases blood flow, compromising glucose and oxygen supply and therefore provoking a dramatic tissue necrotic injury, with massive primary neuronal death at the ischemic core. As a consequence, dying neurons release high amounts of the excitatory neurotransmitter glutamate that generates a second wave of apoptotic neuronal death in peripheral areas surrounding the nucleus of the infarct thus exacerbating the initial injury. The primary cause of this secondary neuronal death is known as **excitotoxicity**. We study **PKD1** (Protein Kinase D1) and **Kidins220** (Kinase D interacting substrate of 220kDa), two molecules we have shown to be key for neuronal survival and neurogenesis and that potentiate neuroprotection from excitotoxicity. We work on elucidating the role of both proteins in neurological diseases associated with dementia and that suffer neuronal loss after acute brain damage or by chronic neurodegeneration. We performed seminal studies on PKD1 function in neurons, and have demonstrated its highly neuroprotective role. We have also identified Kidins220 decreases in stroke and is also altered in human HD, AD and iNPH brain. More recently, we have discovered the molecular mechanisms by which deficiencies in Kidins220 lead to **hydrocephalus**, opening novel pathways for therapeutic intervention of this condition that lacks pharmacological treatment.

PKD1 role in brain function and as neuroprotective molecule

Researchers involved: López C; Sánchez-Miranda, L; Prudencio, M; Sanz, M; González, A.;

Preventing excitotoxicity may confer neuroprotection in a broad range of human neuropathologies. Previous results from our group have shown that PKD1 potentiates a free-radical detoxification pathway that is shut-off by excitotoxicity, and that constitutively active PKD1 is highly neuroprotective in vitro and in vivo. We aim to assay the therapeutic potential of active PKD1 AAVs particles in preclinical studies of neurodegenerative disease mouse models. We are also investigating PKD1 regulation of brain physiological functions and other cerebral pathological processes, such as neuroinflammation, using selective inhibitors and conditional neuronal and astrocytic *Prkd1*-KO mice.

Pathophysiological mechanisms associated with KIDINS220 deficiencies.

Researchers involved: Simón, A; López, C; Prudencio, M; Sanz, M.

Using Kidins220-deficient mice we have found an unanticipated role of Kidins220 as a critical regulator of the SNX27-retromer, a complex that redirects endocytosed cargos to the cell surface, avoiding their lysosomal degradation. We have also demonstrated that the water channel AQP4 is a novel SNX27-retromer cargo. These new data have prompted us to analyse in detail the involvement of Kidins220 in the fine-tuning of endosomal recycling of different cargos crucial for brain function, such as AQP4. Via downregulating SNX27-retromer and AQP4 levels, Kidins220 deficiency induces ventriculomegaly, constituting Kidins220 deficient mice a novel model to analyse hydrocephalus molecular mechanisms and therapies (*Mol. Psychiatry, 2021* and international patent *PCT/EP2022/061794*). Pathogenic variants in *KIDINS220* gene are associated with a novel rare syndrome characterized by spastic paraplegia, intellectual disability, nystagmus and obesity **(SINO syndrome)**. Importantly, SINO syndrome children show **ventriculomegaly** that resembles that of Kidins220 deficient mice. More recently, we have identified Kidins220 as a key player for sensing the availability of growth factors to sustain **Neural Stem Cells** (NSCs) growth and expansion in **adult neurogenesis (Figure 1),** uncovering a molecular link that may help paving the way towards **neurorepair** (Cell Death Dis., 2023). We are investigating SINO syndrome etiopathology in an international collaborative framework.

PUBLICATIONS:

Del Puerto, A.; Lopez-Fonseca, C.; Simón-García, A.; Martí-Prado, B.; Barrios-Muñoz, A.L.; Pose-Utrilla, J.; López-Menéndez, C.; Alcover-Sanchez, B.; Cesca, F.; Schiavo, G.; Campanero, M.R.; Fariñas, I.; Iglesias, T.; Porlan, E. Kidins220 sets the threshold for survival of neural stem cells and progenitors to sustain adult neurogenesis. *Cell Death Dis.* **2023**, *(14)*. DOI. 10.1038/s41419-023-05995-7

Martínez, N.; Gragera, T.; de Lucas, M.P.; Cámara, A.B.; Ballester, A.; Anta, B.; Fernández-Medarde, A.; López-Briones, T.; Ortega, J.; Peña-Jiménez, D.; Barbáchano, A.; Montero-Calle, A.; Cordero, V.; Barderas, R.; Iglesias, T.; Yunta, M.; Oliva, J.L.; Muñoz, A.; Santos, E.; Zarich, N.; Rojas-Cabañeros, J.M. PKD phosphorylation and COP9/Signalosome modulate intracellular Spry2 protein stability. *Oncogenesis*. **2023,** *(12)*. DOI. 10.1038/s41389-023-00465-3

García-Bonilla, M.; Ojeda-Pérez, B.; Shumilov, K.; Rodríguez-Pérez, L.M.; Domínguez-Pinos, D.; Vitorica, J.; Jiménez, S.; Ramírez-Lorca, R.; Echevarría, M.; Cárdenas-García, C.; Iglesias, T.; Gutiérrez, A.; McAllister, J.P.; Limbrick, D.D. Páez-González, P.; Jiménez, A.J. Generation of Periventricular Reactive Astrocytes Overexpressing Aquaporin 4 Is Stimulated by Mesenchymal Stem Cell Therapy. Int. J. *Mol. Sci.* **2023,** *(24).* DOI. 10.3390/ijms24065640)

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

PATENTS:

"Methods and Compositions for the Treatment of Disorders Characterized by a Kidins220 Dysfunction in a Subject". Iglesias T, Campanero MR, del Puerto A, Pose J, Simón A, López C, Sánchez-Miranda L. International Patent PCT/EP2022/061794. Year 2021.

Parkinson, ALS and Tauopathies: New Insights

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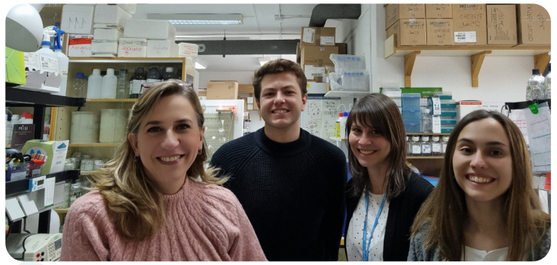
KEYWORDS
Parkinson's disease, ALS, TAU, Neuroinflammation, Oxidative stress, Drug discovery.



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.

Amyotrophic Lateral Sclerosis (ALS): Analysis of RNA Transport and In Situ Protein Translation: STAUFEN 1/2 Involvement?

Researchers involved: Silva-Llanes, I; Lastres-Becker, I

Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disease that affects motor neurons in the spinal cord and cerebral cortex. Patients experience progressive loss of muscle strength and coordination, which increasingly impairs their ability to perform daily activities. Currently, there is no cure for ALS, which is why our laboratory is developing several research lines aimed at addressing this challenge through the design and development of new therapies for ALS treatment. One of the main characteristics of ALS is the presence of alterations in mRNA metabolism and dysregulated axonal transport. For this reason, in a project funded by FUNDELA, we are investigating whether TDP-43 aggregates sequester RNA-binding proteins, preventing them from performing their function. This could mean that mRNA molecules don't reach synaptic connections and cannot be translated, leading to a breakdown in neuron-muscle communication, which ultimately results in the loss of muscle function. Therefore, we are studying RNA granule transport and in situ protein translation in ALS, specifically focusing on determining the involvement of STAUFEN and TDP-43 proteins.

Frontotemporal Dementia: Research on Therapies to Modulate TDP-43 and TAU Proteins Associated with Neurodegeneration

Researchers involved: Silva-Llanes, I; Berrojo-Armisen, A; 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. Currently, there is no approved effective treatment for FTD that can modulate the course of the disease. In recent years, we have identified the transcription factor NRF2 as a key factor in limiting the neurodegenerative process. NRF2 has a very broad spectrum of action in different cellular processes. Therefore, our objective is to repurpose the use of dimethyl fumarate for the treatment of FTD patients, whether TAU or TDP-43-related, and we believe this would greatly benefit these patients who currently lack treatment to slow down the degenerative process.

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

Researchers involved: Silva-Llanes, I; Brakhan, M; 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.

PUBLICATIONS:

Silva-Llanes, I.; Shin, C. H.; Jiménez-Villegas, J.; Gorospe, M.; Lastres-Becker, I. The Transcription Factor NRF2 Has Epigenetic Regulatory Functions Modulating HDACs, DNMTs, and miRNA Biogenesis. Antioxidants *(Basel)* **2023,** *12 (3)*. DOI: 10.3390/antiox12030641.

Brackhan, M.; Arribas-Blazquez, M.; Lastres-Becker, I. Aging, NRF2, and TAU: A Perfect Match for Neurodegeneration? *Antioxidants (Basel).* **2023**,*12 (8)*. DOI: 10.3390/antiox12081564.

Lastres-Becker I. Special Issue "Role of NRF2 in Disease: Novel Molecular Mechanisms and Therapeutic Approaches II". *Biomolecules.* **2023**,*13(5)*,813. DOI: 10.3390/biom13050813.

DOCTORAL THESES AND OTHER WORKS:

Alicia Berrojo Armisen
"Final degree's project: *Análisis del posible efecto neuroprotector del tratamiento con dimetil fumarato en un modelo de demencia frontotemporal TDP-43 dependiente*". Universidad Autónoma de Madrid. Medicina. 2023. 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.

AWARDS:

"Premios del XX Certamen Universitario "Arquímedes" de Introducción a la Investigación Científica a Rodríguez López, Silvia María (accésit) por el Trabajo de Fin de Master".

Biomedical Magnetic Resonance

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

RESEARCH LINES:

Overview

Our laboratory is dedicated to advancing personalized medicine through the innovative use of multimodal biomedical imaging, particularly in oncology, neurology, and obesity. We aim to develop novel, non-invasive biomarkers that offer critical insights into the progression of diseases like obesity and cancer, facilitating tailored therapeutic approaches. With a strong preclinical focus and multidisciplinary expertise, our group utilizes magnetic resonance imaging (MRI) and spectroscopy (MRS) for comprehensive characterization of animal models of neuropathologies in vivo, ex vivo, and in vitro. We combine these with the use of positron emission tomography (PET) for a better understanding of the molecular mechanism of the process studied. Our primary goal is to identify and translate preclinical imaging biomarkers into clinically relevant diagnostic and prognostic tools, as well as for early validation of therapeutic interventions. The non-invasive nature and translational potential of preclinical imaging provide valuable insights into key parameters such as vascularization, cellularity, oxygenation, pH, and metabolism.

Multiparametric and Multimodal Imaging of Neuroinflammation

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

Neuroinflammation is a complex biological process crucial to various neurological diseases, yet accurately assessing it in vivo poses significant challenges. Current methods often rely on invasive techniques, limiting their clinical use. To overcome this, our research focuses on developing a specialized imaging environment using MRI, MRSI, and hybrid PET/MRI techniques. We have successfully implemented protocols for MRI—including T1, T2, T2*, MT, diffusion, perfusion, and functional imaging—as well as MRSI and hybrid MRI/PET techniques. An optimal protocol for characterizing neuroinflammation has been established, excluding perfusion and MRSI while incorporating ex vivo MRS. Additionally, we developed a software platform for automated processing and analysis of imaging data. These efforts aim to provide non-invasive, quantitative methods for assessing neuroinflammation in vivo, enhancing our understanding of its role in disease pathogenesis and progression.

Neuroinflammation in Glioblastoma and Other Neurological Disorders

Researchers involved: Lopez Larrubia, Pilar

Glioblastoma is a highly aggressive brain tumor significantly influenced by its surrounding neuroinflammatory environment, making it essential to understand their interplay for developing effective therapeutic strategies. Neuroinflammation also plays a role in the pathogenesis of various neurological disorders, including obesity and brain trauma. We have examined the role of neuroinflammation in glioblastoma's progression, characterizing the neuroinflammatory response using MRI in both wild-type and transgenic animal models. Our research also explores the relationship between neuroinflammation and obesity within the context of glioblastoma. Furthermore, we have collected and analyzed MRI data from human glioblastoma patients, setting the stage for clinical translation of our findings. These studies aim to provide insights into the mechanisms of neuroinflammation in these diseases and identify potential therapeutic targets.

Interaction between cancer metabolism and cancer acquire immune resistance in triple negative breast cancer models.

Researchers involved: Pacheco Torres, Jesús

Immunotherapies have shown promise in cancer treatment, but tumors like glioblastoma and triple-negative breast cancers often do not respond. We investigated the relationship between tumor metabolism and immune checkpoint expression, focusing on PD-L1. Our findings revealed an inverse relationship between metabolism and PD-L1 across genomic, proteomic, and metabolomic levels, highlighting various inflammation signaling pathways as key factors in this interaction. We identified previously unknown roles for PD-L1 in cancer cell metabolic reprogramming and discovered that downregulation of choline kinase alpha enhances the immunosuppressive effects of PD-L1. These observations offer new insights that could guide the rational design of combinatorial therapies targeting both immune checkpoints and cancer metabolism.

Immunotherapy in glioblastoma: a multimodal approach.

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

Cancer immunotherapies have revolutionized treatment for some cancers, but their effectiveness against glioblastoma (GBM), an aggressive brain tumor, remains limited. GBM's resistance to immunotherapy is likely due to factors like tumor metabolism, an immunosuppressive environment, and high heterogeneity. To overcome these challenges, we are employing a multimodal imaging approach, combining Magnetic Resonance Imaging (MRI), Spectroscopy (MRS), and hybrid MRI-Positron Emission Tomography (PET) with molecular biology techniques. This approach will help us understand the mechanisms behind GBM's resistance to immunotherapy and develop imaging-based biomarkers to guide the design and personalization of more effective combinatorial therapies for patients with GBM.

Cerebral mechanisms underlying obesity development and treatment.

Researchers involved: Lizarbe Serra, Blanca.

Obesity is a chronic disease linked to several comorbidities, including type 2 diabetes, cardiovascular disease, hypertension, certain cancers, and a higher risk of neurodegenerative disorders. Current strategies to combat obesity focus on prevention, promoting healthy lifestyles, and providing palliative medical treatment for its comorbidities. Bariatric surgery has proven to be highly effective in restoring non-obese body mass indexes. It also induces changes in global metabolism, gut microbiota, and brain function. Concurrently, research into anti-obesity pharmaceuticals is showing promising preclinical results. Notably, celastrol, has emerged as a promising anti-obesity and anti-inflammatory agent. Both bariatric surgery and celastrol work by deactivating inflammation in the hypothalamus, a key brain region regulating appetite and energy homeostasis, though the exact mechanisms remain unclear. Other brain areas, such as the mesocorticolimbic and reward centers, also play roles in controlling food intake and exhibit inflammation during obesity. Recent evidence suggests that the initial degree of inflammation may directly influence the success of anti-obesity treatments. Our research develops and implements MRI and spectroscopy methods to show that these techniques can detect appetite and high-fat diet-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 best anti-obesity therapy on an individual basis.

PUBLICATIONS:

Satrustegui, J.; López-Larrubia, P.; Rodrigues, T.B.; Choi, I-Y.; McKenna, M.C. A tribute to Sebastián Cerdán and his key contributions to brain metabolism. *J Neurochem.* **2023**, *00*, 1-6. DOI: 10.1111/jnc.15828.

Pacheco-Torres, I.; Hernández-Sánchez, D.; García-De la Peña, C.; Tarango-Arámbula, LA.; Crosby-Galván, MM.; Sánchez-Santillán, P. Analysis of the Intestinal and Faecal Bacterial Microbiota of the Cervidae Family Using 16S Next-Generation Sequencing: A Review. *Microorganisms*. **2023**, *11(7)*, 1860. DOI: 10.3390/microorganisms11071860.

Marin-Maldonado, F.; Pacheco-Torres, AL.; Gustafson, E. Comparative analysis of onychomycosis in Puerto Rico using molecular and conventional approaches. *J Mycol Med.* **2023**, *33(3)*, 101412. DOI: 10.1016/j.mycmed.2023.101412.

Costa-Pereira, J. T.; Oliveira, R.; Guadilla, I.; Guillen, M. J.; Tavares, I.; Lopez-Larrubia, P. Neuroimaging uncovers neuronal and metabolic changes in pain modulatory brain areas in a rat model of chemotherapy-induced neuropathy - MEMRI and ex vivo spectroscopy studies. *Brain Res Bull.* **2023**, *192*, 12-20. DOI: 10.1016/j.brainresbull.2022.10.018.

Lopez-Larrubia, P.; Santone, A. Editorial: Incorporation of reporting and data systems into cancer radiology. *Front Oncol.* **2023**, *13*,1171171. DOI: 10.3389/fonc.2023.1171171

Ackerstaff, E,; López-Larrubia, P. Editorial: Differentiating brain cancers and glioblastoma through imaging methodologies. *Front Oncol.* **2023**, *13*:1351874. DOI: 10.3389/fonc.2023.1351874.

Guadilla, I.; Gonzalez, S.; Cerdan, S.; Lizarbe, B.; Lopez-Larrubia, P. Magnetic resonance imaging to assess the brain response to fasting in glioblastoma-bearing rats as a model of cancer anorexia. *Cancer Imaging*. **2023**, *23*(1), 36. Doi: 10.1186/s40644-023-00553-y

Ferreira, V.; Folgueira, C.; Garcia-Altares, M.; Guillen, M.; Ruiz-Rosario, M.; DiNunzio, G.; Garcia-Martinez, I.; Alen, R.; Bookmeyer, C.; Jones, J. G.; Cigudosa, J. C.; Lopez-Larrubia, P.; Correig-Blanchar, X.; Davis, R. J.; Sabio, G.; Rada, P.; Valverde, A. M. Hypothalamic JNK1-hepatic fatty acid synthase axis mediates a metabolic rewiring that prevents hepatic steatosis in male mice treated with olanzapine via intraperitoneal: Additional effects of PTP1B inhibition. *Redox Biol.* **2023**, *63*, 102741. DOI: 10.1016/j.redox.2023.102741

Murillo-Cuesta, S.; Lara, E.; Bermúdez-Muñoz, J.M.; Torres-Campos, E.; Rodríguez-de la Rosa, L.; Pilar López-Larrubia, P.; Erickson, S.R.; Varela-Nieto, I. Protection of lipopolysaccharide-induced otic injury by a single dose administration of a novel dexamethasone formulation. *Transl Med Commun.* **2023**, *8*, 23. DOI: 10.1186/s41231-023-00156-6.

DOCTORAL THESES AND OTHER WORKS:

Paula Carretero Navarro
"Final degree's project: *Relationship between the expression of the immunological checkpoint PD-L1, tumor metabolism and acquired immunoresistance in glioblastoma models*". Universidad Autónoma de Madrid. Medicina. 2023. Supervisor/s: Jesús Pacheco Torres, Pilar López Larrubia. Grade: Sobresaliente

Lidia Esteban Merayo
"Final degree's project: *Multiparametric magnetic resonance imaging to assess neuroinflammation in a diet-induced obesity model*". Universidad Autónoma de Madrid. Licenciatura de Bioquímica. 2023. Supervisor/s: Nuria Arias Ramos, Pilar López Larrubia. Grade: Notable.

Laura Alarcón Castellanos
"Final degree's project: *Role of Aquoporin 4 in the neuronal response to stimuli assessed by magnetic resonance imaging in an animal model of obesity*". Universidad Autónoma de Madrid. Licenciatura de Biología. 2023. Supervisor/s: Nuria Arias Ramos, Pilar López Larrubia. Grade: Sobresaliente.

Yolanda Martín Sánchez
"Final degree's project: "*Caracterización del desarrollo de la obesidad inducida por dietas HFHS en ratones mediante parámetros fisiológicos e imagen de resonancia magnética de los cambios cerebrales subyacentes*". Universidad Autónoma de Madrid. Grado en Bioquímica. 2023. Supervisor/s: Blanca Lizarbe. Grade: Sobresaliente.

FUNDING:

"Extraordinary aid for the extension of the MSCA INDIVIDUAL FELLOWSHIP project of the Marie S. Curie program in order to reinforce the scientific excellence of the CSIC in the MARCO program for research and Innovation of the European Union". CSIC – IIB. 2022-2023.

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

"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

department
of **Rare Diseases**

Cilia and Ciliopathies

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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.

First line of research

Researchers involved: Martin-Morales, R; Barbeito, P; Garcia-Gonzalo, FR.

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

Second line of research

Researchers involved: Barbeito, P; Martin-Morales, R; Balverde-Caballero, FB; Garcia-Gonzalo, FR.

Identification of ciliary localization signals (CLSs) in ciliary G protein-coupled receptors (GPCRs), and characterization of their ciliary targeting mechanisms.

Third line of research

Researchers involved: Barbeito, P; 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:

Barbeito, P.; Martin-Morales, R.; Palencia-Campos, A.; Cerrolaza, J.; Rivas-Santos, C.; Gallego-Colastra, L.; Caparros-Martin, JA.; Martin-Bravo, C.; Martin-Hurtado, A.; Sánchez-Bellver, L.; Marfany, G.; Ruiz-Perez, VL.; Garcia-Gonzalo, FR. EVC-EVC2 complex stability and ciliary targeting are regulated by modification with ubiquitin and SUMO. Front. Cell Dev. *Biol.* **2023**, *11*, 1190258. DOI: 10.3389/fcell.2023.1190258.

DOCTORAL THESES AND OTHER WORKS:

Raquel Martín Morales

"Ph.D. thesis: *Mecanismos de control de la localización ciliar de la fosfoinosítido 5-fosfatasa INPP5E implicada en ciliopatías*". Universidad Autónoma de Madrid. Medicina. 2023. Supervisor/s: Francesc García Gonzalo. Grade: Sobresaliente Cum Laude

Florencia Belén Balverde Caballero

"Final degree's project: *Caracterización de los mecanismos de localización ciliar en receptores acoplados a proteínas G*". Universidad de Alcalá. Biología. 2023. Supervisor/s: Francesc García Gonzalo y Pablo Barbeito González. Grade: Sobresaliente

FUNDING:

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

Rare Diseases Associated to Defects in Autophagy

PRINCIPAL INVESTIGATOR
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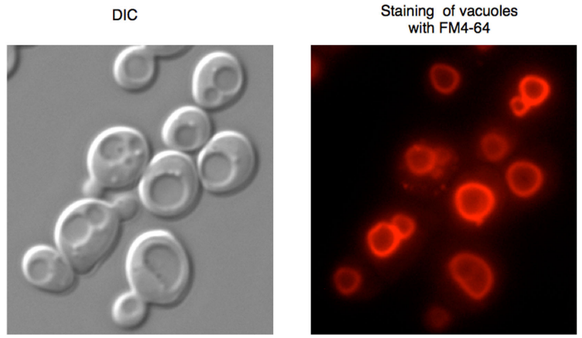
KEYWORDS
Autophagy, BPAN, ChAc, Rare diseases.



Yeast Strain BY4741

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

Researchers involved: Vincent, Olivier; Escalante, Ricardo; Navas, María de los Ángeles; Bueno, Miranda; 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; Bueno, Miranda; 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; Bueno, Miranda; 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:

Tornero-Écija, A.; Zapata-Del-Baño, A.; Antón-Esteban, L.; Vincent, O.; Escalante, R. The association of lipid transfer protein VPS13A with endosomes is mediated by sorting nexin SNX5. *Life Sci. Alliance.* **2023**, *28;6(6)*, e202201852.DOI: 10.26508/lsa.202201852.

Tornero-Écija, A.; Navas, M.A.; Muñoz-Braceras, S.; Vincent, O.; Escalante, R. Effect of rapamycin on lysosomal accumulation in a CRISPR/Cas9-based cellular model of VPS13A deficiency. J. *Cell Mol Med.* **2023**, *27(11)*, 1557-1564. DOI: 10.1111/jcmm.17768.

Mollereau, B.; Hayflick, SJ.; Escalante, R.; Mauthe, M.; Papandreu, A.; Luso, A.; Celle, M.; Aniorte, S.; Issa, AR.; Lasserre, JP.; Lesca, G.; Thobois, S.; Burger, P.; Walter, L. A burning question from the first international BPAN symposium: is restoration of autophagy a promising therapeutic strategy for BPAN?. *Autophagy.* **2023,** *19(12),* 3234-3239. DOI: 10.1080/15548627.2023.2247314.

Bueno-Arribas, M.; Cruz-Cuevas, C.; Navas, MA.; Escalante, R.; Vincent, O. Coiled-coil-mediated dimerization of Atg16 is required for binding to the PROPPIN Atg21. *Open Biol.* **2023,** *13(11),* 230192. DOI: 10.1098/rsob.230192.

DOCTORAL THESES AND OTHER WORKS:

Miranda Bueno Arribas
"Ph.D. thesis: *Bases moleculares del reclutamiento de la maquinaria de la autofagia por las proteínas WIPI"*. Universidad Autónoma de Madrid. 2023. Supervisor/s: Olivier Vincent and Ricardo Escalante. Grade: Sobresaliente Cum Laude. Mención Internacional.

Airam Santiago Roque Guerra
"Master´s thesis: *Estudio del papel de la proteína TipC en el proceso de autofagia usando la ameba social Dictyostelium discoideum*". Universidad Complutense de Madrid. 2023. Supervisor/s: Ricardo Escalante. Grade: Sobresaliente.

Miguel Sánchez Marín
"Final degree's project: *Análisis funcional de un nuevo interactor de la proteína Atg2 en Saccharomyces cerevisiae*". Universidad Politécnica de Madrid. 2023. Supervisor/s: Olivier Vincent. Grade: Sobresaliente.

FUNDING:

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

"Advancing our understanding of the molecular function of VPS13A and development of a human cellular model for preclinical studies. INT-GB/0775". Advocacy for Neuroacanthocytosis Patients (ANP). 2020-2023

Telomeric Diseases and Experimental Therapies

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

RESEARCH LINES:

Overview Telomere Biology Disorders

Researchers involved: Fernández Varas, B.; Guillen Morales, P.; Benitez Buelga, C.; Manguán-García, C.; Carrera Domínguez, 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.; Benítez Buelga, C.; Manguán-García,C.; 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:

Lasaga, M.; Rio, P.; Vilas-Zornoza, A.; Planell, N.; Navarro, S.; Alignani, D.; Fernandez-Varas, B.; Mouzo, D.; Zubicaray, J.; Pujol, R. M.; Nicoletti, E.; Schwartz, J. D.; Sevilla, J.; Ainciburi, M.; Ullate-Agote, A.; Surralles, J.; Perona, R.; Sastre, L.; Prosper, F.; Gomez-Cabrero, D.; Bueren, J. A., Gene therapy restores the transcriptional program of hematopoietic stem cells in Fanconi anemia. *Haematologica* **2023**, *108* (10), 2652-2663. DOI:10.3324/haematol.2022.282418.

DOCTORAL THESES AND OTHER WORKS:

Carlota Carrera Domínguez
"Final degree's project: *Sistemas modelo y nuevas terapias en telomeropatías*". Universidad Autónoma de Madrid. Medicina. 2019. Supervisor/s: Rosa Guerrero López.

FUNDING:

"Integración de las características moleculares, genómicas, morfológicas, y ambientales para mejorar el diagnóstico y tratamiento de precisión en Enfermedades Pulmonares Intersticiales Difusas fibrosantes (Precision-EPID). PMP/22-0083". Instituto de Salud Carlos III. 2023-2025.

"Defining CAF-induced tumor cell plasticity as a positive switch for tumor progression and therapy resistance". Worldwide Cancer Research Grant 23-0272. 2023-2025.

PATENTS:

"Péptido derivado de GSE24.2 y usos del mismo". Perona R., Sastre, L. Guillen-Morales, P. Guerrero-López, R. Fernández-Varas, B. Gutiérrez-Rodríguez, M. Martín-Martínez, M. Presentation 17 de Marzo de 2023, PCT/ES2023/070172. Licenced to Mediczen Global LLC, Mediczen Global Europa, S.L

Genetics and Pathophysiological Mechanisms of Congenital Anomalies

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SENIOR TECHNICAL SPECIALIST
Flores Mauriz, Carmen Lisset

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 procollagen type I. However, there is also a small fraction of OI cases that have mutations in other genes. Our goal in this line of research is to identify new causes and molecular mechanisms that lead to OI or to other forms of bone fragility.

PUBLICATIONS:

Barbeito, P.; Martin-Morales, R.; Palencia-Campos, A.; Cerrolaza, J.; Rivas-Santos, C.; Gallego-Colastra, L.; Caparros-Martin, J. A.; Martin-Bravo, C.; Martin-Hurtado, A.; Sanchez-Bellver, L.; et al. EVC-EVC2 complex stability and ciliary targeting are regulated by modification with ubiquitin and SUMO. *Front Cell Dev Biol.* **2023**, *11*, 1190258. DOI: 10.3389/fcell.2023.1190258.

Maroofian, R.; Efthymiou, S.; Suri, M.; Rahman, F.; Zaki, M. S.; Maqbool, S.; Anwa, N.; Ruiz-Perez, V. L.; Yanovsky-Dagan, S.; Elpeleg, O.; et al. Consolidating the association of biallelic MAPKAPK5 pathogenic variants with a distinct syndromic neurodevelopmental disorder. *J Med Genet.* **2023**, *60* (8), 791-796. DOI: 10.1136/jmg-2022-108566.

Piceci-Sparascio, F.; Micale, L.; Torres, B.; Guida, V.; Consoli, F.; Torrente, I.; Onori, A.; Frustaci, E.; D'Asdia, M. C.; Petrizzelli, F.; et al. Clinical variability in DYNC2H1-related skeletal ciliopathies includes Ellis-van Creveld syndrome. *Eur J Hum Genet.* **2023**, *31* (4), 479-484. DOI: 10.1038/s41431-022-01276-7.

Alvarez, L. F. G.; Tenorio-Castano, J.; Poletta, F. A.; Santos-Simarro, F.; Arias, P.; Gallego, N.; Orioli, I. M.; Mundlos, S.; Castilla, E. E.; Martinez-Glez, V.; et al. A large, ten-generation family with autosomal dominant preaxial polydactyly/triphalangeal thumb: Historical, clinical, genealogical, and molecular studies. *Am J Med Genet A.* **2023**, *191* (1), 100-107. DOI: 10.1002/ajmg.a.62994.

DOCTORAL THESES AND OTHER WORKS:

Aixa Horcajo Arbosa

"Master´s thesis: *Análisis funcional de mutaciones en PRKAC-B y su efecto en la vía de señalización de Hedgehog*". Universidad Complutense de Madrid. 2023. Supervisor/s: Víctor L Ruiz Pérez y Estefanía Anguita Espinosa. Grade: Sobresaliente

FUNDING:

"Expanding Knowledge on the Genetics and Molecular Basis of Rare Congenital Disorders. PID2019-105620RB-I00". MICINN. 2020-2023

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

AWARDS:

"Early career poster award "Best Clinical Research" to Asier Iturrate, ESHG annual conference, Glasgow 2023.

"Premio a la Mejor Presentación Oral, reunión anual CIBERER" 2023.

"Premio Beca Dr. Luis Álvarez Modalidad 4, a la publicación de artículos científicos de alto impacto", IdiPaz 2023

Molecular Mechanisms of Mitochondrial Pathophysiology

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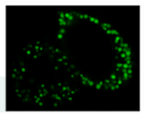
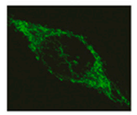
KEYWORDS
Mitochondria, OXPHOS, mtDNA, Physiopathology, Animal Models.




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 other essential processes at the level of the cell, tissue and organism such as lipid metabolism, calcium buffering, apoptosis, the assembly of iron-sulphur clusters or the biosynthesis of nucleotides, cholesterol and amino acids, etc. In addition, currently they are considered a central element in cell signaling and physiology.

Mitochondria have their own genome (mtDNA), a small circular molecule encoding 13 proteins, all of them structural subunits of the OXPHOS system, as well as 2 rRNAs and 22 tRNAs necessary for their translation. The approximately 1100 remaining mitochondrial proteins are encoded by the nuclear DNA and are imported into the organelle. Mitochondrial biogenesis is a complex process leading to the formation of the suitable mitochondrial mass with a defined degree of differentiation to satisfy the energy needs of each cell type. This process requires a precise coordination of the expression of both genomes. An important aspect at this level is the complex machinery and processes responsible for mtDNA maintenance and decoding, which requires the control of the replication to reach the precise copy number of molecules per cell, the almost complete transcription of both strands in two polycistronic RNAs and their processing, maturation and translation in the mitochondrial ribosome.

Thus, mutations in nuclear or mitochondrial genes encoding proteins related to the OXPHOS system biogenesis cause the so-called mitochondrial diseases (MDs). Although individually considered MDs are rare diseases, as a whole, they constitute the largest group of inborn errors of metabolism. MDs are genetically and clinically very 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.

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 reduced group of proteins involved in the synthesis of singular mitochondrial tRNAs.
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 mitochondrial Gln-tRNAGln, whose functional defects provoke devastating phenotypes leading to death in the first weeks of life.

Line 3. To further understand the relationship of mtDNA and tumorigenesis.
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, centro mixto 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.
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.

PUBLICATIONS:

Povo-Retana, A.; Fariñas, M.; Landauro-Vera, R.; Mojena, M.; Alvarez-Lucena, C.; Fernández-Moreno, M. A.; Castrillo, A.; Medina, J. V. de la R.; Sánchez-García, S.; Foguet, C.; Mas, F.; Marin, S.; Cascante, M.; Boscá, L. Immunometabolic Actions of Trabectedin and Lurbinectedin on Human Macrophages: Relevance for Their Anti-Tumor Activity. *Front. Immunol*. **2023**, *14*, 1211068. DOI: 10.3389/fimmu.2023.1211068.

DOCTORAL THESES AND OTHER WORKS:

Sophie Jane Fitch Clark
"Ph.D. thesis: *Caracterización de la glutamil-ARNtGln amidotransferasa mitocondrial (GatCAB) como nuevo modelo de patologías de la traducción mitocondrial*". Universidad Autónoma de Madrid. Facultad de Medicina. 2023. Supervisor: Miguel Ángel Fernández Moreno. Grade: Sobresaliente Cum Laude

FUNDING:

"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.

Neuropathology of Hearing and Myelinopathies

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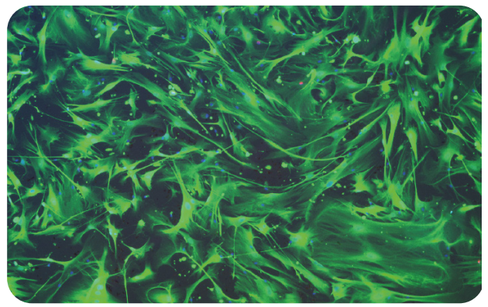
KEYWORDS
Hearing, Hearing loss, Vestibular schwannoma, IGFs, Cellular senescence, Inflammation.

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.

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.



Primary human vestibular schwannoma in culture. Vestibular schwannoma cells are labelled in green (S-100), nuclei in blue (DAPI) and proliferating cells in red (EdU).

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.

Line 1. 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.

Line 2. 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.

Line 3. 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.

Line 4. 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).

Line 5. 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 La Paz, 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:

García-Mato, Á.; Cervantes, B.; Rodríguez-de la Rosa, L.; Varela-Nieto, I. IGF-1 Controls Metabolic Homeostasis and Survival in HEI-OC1 Auditory Cells through AKT and mTOR Signaling. *Antioxidants (Basel).* **2023**, *12(2),* 233. DOI: 10.3390/antiox12020233.

de Lope, C.; García-Lucena, R.; Magariños, M.; León, Y.; Casa-Rodríguez, N.; Contreras, N.; Escudero-Iriarte, C.; Varela-Nieto, I.; Maire, P.; Palmero, I. Dysfunction of programmed embryo senescence is linked to genetic developmental defects. *Development.* **2023**, *150(9),* dev200903. DOI: 10.1242/dev.200903.

Murillo-Cuesta, S.; Lara, E.; Bermúdez-Muñoz, J.M.; Torres-Campos, E.; Rodríguez de la Rosa, L.; López-Larrubia, P.; Erikson, S.; Varela-Nieto, I. Protection of lipopolysaccharide-induced otic injury by a single dose administration of a novel dexamethasone formulation. *Transl Med Commun* **2023**, *8(23).* DOI:10.1186/s41231-023-00156-6

DOCTORAL THESES AND OTHER WORKS:

Ángela García Mato
"Ph.D. thesis: *Mecanismos moleculares implicados en la regulación de la homeostasis coclear. Papel del IGF-1 y de la G6PD*". Universidad Autónoma de Madrid. Departamento Biociencias Moleculares. Facultad de Medicina. 2023. Supervisor/s: Isabel Varela Nieto, Lourdes Rodríguez de la Rosa. Calificación: Sobresaliente Cum Laude

Inés Méndez Grande
"Master´s thesis: *Establecimiento de técnicas para la generación de esferoides a partir de células de schwannoma vestibular*". Universidad CEU San Pablo. Medicina. 2023. Supervisor/s: José Miguel Cosgaya Manrique. Grade: Sobresaliente

Jorge Caballero Lombraña
"Final degree's project: *Caracterización de la respuesta de células de schwannoma vestibular a agentes senogénicos y senolíticos*". Universidad Autónoma de Madrid. Biología. 2023. Supervisor/s: Ana María Jiménez Lara. Grade: Sobresaliente

Irene Pérez Ruiz
"Final degree's project: *Modelo de daño acústico por ruido blanco de banda ancha en rata*". Universidad Complutense de Madrid. Veterinaria. 2023. Supervisor/s: Rafael Cediel, Silvia Murillo. Grade: Sobresaliente

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

"Industrial Doctorate of the Community of Madrid - Alodia Pharmaceutical SL (IND2020/BMD-17454)". CAM. 2021-2023

"New treatments for the prevention and treatment of hearing loss (ER-P17PE12)". CIBERER-Spiral Therapeutics. 2021-2023

"Conexion Nanomedicina del CSIC. NANOMED-CSIC". CSIC. From 2021

"MINA-CM Madrid Innovative Neurotech Alliance. P2022/BMD-7236". CAM. 2023-2026

"Desarrollo e implementación de métodos alternativos en investigación auditiva. PIE-CSIC-202320E217". CSIC. 2023-2024

"Red de investigación en inflammasoma y piroptosis en enfermedades crónicas y cáncer. RED2022-134511-T". MICINN. 2023-2026

"Plataformas ISCIII de apoyo a la I+D+I en Biomedicina y Ciencias de la Salud de la Acción Estratégica en Salud 2017-2020 (PT20/00118 UNIDAD CIBERER)". ISCIII. 2020-2023

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. PCT/EP2023/070340 (21/07/2023)

AWARDS:

"BBVA Foundation-SEBBM Young Researcher Award (Sandra Franco Caspueñas): Induction of cellular senescence and senolysis as a potential threpeutic strategy for vestibular Schwannoma". 2023

New Mechanisms and New Models of DNA Replication and Repair

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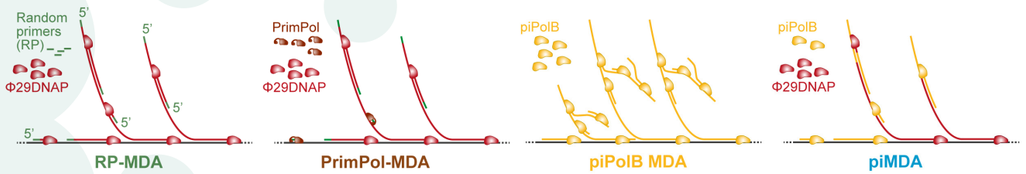
KEYWORDS
DNA polymerase, DNA amplification, mobile genetic elements, bacterial genomics



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. Moreover, as detailed below, we have addressed the biological role of piPolB and its application to develop new DNA amplification methods.

Developing of new methods for whole (meta) genome amplification

Researchers involved: Mayoral Campos, Carmen; Mateo-Cáceres, Víctor; Redrejo-Rodríguez, Modesto

The unique features of piPolB suggest its applicability in isothermal DNA amplification, leading us to develop a novel method for whole (meta)genome DNA amplification with various biotechnological and biomedical applications (Ordóñez et al. 2023). Specifically, we have optimized a new isothermal multiple displacement DNA amplification (MDA) method, known as piMDA, which combines piPolB with the superior processivity of Φ29 DNA polymerase (Φ29DNAP), enabling efficient and accurate whole genome and metagenome amplification.

High-deep sequencing of amplification product reactions indicated that piMDA surpasses commercial methods in amplifying genomes and metagenomes with high GC content, demonstrating superior performance in terms of amplification bias, error rate, and variant identification.

However, piMDA, as expected in exponential amplification processes, sequence bias re-

mains still higher compared to non-amplified control samples. Linear amplification reactions might help achieve less biased sequences compared to exponential processes. Given the moderate processivity of piPolB, we hypothesized that solo piPolB MDA reactions (piPolB-MDA) might produce amplification products with reduced sequence bias. The amplification rate in piPolB-MDA was low, with a high ratio of unidentified DNA sequences that could only result from ab initio DNA synthesis. Primer-less and untemplated ab initio DNA synthesis has been observed in various DNA polymerases since the 1960s and 1970s, though it has been a subject of debate and is often overlooked in scientific literature. Our current research aims to investigate the mechanisms of piPolB ab initio DNA synthesis to gain a better understanding, reduce its occurrence in MDA protocols, and explore potential new applications of piPolB.

Escherichia coli piPolB promotes survival of persister cells after exposure to DNA crosslinking agents

Researchers involved: Arredondo Lamas, J.J.; Solar Venero, E., Mateo Cáceres, V; Redrejo Rodríguez, M.

Previous results from our lab indicate that this polymerase might provide an evolutionary advantage by increasing persister survival rate possibly by their interaction or collaboration with DNA repair mechanisms against genotoxic agents. Our results indicate that piPolB does not contribute to resistance against mitomycin C (MMC) or nitrogen mustard but to persistent survival, and that the protein expression is induced in the presence of distinct DNA replication blocking agents. While a mutant lacking piPolB (ΔpiPolB) showed reduced persistent survival compared to the wild type strain, the complemented ΔpiPolB+piPolB strain presents similar persistent survival ratios to wild type. Overall, our results support a biological role of piPolB in DNA damage tolerance and suggests its possible collaboration with DNA repair pathways such as SOS response. We propose the study of piPolB participation along other DNA repair mechanisms through epistasis assays. Finally, the biological role of piPolB and its wide distribution among diverse bacteria paves the way for future research lines towards the development of new treatments against resistant and persistent pathogens.

Prevalence and genomic characterization of pipolins and other mobile genetic elements

Researchers involved: Solar Venero, E; Mateo Cáceres, V; Redrejo Rodríguez, M.

We conducted a comprehensive screening of pipolins in all available bacterial genomes in the Genbank database using ExplorePipolin, a custom pipeline previously developed in our lab to identify and reconstruct pipolins from draft genomes. The analysis indicated that pipolins are integrative elements commonly flanked by direct repeats in Gammaproteobacteria genomes, often occupying integration hotspots of known mobile elements. Integrase dynamics correspond with alternative integration sites, enabling diverse lifestyles ranging from integrative to mobilizable and plasmid pipolins, as observed in genera such as *Limosilactobacillus, Pseudosulfitobacter,* and *Staphylococcus.* Detailed examination of identified pipolins revealed their role in maintaining antiviral defense systems through frequent exchanges with other mobile genetic elements (MGEs). Consequently, pipolins serve as reservoirs of adaptive traits primarily related to defense functions. Pipolins exhibit a bimodular structure with various cargo genes and a minimal set of core genes, with a primer-independent DNA polymerase (piPolB) being the only universal hallmark. We propose that these MGEs, along with potentially overlooked elements, form a novel superfamily within the bacterial mobilome, providing dynamic platforms for exchanging defense systems. These antiviral genes may be incorporated by ciMGEs, plasmids, and other gene transfer machinery-bearing elements, which benefit from the defense gene reservoir provided by pipolins and other mobile genetic elements.

Additionally, in collaboration with Dr. Miguel de Vega's laboratory (CBMSO), we examined the prevalence of the Escherichia coli insertion sequence-excision enhancer in enterohemorrhagic strains (Calvo et al. 2023). Although IEE had been identified in the most pathogenic serotypes of E. coli, its biochemical features that might explain its role in IS excision remain unclear. Our findings indicate that IEE is present in over one third of all available E. coli genome assemblies and is highly conserved and abundant within enterohemorrhagic, enteropathogenic, and enterotoxigenic genomes.

PUBLICATIONS:

Calvo, P.A.; Mateo-Cáceres V.; Díaz-Arco S., Redrejo-Rodríguez M., de Vega M. The enterohemorrhagic Escherichia coli insertion sequence-excision enhancer protein is a DNA polymerase with microhomology-mediated end-joining activity. *Nucleic Acids Res.* **2023**, *51*, 1189-1207. DOI: 10.1093/nar/gkad017

Ordóñez, C.D.; Mayoral-Campos, C.; Egas, C.; Redrejo-Rodríguez, M. A primer-independent DNA polymerase-based method for competent whole-genome amplification of intermediate to high GC sequences. *NAR Genom Bioinform.* **2023**, *5*, lqad073. DOI: 10.1093/nargab/lqad073

Ordóñez, C.D.; Redrejo-Rodríguez, M. DNA Polymerases for Whole Genome Amplification: Considerations and Future Directions. *Intl J Mol Sci.* **2023**, *24*, 9331. DOI: 10.3390/ijms24119331

DOCTORAL THESES AND OTHER WORKS:

Diego Duarte Zara
"Master´s thesis: *Escherichia coli primer-independent PolB is involved in persistence against DNA crosslinking agents*". Master's Degree in Biotechnology, Universidad Autónoma de Madrid. 2023. Supervisor/s: Juan J. Arredondo Lamas and Modesto Redrejo Rodríguez. Grade: Sobresaliente 9

Alba Lozano Rubio
"Final degree's project: *Purificación y caracterización preliminar de la DNA polimerasa del bacteriófago YERA41*". Universidad Complutense de Madrid. BSc in Biology. 2023. Supervisor/s: Modesto Redrejo Rodríguez. Grade: Sobresaliente 9.5

FUNDING:

"Insights into Pipolins diversity and dynamics in a wide range of pathogenic bacteria. SI3-PJI-2021-00271". UAM-CAM. 2022-2023

"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

PATENTS:

"Primer-independent DNA polymerases and their use for DNA synthesis." Margarita Salas, Modesto Redrejo-Rodríguez, Mart Krupovic y Patrick Forterre. EU Patent Application EP18789144B1 (Application 20/10/2017, Granted 15/6/2023). USA Ref. 16/756,812 (1/5/2020). Patent licensing: 4BaseBio Inc (2022-).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