SCIENTIFIC REPORT

2019 2020

Instituto de Investigaciones Biomédicas Alberto Sols

CSIC
Consejo Superior de Investigaciones Científicas

UAM

Welcome from the director

It is a pleasure to present the scientific report of the Institute of Biomedical Research Alberto Sols (IIBM) for the period 2019-2020. The IIBM belongs jointly to the Spanish Council for Scientific Research (Consejo Superior de Investigaciones Científicas, CSIC) and the Department of Biochemistry of the School of Medicine of the Autonomous University of Madrid (UAM). Distributed in four departments, scientists at the IIBM investigate the molecular mechanisms that lead to the generation of disease, encompassing a broad spectrum of basic science from simple organisms to translational medicine. Important aspects of biomedical processes including metabolic regulation, cancer, immune response, cell communication, neurodegeneration, aging and rare diseases are the focus of our research.

The 2019-2020 biennium has been a period of changes. On the one hand, in January 2020, Lisardo Boscá ended his mandate as Director of the IIBM. I would like to thank him for all his continued efforts and express our recognition to his dedication during many years, having to face at times very difficult situations.

On the other hand, the arrival of the COVID-19 pandemic hit our society at large in an unprecedented manner in modern times. The IIBM reacted to that immense challenge by setting up a SARS-CoV-2 detection platform by PCR, once the pertinent authorizations were obtained. This was accomplished with the altruistic help of volunteers from the staff of the CSIC, the UAM, and the Madrid Science Park Foundation. This effort was carried out in collaboration with the Department of Preventive Medicine, Public Health and Microbiology of the UAM School of Medicine and the VISAVET Health Surveillance Center of the Complutense University of Madrid. The target population were the elderly and disabled residents of institutions within the Community of Madrid. A special mention is due to the important and altruistic financial support of Alantra, an investment company who donated 300.000 euros for the acquisition of reagents and equipment. These were essential to accomplish our goal of 10.000 reactions, and we remain deeply indebted to them. From the beginning of May to the end of the State of Alarm lockdown in June a total of 10.165 PCRs were carried out in the IIBM to analyze samples from 8.772 individuals (4.409 residents and 4.363 employees). Around 6,5% yielded results compatible with the presence of SARS-CoV-2. The IIBM is proud of having been able to contribute to the fight against the COVID-19 pandemic during those difficult times, and I would like to thank all of those involved for their help, dedication and unconditional effort.

The return to the “new normality” after the lockdown, imposing restricted numbers of personnel within our premises and the use of digital technologies and virtual seminars to interact, inevitably translated into a need to accomplish our scientific goals with a drastic change in working habits. Despite these difficulties, this was carried out successfully due to the enthusiastic and professional dedication of our scientists and staff.

I would like to thank all the personnel in administration, coordinated by the IIBM executive manager Isabel Ocaña, as well as the staff of general and core services and maintenance, whose dedication is essential to keep the IIBM running. Also, my gratitude and recognition to Aurora Sánchez Pacheco, who accepted to continue as Vice-director of the IIBM after serving in the same position with Lisardo Boscá. Her experience has been a great asset to handle many of the managerial issues required to solve many of our day-to-day activities.

Finally, I would like to devote a few words to express our recognition to Prof. Carlos Gancedo, who retired at the end of 2020 after 57 years of uninterrupted service in our institute. Dr. Gancedo started his research career as a student with Alberto Sols, and was part of his team when they moved to the newly created Department of Biochemistry at UAM in the late sixties of the previous century. Since then, he heartedly dedicated his professional life to develop a scientific career, during which he became an internationally recognized authority in the field of yeast biochemistry. We are proud and privileged to have had him as a colleague at IIBM, and wish him all the best in his new endeavors.

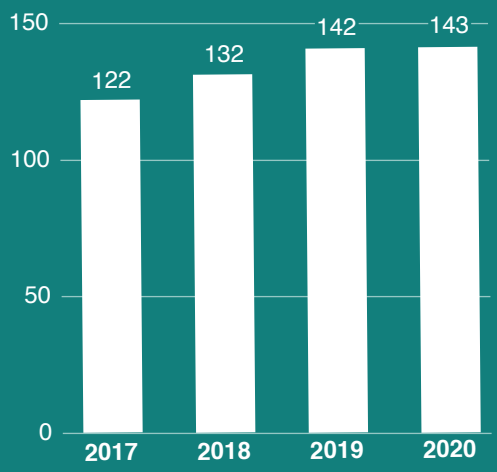
Our goal is to continually improve the image of the IIBM as a reference center in Biomedical Research. The IIBM is well prepared to confront the new challenges imposed by an ever-increasing competitive scenario in modern science. We are ambitious in our objectives, and we tackle them with determination and confidence that the effort will be worthwhile.

Mario Vallejo Fernández de la Reguera
Director, IIBM

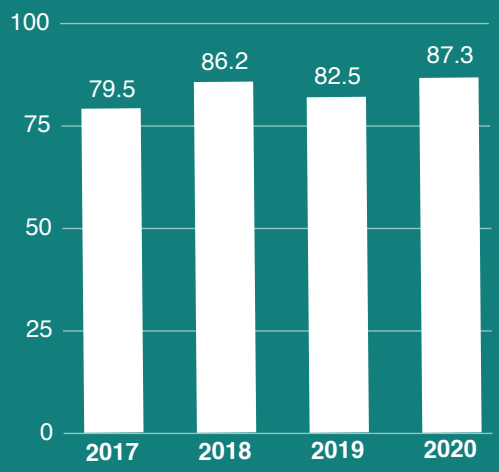
DATA SUMMARY

FUNDING RECRUITMENT

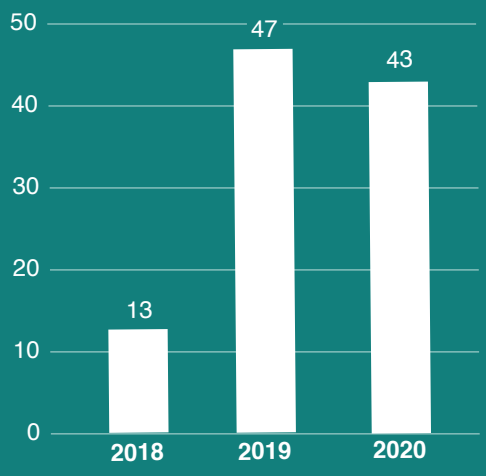
PUBLICATIONS



PUBLICATIONS IN Q1 (%)



OUTREACH ACTIVITIES



Research departments

2019-2020

1 Cancer Biology

2019-2020

1 Cancer Biology

Department of Cancer Biology

Cancer is not a single disease but rather a combination of more than 200 pathologies that arise from the normal cells in our bodies, through complex genetic and molecular alterations in a stepwise manner. The laboratories of the Cancer Biology Department are focused on understanding these molecular, cellular, and genetic alterations that lead to oncogenic events, intending to describe the biological mechanisms involved in the initiation, development, and progression of malignant tumors. The research in the Cancer Biology Department allows us to discover new biomarkers for diagnosis, prevention, and therapeutic intervention in cancer, facilitating the translation of our discoveries to a clinical context. Hence, we combine a high knowledge in basic research with a strong translational vocation, to produce better treatments, potential cures, and prevention strategies for many different types of cancer, one of the leading causes of death in developed countries.

Our research teams have come together around common cancer types affecting our society, such as colon, breast, prostate, pancreatic, and lung cancers. Transversely to the different cancer types, we investigate key molecular oncogenic processes such as epithelial-mesenchymal transition, angiogenesis, cell cycle regulation, and cell migration and invasion. In addition, great effort is also devoted to understanding the molecular mechanisms by which tumor cells develop resistance to anti-tumor drugs. This research requires a multidisciplinary approach that combines the use of animal models, innovative technologies in cellular and molecular biology, supported by computational biology. Finally, we have established numerous scientific collaborations between IIBM researchers and clinical investigators, and physicians that facilitate the transfer of the basic knowledge generated into clinical practice.

In the following pages, we describe the specific projects developed in the Cancer Biology Department laboratories during the years 2019-20.

2019-2020

Cell signaling and cancer

PRINCIPAL INVESIGATOR
Sánchez Prieto, Ricardo
Belandia Gómez, Borja

ASSOCIATE INVESTIGATORS
Ortega Muelas, Marta
Pascual Serra, Raquel

MASTER STUDENTS
Albandea Rodríguez, David
Raposo Gutiérrez, Irene

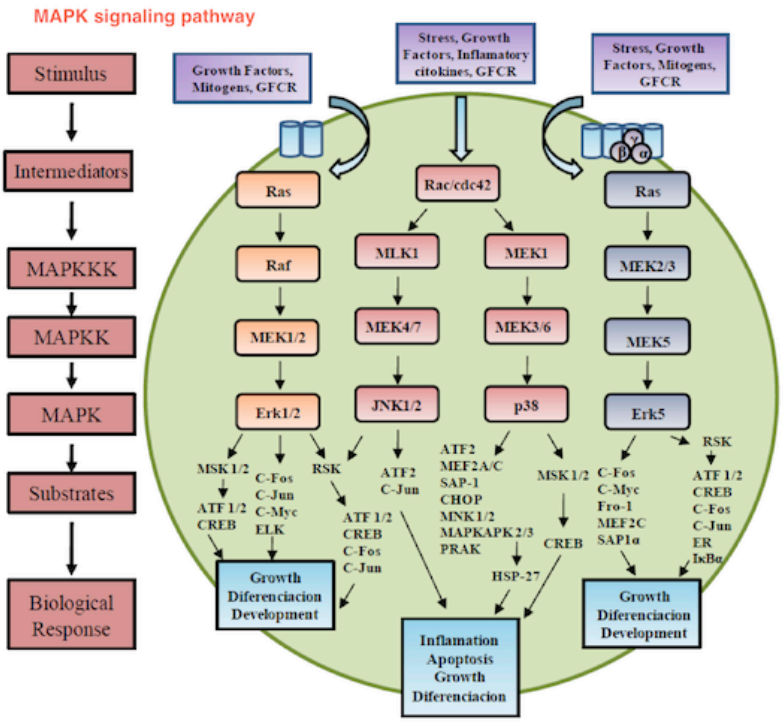
PREDOCTORAL
Arconada Luque, Elena

Keywords: MAPK, Chemotherapy, Radiotherapy, Kinase Inhibitors, p53, autophagy.

Research Lines

Role of cellular signaling in transformation

Our group is interested in studying how cellular signaling (vg. MAPK, AKT) can affect the process of cellular transformation. To this end, we have developed several lines of research including chemical and genetic approaches applied to in vitro models of cancer cell lines and in vivo mouse models. For example, in the case of MAPK we have been able to uncover the implication of erk1/2 in the process of viral oncogenesis. We are currently focusing on the study of the role of MAPK-dependent signaling in human sarcoma, a poorly studied pathology but with high incidence in childhood. By using an experimental mouse model based on chemical carcinogenesis we expect to clarify MAPK´s role in the development of soft tissue sarcomas.



Role of cellular signaling in cancer therapy

Role of cellular signaling in the response to chemotherapy.
Our study encompasses conventional chemotherapy as well as novel compounds used in targeted therapy, such as Imatinib, Palbociclib or Sorafenib, among others. The main goal is to understand how cellular signaling can affect the response to cancer therapy and its involvement in the development of resistant phenotypes. On this subject, our group has great expertise in the study of how p38 MAPK or ERK1/2 modulate the cellular responses to several antitumor agents including Cisplatin, 5-FU, Sorafenib or Imatinib.

Role of cellular signaling in the response to radiotherapy.

In the case of ionizing radiation, we are focused on elucidating the role of cellular signaling in the development of cancer cell radioresistance. Also, our group has great interest in the role of cellular signaling in the radiosensitizer potential of several chemotherapeutics agents such as 5-FU or Palbociclib. In addition, our laboratory is also studying the interplay between key proteins in DNA damage response (vg. ATM, p53) and cellular signaling.

Role of autophagy in cancer therapy.

Finally, our group is also interested in the role of autophagy in the cellular responses to different antitumor treatments. In this sense, Autophagy has become a key player in the development of chemo and radioresistance. On the other side, Autophagy induction could be an alternative mechanism for treating tumors resistant to apoptotic responses induced by chemo and radiotherapy, suggesting a novel therapeutic potential for the modulation of this interesting biological process.

Publications

(2020). *p38β and Cancer: The Beginning of the Road.* Int J Mol Sci. 21(20): 7524.

Pascual-Serra, R., Fernández-Aroca, DM., Sabater, S., Roche, O., Andrés, I., Ortega-Muelas, M., Arconada-Luque, E., Garcia-Flores, N., Bossi, G., Belandia, B., Ruiz-Hidalgo, MJ., Sánchez, R. (2020). *p38β (MAPK11) mediates gemcitabine-associated radiosensitivity in sarcoma experimental models.* Radiother Oncol.

(2019). *Blockage of autophagic flux is associated with lymphocytosis and higher percentage of tumoral cells in chronic lymphocytic leukemia of B cells.* Clin Transl Oncol. 21(9): 1280-1285.

(2019). *HIF1α Suppresses Tumor Cell Proliferation through Inhibition of Aspartate Biosynthesis.* Cell Rep. 26(9): 2257-2265.

(2019). *P53 pathway is a major determinant in the radiosensitizing effect of Palbociclib: Implication in cancer therapy.* Cancer Lett. 451: 23-33.

Doctoral theses and other works

Raquel Pascual Serra

"Papel de la ruta de p38 MAPK en modelos de patología sarcomatoide: implicaciones en la biología tumoral y terapia".
Universidad de Castilla la Mancha. Medicina. 2019. Director/es: Ricardo Sánchez. Calificación: Sobresaliente cum laude

Marta Ortega Muelas

"La ruta de ERK5 como nueva diana de sorafenib".
Universidad de Castilla la Mancha. Medicina. 2019. Director/es: Ricardo Sánchez. Calificación: Sobresaliente cum laude

Funding

"Papel de MAPKS en sarcoma; implicaciones en diagnóstico y terapia."
Financiado por: Agencia Estatal de Investigación. Año 2019-2021

Molecular characterization of tumorigenesis and vascular pathogenesis

Principal Investigator
Campanero García, Miguel

Investigators under contract
Sierra Cruz, Marta
Punzón Jiménez, Paula

Predoctoral
Chiodo-, Yuri
Kourani Méndez, Omar
Martín Cortázar, Carla
Hernández Alcántara, Alberto

Support Personnel
Martínez Nuñez, Patricia

Undergraduate Students
Tomero Sanz, Henar
Medina Dome, Gonzalo Antonio
Fuentenebro Navas, David Chica
Pineda, Manuela

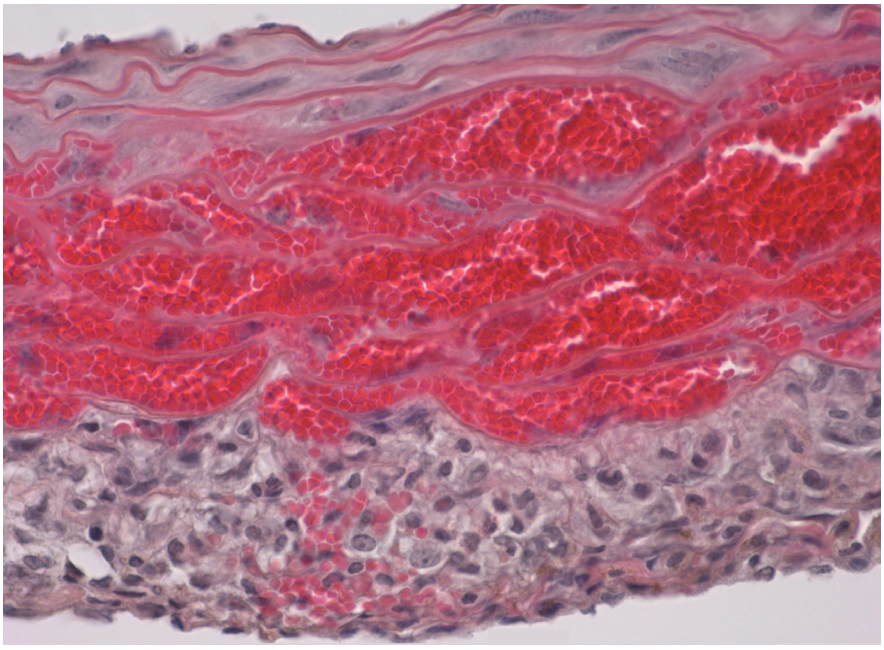
Master Students
Murzakhmetova-, Sabina

Keywords: MAPK, Chemotherapy, Radiotherapy, Kinase Inhibitors, p53, autophagy.

Research Lines

We are interested in uncovering novel mediators of tumorigenesis and cardiovascular pathogenesis, the two major causes of death worldwide, because these mediators might become novel targets for therapeutic intervention. To this end, we use a combination of in vitro and in vivo experimental approaches involving cellular and molecular analysis, mouse models, and advanced imaging techniques.

We had thus uncovered a critical role for E2F1 and E2F4 in Burkitt lymphoma (Cancer Res 2009; Leukemia 2012) and now discovered that members of the MAZ family of transcription factors are essential for the transcriptional induction of the MYB oncogene (Nucleic Acids Res 2017). By using transcriptomics to compare gene expression profiles of lymphoma cells and immortal, but non-tumoral lymphocytes, we have recently identified >1,600 differentially expressed genes. We used a combination of in vivo and in vitro experimental approaches to demonstrate that one of these genes, CDCA7, is essential for lymphoma cell migration and invasion (Haematologica 2019) and for anchorage-independent growth and lymphomagenesis (Haemtatologica 2018). These results therefore urge to evaluate the potential of CDCA7 as a therapeutic target for lymphoid tumors. We are currently assessing the contribution of additional differentially expressed genes to



Microscopic image of an intramural hematoma

lymphomagenesis. We are also investigating the molecular mechanisms underlying anchorage-independent growth of lymphoid tumors, a treat almost exclusive to tumor cells rarely investigated with lymphoid cells.
In collaboration with the group of Dr. J.M. Redondo (CNIC), we also search for genes mediating pathological vascular wall remodeling, a key process in the development of hypertension and arterial diseases such as atherosclerosis and aneurysm. We had determined that calcineurin and its downstream effector Rcan1 are essential mediators of abdominal aorta aneurysm, atherosclerosis, and restenosis (J Exp Med 2011; EMBO Mol Med 2013). Much of our recent effort focused on the identification of novel genes that mediate aortic diseases. Our studies allowed the identification of new pathophysiological mechanisms and new therapeutic targets in aortic diseases.

Using tissue-specific inducible knockout mice, we have uncovered a homeostatic role for Rcan1 in the aorta and that its genetic inactivation in the adult mouse predisposes to hypertension-induced intramural hematoma and subsequent aneurysm through mechanisms involving GSK-3b, ROCK and smooth muscle Myosin (Nat Commun 2018). These results have opened new exciting avenues of research into the pathogenesis of aortic diseases.

Our studies showing that deficiency of the metalloproteinase Adamts1 leads to thoracic aorta aneurysm (TAA) in mice due to increased Nos2-dependent NO production (Nat Med 2017) are particularly pioneering. We also showed that TAA was reversed using pharmacological NOS2 inhibition in mouse models, raising the possibility that blocking NO signaling could be a novel treatment for TAA. Indeed, we are pursuing clinical trials with NOS2 inhibitors in Marfan syndrome, a heritable life-threatening disease in which TAA accounts for over 90% of its mortality. These findings changed our view of the pathophysiology of TAA and prompted us to further explore the molecular and biomechanical mechanisms of TAA and identify additional mediators of these aortic diseases.

Role of Snail1, E47 and Lysyl Oxidase-like 2 and 3 (LOXL2/LOXL3) in tumour progression and metastasis

Principal Investigator
Cano García, Amparo

Co-Principal Investigator
Portillo Pérez, Francisco

Associate Investigators
Eraso Mazmela, Pilar
Mazón Calpena, María Jesús
López Menéndez, Celia

Investigators under contract
González Santamaría, Patricia

POSTDOCTORAL
González Santamaría, Patricia
Majuelos Melguizo, Jara

Predoctoral
Bustos Tauler, José
Vázquez Naharro, Alberto

Support Personnel
Santos Fernández, Vanesa
Zamora Cañadas, Carmen

Undergraduate Students
Barahona Santervás, Henar
Moreno Palomares, Rocio

Master Students
González Masa, Andrea
Ramos Nevot, Carmen

Undergraduate Student
Pizarro, Patricia

Keywords: MAPK, Chemotherapy, Radiotherapy, Kinase Inhibitors, p53, autophagy.

Research Lines

Role of Snail1, E47 and Lysyl Oxidase-like 2 and 3 (LOXL2/LOXL3) in tumour progression and metastasis

Snail1 and E47 are two Epithelial-Mesenchymal Transition factors (EMT-TFs) described two decades ago. While the in vivo function of Snail1 in invasion and metastasis is well characterized, much less is known on the in vivo action of E47. LOXL2 and LOXL3 are two members of the lysyl oxidase family that catalyze oxidative de-amination of lysine residues promoting covalent cross-linkages. Accumulating evidence indicates the participation of lysyl oxidases in diverse extra- and intra-cellular functions ranging from extracellular matrix maturation to tumorigenesis and metastasis. We previously showed that intracellular LOXL2 and LOXL3 regulate Snail1 stability and induce EMT independent of its catalytic activity. LOXL2 plays also Snail1-independent roles in EMT and cooperates with other EMT-TFs, like E47. Besides, a link between intracellular LOXL2, the UPR pathway and EMT induction has been established. Increased intracellular LOXL2 expression is a poor prognosis factor in human squamous cell carcinomas and is associated to metastatic basal breast carcinomas (BBC). Regarding LOXL3, we identified LOXL3 overexpression in human melanoma cells and characterized its essential role for melanoma cell survival. Our main research interest focus on further understanding of the role of Snail1, E47 and LOXL2/LOXL3 in tumorigenesis and metastasis; we aim to dissect the contribution of intracellular functions of LOXL2/LOXL3 and their interrelation with EMT-TFs in initiation and progression of breast carcinoma and melanoma. To this end, we developed genetically modified models (GEMs) for conditional deletion or overexpression of Loxl2 and Loxl3 in breast and melanoma cancer models, and tumor-derived cell lines manipulated for Loxl2 or Loxl3 expression. We also generated GEM with conditional deletion of E2A (coding for E47) in the context of PyMT breast cancer. During the last two years (2019 and 2020), we addressed the following objectives:

1. Characterization of LOXL2 action in breast lung metastasis. Loxl2 induces invasion and metastasis by regulating Snail1 stability and favoring generation of the lung pre-metastatic niche.

2. Characterization of in vivo LOXL3 action in melanoma. Deletion of Loxl3 in the specific mouse melanoma models delays melanoma initiation and impairs lymph node metastasis, supporting Loxl3 contribution to melanomagenesis and metastasis. Generation of primary melanoma cell lines, wt and Loxl3-KO, is allowing mechanistic characterization of Loxl3 action in melanoma.

3. Characterization of E47 action in breast cancer initiation and metastasis. The conditional PyMT-E2A-KO model provided evidence for a key action of E47 in breast cancer initiation and lung metastasis, and for its contribution to self-renewal of tumor initiating cells and maintenance of undifferentiated tumor phenotype. E47 actions are mediated by transcriptional upregulation of Snail1 and interaction with Lox2. High E2A and SNAI1 co-expression occurs in BBC, highlighting the biological relevance of the E2A-Snail1 axis in human tumors.

Publications

(2020). *Looking for a Better Characterization of Triple-Negative Breast Cancer by Means of Circulating Tumor Cells.* J Clin Med. 9(2): 353.

(2020). *Guidelines and definitions for research on epithelial-mesenchymal transition.* Nat. Rev. Mol. Cell Biol. 21(6): 341-352.

(2020). *G-protein-coupled receptor kinase 2 safeguards epitelial phenotype in head and neck squamous cell carcinomas.* Int J Cancer. 147: 218-229.

(2020). *IMPACT OF NOTCH SIGNALING ON THE PROGNOSIS OF PATIENTS WITH HEAD AND NECK SQUAMOUS CELL CARCINOMA.* Oral Oncol. 110: 105003.

(2020). *CDH1 and SNAI1 are regulated by E7 from human papillomavirus types 16 and 18.* Int J Oncol. 57(1): 301-313.

(2019). *Intracellular Delivery of an Antibody Targeting Gasdermin-B Reduces HER2 Breast Cancer Aggressiveness.* Clinical Cancer Research. 25: 4846-4858.

(2019). *Contribution of epithelial plasticity to therapy resistance.* Journal of Clinical Medicine. 8(5): pii: E676.

(2019). Lysyl *Oxidase-Like 2 Protects against Progressive and Aging Related Knee Joint Osteoarthritis in Mice.* Int J Mol Sci. 20(19): 4798.

Doctoral theses and other works

José Bustos Tauler

"Relevancia de la proteína lisil oxidasa-like 3 en el desarrollo embrionario y en la iniciación y progresión del melanoma".
Autónoma de Madrid (UAM). Ciencias. 2019.
Director/es: Amparo Cano. Calificación: Sobresaliente cum laude

Funding

"Contribution of LOXL2 and LOXL3 to tumour progression and metastasis."
Financiado por: Ministerio de Economía y Competitividad. Año 2017-2020

"Consorcio Ciber. Area temática de cáncer (CIBERONC)."
Financiado por: Insituto de Salud Carlos III.
Año 2017-2021

Awards

"ASEICA Research Award." Año 2020

Cell cycle and cancer biomarkers

Principal Investigator
de Cárcer Díez, Guillermo

Investigators under contract
Colomo del Pino, Sara
López Pernas, Gema

Predoctoral
Monfort Vengut, Ana

Marugán Seseña, Carlos
Martínez Alonso, Diego

Support Personnel
Ortigosa Fernández, Beatriz

Undergraduate Students
González Rodríguez, Marta
Jurado Muñoz, Andrea

Master Students
Tercero Malo, Rut
Rio Vilariño, Anxo
Martínez Sánchez, María del Mar
Monfort Vengut, Ana

Keywords: Mitosis, Plk1, cell cycle, cancer, biomarkers, oncogene, tumor suppressor, mitotic kinases, genomic instability, chromosome instability, aneuploidy, polyploidy, CRISPR screens.

Research Lines

Cancer is one of the main causes of mortality and its complexity grows as different types of cancer can be further classified into different subtypes. To deepen the knowledge concerning the molecular mechanisms associated with cancer is essential for the development of new therapeutic strategies, with the final goal to reach the concept of **"personalized medicine"**.

Alterations in cell cycle progression, high rates of cell proliferation and chromosomal instability are common features of multiple tumors. Numerous cell cycle regulators are overexpressed in tumors and very often this correlates with poor clinical prognosis. Nevertheless, **proper identification of reliable biomarkers related to cell cycle, which not only correlate to prognosis but also provide a therapeutic value, is still lacking.**

Classical chemotherapy, although effective in a percentage of cases, is associated with severe cytotoxic side effects. As an alternative, pharmaceutical companies are developing new inhibitors against specific molecular targets of high therapeutic value. Unfortunately, the newly designed drugs, although effective in certain types of tumors are not as efficient as initially thought. Therefore, **to increase the knowledge about the molecular basis of certain tumors**, as well as to **identify mechanisms of sensitivity and resistance to newly developed drugs**, is of great interest to the medical community and for upcoming cancer patients.

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 to translate this knowledge to the clinic.

Current Research Projects

Deregulation of cell division is a common feature in multiple types of tumors. Tumor cells cancel the control mechanisms of the cell cycle, resulting in the accumulation of genetic aberrations. In recent decades, as an anticancer strategy effort, researchers and pharmaceutical companies have developed a collection of drugs to stop cell division,

in order to kill tumoral cells, by inhibiting the main mitotic kinases such as the **Cycle-Dependent Kinases (CDKs)**, the family of **Aurora kinases** and the family of **Polo-like kinases (PLKs)**.

We pretend to use cell cycle regulators as biomarkers form 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.

We address this by three different approaches:

1. **Drug sensitivity screenings:** Cell division genes are often overexpressed in tumors, and this commonly confers poor prognosis to the patients. We want to evaluate if the expression of certain mitotic genes can predict sensitivity to different pharmacologic drugs.
   - In collaboration with the pharmaceutical company **Lilly**.
   - Sponsored by the **AECC** (Spanish Association Against Cancer)
2. **Cell Cycle Drugs Resistance Mechanisms:** A recurring problem in therapies with kinase inhibitors is the appearance of drug resistance mechanisms and therefore the loss of efficacy over time. Given the recent emergence of a new generation of cell division cancer drugs, the need to identify new resistance mechanisms increased substantially.
   - Sponsored by the **AECC** (Spanish Association Against Cancer)
   - Sponsored by the **"MCIU"** (Spanish Ministry of Science and Innovation)
3. **Mitotic regulators: Oncogenes or tumor suppressors?**: 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 during 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?**
   - Sponsored by the "MCIU" (Spanish Ministry of Science and Innovation)
   - Sponsored by the "CSIC" as core starting funding.

Publications

(2020). *Squamous differentiation requires G2/mitosis slippage to avoid apoptosis.* Cell Death & Differentiation. 27(8): 2451-2467.

(2019). *A Chemical Screen Identifies Compounds Capable of Selecting for Haploidy in Mammalian Cells.* Cell Reports. 28(3): 597-604.

(2019). *The Mitotic Cancer Target Polo-Like Kinase 1: Oncogene or Tumor Suppressor?* GENES. 10(3).

Funding

"IDENTIFICACION DE LOS MECANISMOS ONCOGENICOS ASOCIADOS A LA QUINASA MITOTICA PLK1."
Financiado por: Ministerio de Ciencia, Tecnologia y Universidades. Año 2019-2021

"IDENTIFICACIÓN DE NUEVOS BIOMARCADORES PARA CANCER DE MAMA: MECANISMOS DE SENSIBILIDAD Y RESISTENCIA A DROGAS DE CICLO CELULAR."
Financiado por: Fundación Científica AECC. Año 2017-2020

"IDENTIFICACIÓN DE LOS MECANISMOS ONCOGÉNICOS ASOCIADOS A LA QUINASA MITÓTICA PLK1"
Financiado por: CSIC. Año 2018-2019

Bioinformatics and computational biology

Principal Investigator
Díaz Uriarte, Ramón

Postdoctoral
Díaz Colunga, Juan

Support Personnel
Miguel González, Juan Antonio

Keywords: Statistics, computational biology, bioinformatics, evolution, ecology, cancer, cancer progression models, computer simulation.

Research Lines

Our main research area is bioinformatics, computational biology, computational statistics and evolutonary biology applied to the analysis of "high-throughput" data, mainly in cancer. Part of our work ranges from the application of standard techniques to the development of new statistical approaches, with special emphasis in their implementation using high performance computing. Our main current work focuses on trying to understand the sequence of driver genetic events and predict tumor evolution using methods that take ideas from phylogenetic methods and probabilistic graphical models which has also led us to develop software for simulating clonal evolution processes. This area is actually our main major focus: cancer progression models and evolutionary models of cancer (applying ecology and evolutionary biology to cancer).

Publications

Díaz, R., Vasallo, C. (2019). *Every which way? On predicting tumor evolution using cancer progression models.* PLoS Comput. Biol. 15(8): e1007246.

Hosseini, S., Díaz, R., Markowetz, F., Beerenwinkel, N. (2019). *Estimating the predictability of cancer evolution.* Bioinformatics. 35(14): i389-i397.

Funding

"IDENTIFICACION DE RESTRICCIONES EN EL ORDEN DE ACUMULACION DE MUTACIONES DURANTE LA PROGRESION TUMORAL: METODOS DE INFERENCIA Y SOFTWARE DE SIMULACION."
Financiado por: MINECO. Año 2015-2020

"TERAPIA ANTITUMORAL ADAPTATIVA USANDO DATOS TRANSVERSALES Y PREDICCIONES DE MODELOS DE PROGRESION TUMORAL."
Financiado por: AEI. Año 2020-2024

Cancer Signalling and Therapy

PRINCIPAL INVESTIGATOR
Wolfgang Alexander Link

MASTER STUDENTS
Calissi-, Giampaolo

Keywords: Cancer, Therapy resistance, FOXO transcription factors, human longevity, target and drug discovery.

Research Lines

The focus of our research has been the PI3K/AKT/FOXO signaling which is considered as the most frequently activated pathway in cancer. FOXO is the major transcriptional effector of this signaling pathway and inactivated in many tumours[1]. FOXO3a is the second most replicated gene associated with extreme human longevity (Figure1) [2].

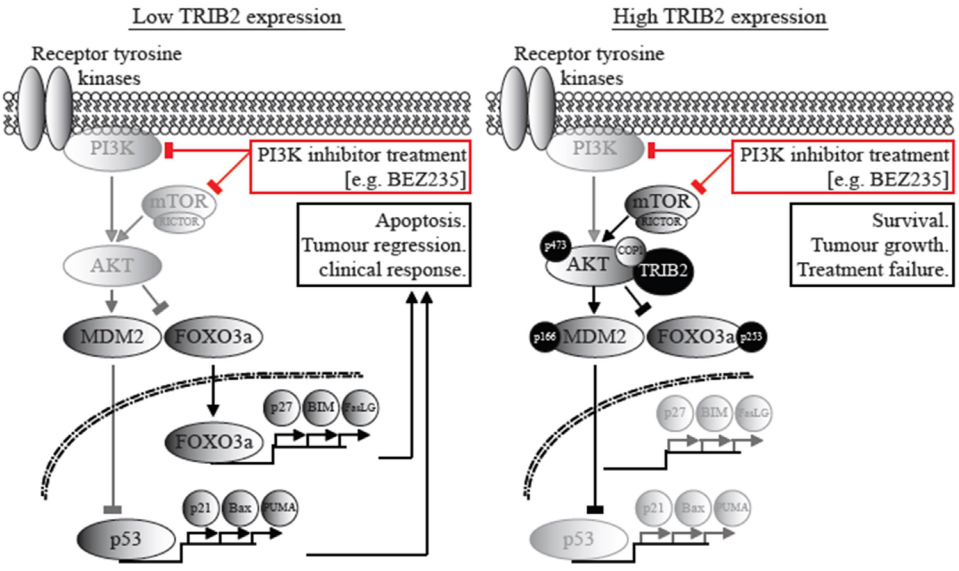


Figure 2. *Cell model of TRIB2-mediated drug resistance adapted from [11]. TRIB2-mediated AKT activation leads to the inactivation of FOXO and p53, well-known tumour suppressors, and effectors of anti-cancer drug action*

FOXO activating compounds

Our Team is unique in the world because we have a collection of over 300 previously identified FOXO activator molecules[3,4]. Among these compounds are several kinase inhibitors[1,4,5], nuclear export inhibitors[4,5,6,7]and orphan natural products[8]. These molecules have great potential to be developed as drugs against cancer and aging.

Mechanisms of therapy resistance

Resistance to therapy is the fundamental reason for treatment failure and represents the major barrier to improve survival of cancer patients. Understanding how cancer cells manage to escape treatment is essential to develop therapies that block escape routes. A major breakthrough has been accomplished with the discovery of the FOXO repressor protein TRIB2 as a novel oncogene in melanoma[9]. TRIB2 is overexpressed in melanoma and correlate with poor response to treatment[10]. Our research group has discovered a novel, clinically relevant TRIB2-mediated mechanism of drug resistance. Therapy resistance is mediated by direct interaction of TRIB2 with AKT. TRIB2/AKT-interaction promotes AKT activation resulting in FOXO inhibition and p53degradation[11]. As a consequence, the expression of FOXO and p53 target genes which lead to drug-induced apoptosis, is attenuated by TRIB2 (Figure2). We plan to translate our results into clinically useful tools, namely TRIB2 inhibitors to overcome therapy resistance and TRIB2-based biomarkers to predict treatment response and therefore to stratify patients into responders and non-responders.

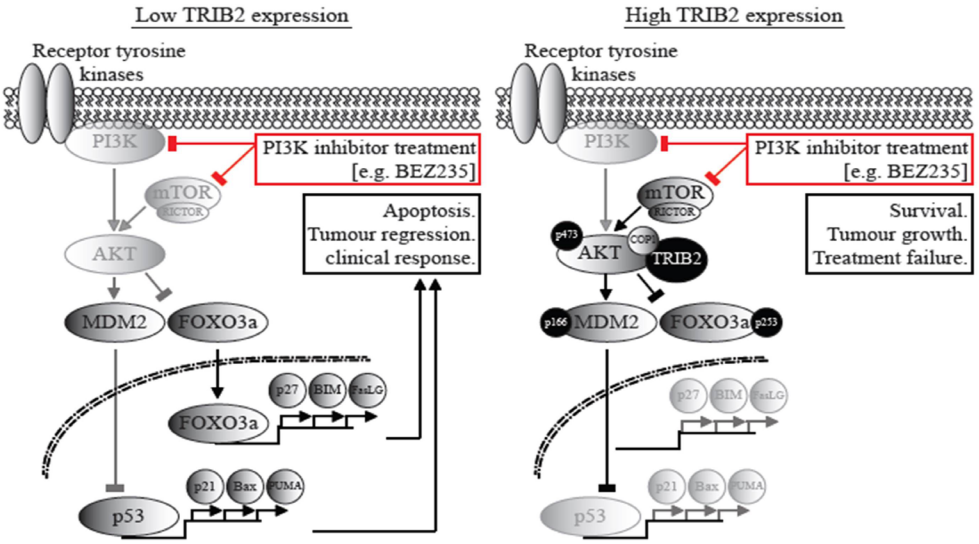


Figure 2. *Cell model of TRIB2-mediated drug resistance adapted from [11]. TRIB2-mediated AKT activation leads to the inactivation of FOXO and p53, well-known tumour suppressors, and effectors of anti-cancer drug action*

Private appropriation of publicly financed research

The way new medicines are discovered has changed dramatically over the past few decades[12]. Modern drug development relies on the molecular understanding of the disease which is often the result of decades of publicly financed research (Figure3).

Figure 3. *Flow chart of past and present drug discovery and development process. In the past, a drug was found before its molecular target was identified. Modern drug discovery is based on the molecular understanding of the disease and starts out with a validated molecular target. Figure adapted from [12].*

In an environment of shrinking science funding and increasing knowledge-dependence of drug discovery, private appropriation of publicly financed research without efficient return mechanisms threatens the pace of discovery and generation of future medicines[13]. In addition, novel therapeutic options including targeted therapy and immunotherapy are extremely expensive and broad access to them won´t be sustainable for the public health care systems within the near future in many countries. We are collaborating with economists to measure the contribution of public funding to innovative drugs.

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Src Family of Kinases and Breast Cancer: The functional role of Src kinases in breast cancer: contribution of the adapter and catalytic domains

Principal Investigator	Associate Investigator	Postdoctoral
Martín Pérez, Jorge	**Calcabrini, Annarica**	**Mayoral Varo, Víctor**

Keywords: c-Src, SH2 and SH3 c-Src adapter domains, breast cancer, cancer stem cells, glucose metabolism.

Research Lines

c-Src, the prototype of the Src Family of non-receptor tyrosine Kinases (SFKs), contains three major functional domains, the adapters SH2 and SH3, and the catalytic domains, which determine the cellular role of c-Src. Its expression and/or its kinase activity is increased in several tumors, including breast cancer. c-Src is associated with and activated by, receptors for growth factors and cytokines relevant in mammary gland physiology and in human breast cancer. Thus, the goal of this project is to define the importance of the SH2, SH3 and catalytic functional domains in metastatic human breast cancer. To this end, we are analyzing the conditional expression (Tet-On System) of c-Src variants with inactivating point-mutations of each of these three mayor domains in human metastatic breast cancer cell lines MDA-MB-231 or SUM159PT. We evaluated the effects of c-Src variants expression on cell proliferation, migration, invasion, anchorage-independent growth, the capability breast cancer stem cell renewal, exosomal production, etc.

Several scientific pieces of evidence show that c-Src is associated with the composition and functionality of exosome, suggesting its possible role in intercellular communication. In cancer, exosomes can facilitate the pre-metastatic niche where circulating tumor cells will finally nest. We have previously shown that c-Src can modulate the expression of certain proteins in exosomes. In addition, c-Src can facilitate the production and secretion of exosomes. The question raised as to whether the adapter domains of c-Src will modulate the number of exosomes, their composition, and consequently, the pre-metastatic niche of the triple negative breast cancer cells.

Presently, c-Src catalytic inhibitors are used in clinical oncology, if the c-Src adapter SH2 and/or SH3 domains are also crucial for metastatic breast cancer, the generation of inhibitors for the SH2 and/or SH3 could be employed in combination with those for the catalytic to completely block c-Src functionality in metastatic breast cancer. Moreover, since c-Src is also involved in other metastatic tumors such as colorectal, prostatic, and pancreatic, the results obtained in this study could eventually be extrapolated to other human cancers.

Results so far showed that in MCF7 (luminal A human breast cancer cells), which conditionally express SrcDN (c-Src-K295M/Y527F, dominant negative variant), the protooncogene controls the renewal of the subpopulation of breast cancer stem cell. This effect is due to the regulation of glucose metabolism.

Moreover, using conditional expression of c-Src variants, as well as aptamers specifically directed to the c-Src-SH2, we have observed that the SH2 domain of c-Src plays a relevant role in this protooncogene functionality both in MDA-MB-231 and in SUM159 triple negative breast cancer cell lines, which in turns controls pathways that regulates the renewal of the breast cancer stem cell subpopulation, as well as total cell proliferation, colony formation, migration and invasion, which are essential functions for tumorigenesis.

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Doctoral theses and other works

Víctor Mayoral Varo

"Papel de los dominios adaptadores de c-Src en cáncer de mama". Universidad Autónoma de Madrid. Medicina. 2019. Director/es: Jorge Martín. Calificación: CUM LAUDE

Funding

"Papel de los dominios adaptadores SH2 y SH3 de c-Src en la metástasis mamaria."
Financiado por: Ministerio de Ciencia, Innovación y Universidades. Año 2016-2020

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

PRINCIPAL INVESTIGATOR
Moreno Bueno, Gema

POSTDOCTORAL
Sarrió López, José David
Oltra Sanchis, Sara
Perez Lopez, Maria
Laura Sin Diaz

PREDOCTORAL
Gámez Chiachio, Manuel
Colomo del Pino, Sara
Ramos Nebot, Carmen

SUPPORT PERSONNEL
Diaz, Eva
Martínez Sánchez, Lidia
Morales Dolores, Saleta

Keywords: Breast and gynecological cancer, prognosis, molecular classification, immunotherapy, targeted therapies.

Research Lines

During the last years, the main objective of our group has been the study of the mechanisms underlying tumor progression together with the identification of new targets related to the response to treatment, using an approach based on pharmacogenomics and high-performance sequencing techniques.

Our group has also deepened in the study of the molecular mechanisms of the Mesenchymal Epithelium Transition (TEM) as crucial events in tumor progression. Specifically, we have focused on the analysis of basal phenotype breast tumors as well as endometrial carcinomas and high grade ovarian serous.

Another line of research of the group has focused on advancing the molecular mechanisms that lead to failures. On the other hand, from a clinical point of view one of the failures in the treatment in breast cancer is the innate or acquired chemoresistance that patients develop. Studies carried out in the laboratory have revealed the existence of possible markers involved in resistance to Trastuzumab. In this line of work, new markers are searched with predictive and prognostic capacity, markers of alternative therapies in ovarian cancer and endometrial carcinomas.

The concrete objectives are:
a) Analysis of the gene expression profile in tumor and cell samples, related to TEM processes.
b) Identification of breast carcinomas of basal phenotype and / or carcinosarcomas as candidates to undergo TEM processes.
c) Identification of tumor markers in breast and gynecological cancer through high-throughput mass sequencing.
d) Pharmacogenomic study in breast cancer. Identification of drug resistance mechanisms.
e) Role of Gasdermin B in HER2+ breast cancer as a new resistance mechai¡nism to antiHER2 therapies.

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Doctoral theses and other works

María Pérez López

"Decoding the molecular role of Gasdermin B in inflammatory diseases".
Universidad Autónoma de Madrid. Facultad de Medicina. 2020. Directores: Gema Moreno, José David Sarrió. Calificación: Sobresaliente cumlaude

Jeanette Martinez del Val

"Study of the involvement of Gasdermina B in resistance to chemotherapy and validation of new targeted compounds in the Dario Rerio preclinical model."
Universidad de Santiago de Compostela. Facultad. 2019. Directoras: Gema Moreno, Laura Sánchez Piñon. Calificación: Sobresaliente

"Molecular characterization of Gasdermin B cytotoxic activity and its potential application for the treatment of cancer (GB-citoTOX)."
Financiado por: Proyectos I+D+i» 2019-Modalidad «Retos Investigación». Plan Estatal de Investigación Científica y Técnica y de Innovación 2017-2020. Ministerio de Ciencia e Innovación. Ministerio de Universidades. Año 2020-2023

"Targeting Gasdermin-B overexpression as a new therapeutic approach in Her2+ cancers."
Financiado por: Fundación Científica de la Asociación Española Contra el Cáncer. Año 2019-2022

"Grupos coordinados clínicos AECC-2018. Targeting the most aggressive type of endometrial carcinoma."
Financiado por: Fundación Científica de la Asociación Española Contra el Cáncer. Año 2018-2023

"Gasdermina B: mediador de respuesta inflamatoria y terapéutica en cáncer de mama y digestivo."
Financiado por: Instituto de Salud Carlos III. Cofinanciado por la Unión Europea a través del Fondo Europeo de Desarrollo Regional (FEDER). Año 2017-2019

"Consorcio CIBERONC. Área Cáncer de mama."
Financiado por: Instituto de Salud Carlos III. Cofinanciado por la Unión Europea a través del Fondo Europeo de Desarrollo Regional (FEDER) y del Fondo Social Europeo (FSE). Año 2020-2021

Vitamin D and colon cancer

PRINCIPAL INVESTIGATOR
Muñoz Terol, Alberto

CO-PRINCIPAL INVESTIGATORS
Larriba Muñoz, María Jesús
González Sancho, José Manuel

POSTDOCTORAL
Barbáchano Becerril, Antonio
Fernández Barral, Asunción
Ferrer Mayorga, Gemma
Costales Carrera, Alba

PREDOCTORAL
Bustamante Madrid, Pilar

Albandea Rodríguez, David

Keywords: Colon cancer, organoids, stromal fibroblasts, vitamin D, Wnt.

Research Lines

Colon/colorectal cancer (CRC) is a leading neoplasia worldwide in terms of incidence and mortality. In Spain, CRC is the most frequent cancer both genders considered. Many epidemiological studies associate vitamin D deficiency with high risk and poor prognosis of CRC. In the last two decades, our group and others have shown that the active vitamin D metabolite 1alpha,25-dihydroxyvitamin D3 (calcitriol) inhibits proliferation, promotes differentiation and attenuates the migratory and invasive phenotype of colon carcinoma cells. We were first to report that part of these effects was due to the antagonism of the Wnt/beta-catenin signaling pathway whose abnormal activation is an initial and crucial event in CRC.

Today it is well accepted that the tumor microenvironment or stroma plays a crucial role in colon tumorigenesis. Fibroblasts are the major cellular component of tumor stroma. Recently, we have described that calcitriol exerts its antitumor action in CRC not only by acting on carcinoma cells but also through the inhibition of the protumoral capacities of cancer-associated fibroblasts (CAFs). Thus, high expression of vitamin D receptor (VDR) and of a calcitriol-associated gene signature in tumor stromal fibroblasts is significantly associated with longer patient survival. These findings have a clear clinical impact as indicate that the therapeutic action of calcitriol can extend to CRC patients who express VDR in CAFs even in the absence of VDR expression in carcinoma cells, which occurs in a relevant percentage of patients.

Based on these data, we have studied the effects of calcitriol and the canonical Wnt3A factor, alone or in combination, on CCD-18Co human colon myofibroblasts. Our data show that calcitriol and Wnt3A have additive and partially overlapping modulatory effects on the gene expression profile and phenotype of colon fibroblasts. Both agents inhibit fibroblast proliferation and migration, while calcitriol reduces but Wnt3A increases their capacity to remodel the extracellular matrix.

Organoids are a novel technology of three-dimensional culture of normal or tumor stem cells and their progeny that allow the generation of structures resembling the organ they derive from and, thus, reproduce the in vivo situation better than classical two-dimensional cell cultures. We have established a living biobank of colon normal and tumor organoids from surgical biopsies of CRC patients. In these organoids we have studied the effects of calcitriol on cell gene expression, proliferation and phenotype. In addition, we have optimized an assay to test the activity of antitumor drugs against CRC in these patient-derived organoids. Finally, we have also established a living biobank of rectum normal and tumor organoids from endoscopic biopsies to compare: a) colon and rectum normal stem cells, b) colon and rectum tumor stem cells, and c) their respective response to calcitriol.

Our group collaborates with colleagues from different scientific institutions: Drs. Prieto, Cantero, Burgos and Guerra (Hospital Universitario La Paz), García-Olmo and Rojo (Fundación Jiménez Díaz), Batlle (IRB), Real (CNIO), Lafarga (Universidad de Cantabria), Peña (Hospital Universitario Ramón y Cajal), Sánchez-Puelles (CIB), and del Peso (our Institute).

Publications

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Doctoral theses and other works

Alba Costales Carrera

"Patient-derived normal and tumor colorectal organoids: studies on gene expression, vitamin D and drug activity".
Universidad Autónoma de Madrid. Facultad de Medicina. 2019. Director/es: Alberto Muñoz, Antonio Barbáchano. Calificación: Sobresaliente cum laude

Funding

Alberto Muñoz Terol

"Consorcio CIBER Área Temática de Cáncer (CIBERONC)."
Financiado por: Instituto de Salud Carlos III. Año 2017-2021

"Cáncer de colon: efecto de la vitamina D sobre organoides normales y tumorales derivados de pacientes y sobre el microambiente tumoral."
Financiado por: Ministerio de Economía y Competitividad. Año 2017-2020

"Red Temática de Receptores Nucleares en Cáncer, Metabolism e Inflamación (NuRCaMeIn2)."
Financiado por: Ministerio de Economía y Competitividad. Año 2018-2021

"Estudio de los efectos de la vitamina D en la fisiología y patología del colon humano usando organoides y cultivos primarios de fibroblastos derivados de pacientes."
Financiado por: Ministerio de Ciencia e Innovación. Año 2020-2023

"Ensayo de drogas antitumorales en organoides colónicos humanos. Efecto de la vitamina D."
Financiado por: Farmasierra Manufacturing, S.L. Año 2020-2021

"Cáncer de colon: efecto de la vitamina D sobre el microambiente tumoral."
Financiado por: Consejo Superior de Investigaciones Científicas. Año 2020-2021

María Jesús Larriba Muñoz

"Efectos de la vitamina D sobre el microentorno tumoral en cáncer de colon."
Financiado por: Consejo Superior de Investigaciones Científicas. Año 2018-2019

Cytoskeleton and Metastasis

Principal Investigator
Orgaz Bueno, José Luis

Keywords: Melanoma, Myosin, cytoskeleton, metastasis, therapy esistance.

Research Lines

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

Rho GTPase signalling controls the cell cytoskeleton through regulation of actin polymerization and actomyosin contractility; both 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) inactivates the myosin light chain 2 (MLC2) phosphatase (MYPT), which leads to increased phosphorylation of MLC2 (p-MLC2) and NMII activity, which drives contractile forces required for migration, invasion and metastasis.

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 metastasize. 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. Most melanomas carry mutations in the mitogen activated protein kinase (MAPK) pathway (RAS-BRAF-MEK-ERK), in particular in BRAF ($BRAF^{V600E}$ being the most common, 50% patients) and RAS (20% patients). Mutant BRAF constitutively activates ERK signalling that drives cancer cell proliferation and tumour progression. Targeted therapies using $BRAF^{V600E}$ inhibitors (BRAFi) and also in combination with MEK inhibitors increase survival of $BRAF^{V600E}$ melanoma patients. Unfortunately, responses are temporary and patients relapse due to acquired drug resistance in less than a year. Most mechanisms of resistance to MAPK inhibitors (MAPKi) involve reactivation of ERK.

Dynamic transcriptional rewiring and epigenetic changes enable rapid adaptation to MAPK-targeted therapy and later development of resistance. In fact, transcriptional alterations in pathways controlling metastatic abilities have been suggested to drive resistance to targeted therapies. One such pathway is the ROCK-NMII pathway, which is positively regulated by MAPK. Resistance to MAPKi in melanoma involves extensive cytoskeletal remodeling and NMII reactivation. 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 lab is to understand how the cytoskeleton, in particular NMII, is regulated during cancer progression and during adaptation to therapy, in particular in 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.

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Funding

"Understanding Myosin II regulation during adaptation to targeted therapies in melanoma and potential therapeutic interventions to delay therapy resistance."
Financiado por: Comunidad de Madrid. Año 2020-2024

Cell senescence and tumor suppression

Principal Investigator
Palmero Rodríguez, Ignacio

Predoctoral
de Lope García, Cristina
López Antona, Irene

Support Personnel
Benito Hernández, Samantha
Pérez Tablado, Silvia

Undergraduate Students
Escudero Iriarte, Carmen
González Méndez, Paula

Master Students
González Molina, Mª del Pilar
Martín Alonso, Samara
Luque Martín, Laura

Keywords: Senescence, tumor suppression, cell plasticity.

Research Lines

SIX1 and senescence in cancer

We have recently shown that the SIX1 homeoprotein is an essential repressor of cellular senescence, mainly via the regulation of the senescence effector p16INK4A (Adrados et al, Oncogene, 2016. PMID 26500063). SIX1 is also an oncogene, frequently activated in different types of human tumors such as lung and brain cancer or sarcomas. We have explored the role of senescence in the protumorigenic role of SIX1, studying mouse fibrosarcoma tumors derived from transformed fibroblasts. Our results show that SIX1 has a protumorigenic effect in this model, which is accompanied by the blunting of the expression signature associated to SIX1 in senescence. This is consistent with the role of cell senescence as a tumor barrier and suggests that opposing senescence plays a role in the oncogenic role of SIX1. These studies have also shown that SIX1 promotes an undifferentiated phenotype in these tumors, mainly mediated by the transcrptional activation of the stemness regulator SOX2. Our results have identified new mechanisms that may underpin the oncogenic role of SIX1: blocking the tumor suppressive action of senescence and promoting stem-cell features via SOX2 (De Lope et al, Scientific Reports, 2019. PMID 30723235).

SIX1 and senescence in development

Developmental senescence is a form of programmed, physiological senescence that occurs transiently in specific embryo locations and contributes to patterning during embryonic development. SIX1 is a key transcriptional regulator of development, essential for the formation of organs such as inner ear, kidney, muscle and thymus, among others. Interestingly, alterations in the SIX/EYA pathway are linked to the Branquio-Oto-Renal (BOR) syndrome, a human rare congenital disorder with defects in the ear and kidney and branchial arch anomalies. Based on our recent finding that SIX1 is a senescence regulator, we are currently testing the hypothesis that aberrant activation of senescence can underpin the developmental defects associated with SIX1 deficiency in mice and humans.

Senescence and cell plasticity

One of our interests is to understand the link between cell senescence and plasticity. Emerging evidence has uncovered a complex connection between senescence, differentiation and regeneration in different contexts, with examples of both inhibitory and activating effects of senescence. In this context, our recent results have identified a gene signature related to differentiation in cellular models of senescence triggered either by oncogenic stress or SIX1 loss (Adrados et al, Oncogene, 2016. PMID 26500063). To investigate further this link, we are currently exploring the impact of senescence on the potential of primary fibroblasts to transdifferentiate to different cell types. We hope that this work will shed light on the connection between cell senescence and differentiation.

Publications

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Doctoral theses and other works

Irene López-Antona

"Regulación del fenotipo miofibroblástico en senescencia".
Universidad Autónoma de Madrid, 2019.
Director: Ignacio Palmero.
Calificación: Sobresaliente cum laude

Funding

"Senescencia y plasticidad celular en fisiología y enfermedad."
Financiado por: AEI. Año 2019-2021

"Red de Excelencia "Senescencia celular: mecanismos y terapias (Senestherapy III)."
Financiado por: AEI. Año 2020-2022

Hypoxia and Angiogenesis

Principal Investigators
Peso Ovalle, Luis del
Jiménez Cuenca, Benilde

Investigators under contract
Acosta Iborra, Bárbara

Predoctoral
Puente Santamaría, Laura

Support Personnel
Hernández Sierra, Rosana

Undergraduate Student
Alonso Galicia, Leire
González Serrano, Bárbara Pilar

Master Students
Puente Santamaría, Laura

Keywords: Hipoxia, genómica, bioinformática, angiogénesis.

Research Lines

The elucidation of the cellular and molecular adaptive responses to hypoxia constitutes an important research topic due to the relevance of this process in physiology and high-incidence pathologies such as cancer and cardiovascular diseases. The Hypoxia Inducible Transcription Factors (HIFs) play a pivotal role in this response by controlling the expression of most of the genes involved in adaptation to hypoxia. The aim of our group is to contribute to the understanding of the transcriptional response to hypoxia and the cellular and molecular mechanisms underlying central adaptation responses like angiogenesis. Our long term goal is to exploit this knowledge to improve clinical management of pathologies in which development of tissue hypoxia is a common feature.

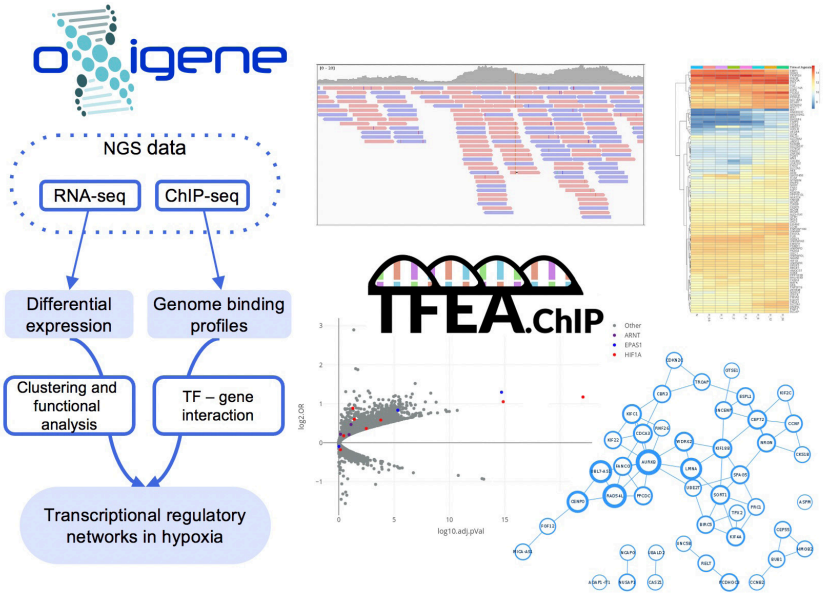
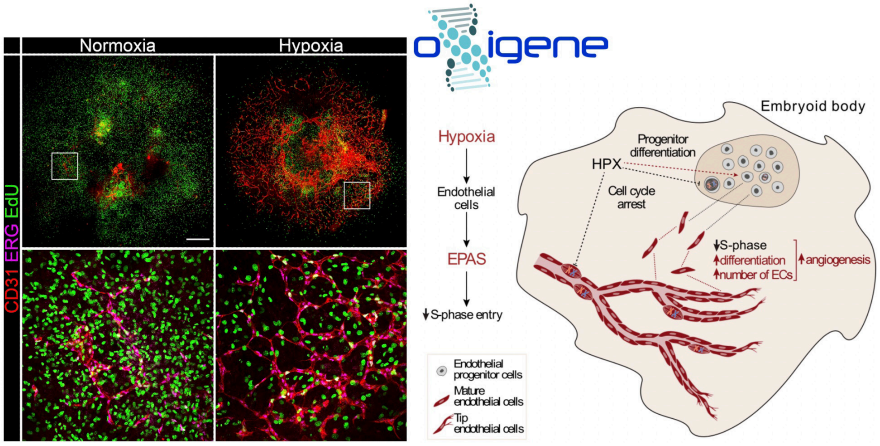


Figure 1
Characterization of the transcriptional response to hypoxia.

Ablation of HIF prevents both gene upregulation and repression triggered by hypoxia. However, genome-wide profiling of HIF-binding sites indicates that only gene induction is directly regulated by HIF. To identify the mechanisms responsible for gene repression during hypoxia, we developed a computational approach that, exploiting the vast amount of publicly available ChIP-seq datasets, identified enriched transcriptional regulators. In addition to the identification

Genomics and Bioinformatics

of factors that significantly affected hypoxia-repressed genes, this work led to the development of a general tool for the identification of enriched transcriptions in sets of differentially expressed genes (Puente-Santamaria Bioinformatics. 2019).



Stem Cell Models of angiogenesis

Figure 2

Identification of polymorphisms affecting HIF binding sites and their contribution to disease inter-individual variability.

The vast majority of human genetic variation lies in non-coding regions of the genome, but its functional impact in phenotype and disease is almost unknown. We have described that several common SNPs affect the transcriptional response to hypoxia. However, a global analysis of common genetic variability on the response to hypoxia is still lacking. Aiming to fill this gap, we designed an unbiased approach to identify SNPs having a functional impact on HIF binding to chromatin. Using this strategy, we have identified over 300 variants conferring allele-specific binding, most of them outside HREs (Martínez et al., Manuscript in preparation).

Understanding the role of HIFs in endothelial cells during angiogenesis induced by hypoxia.

Angiogenesis is the main mechanism that allows vascular expansion and a fundamental adaptive response to hypoxia in physiology and disease. Knowledge of the role of HIFs in the control of angiogenesis is still incomplete. By analyzing changes in the transcriptomic profile of endothelial cells (ECs) under hypoxia we uncovered that the repression of cell cycle entry and DNA replication stand as central responses in the early adaptation of ECs to low oxygen tension (Acosta et al, FASEB J 2020). Accordingly, hypoxia imposed a restriction in S-phase in ECs that is mediated by Hypoxia-Inducible Factors. Our results indicate that the induction of angiogenesis by hypoxia in embryoid bodies generated from murine stem cells is accomplished by the compensation of decreased S-phase entry in mature ECs with induction of differentiation of progenitor cells.

Publications

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Funding

"Hipoxia y angiogénesis: mecanismos básicos en fisiología y enfermedad."
Financiado por: MINECO. Año 2018-2020

"Implementación de procedimientos para análisis de datos derivados de técnicas de secuenciación de alto rendimiento."
Financiado por: CAM. Año 2020-2022

Epithelial Carcinogenesis

Principal Investigators
Quintanilla Ávila, Miguel
Martín Villar, Ester

Postdoctoral
Montero Montero, Lucía

Predoctoral
Montero Montero, Lucía

Support Personnel
Beltrán Álvarez, Santiago

Master Students
Beltrán Álvarez, Santiago
Jarcovsky González, Rocío

Collaborations
Bernabéu Quirante, Carmelo

Research Lines

Relevance of podoplanin-CD44 intreraction for carcinoma cell invasion

Podoplanin (PDPN, also called PA2.26, Aggrus, D2-40) is a mucin-like transmembrane glycoprotein expressed in a variety of cancer types, including squamous cell carcinomas (SCCs), that plays an important role in malignant progression. Our previous studies found that podoplanin is a component of invadopodia, in which promotes its stability and an efficient degradation of the extracellular matrix (ECM). Invadopodia are plasma membrane protrusions with proteolytic activity that are present in cells from SCCs and other tumors, and are involved in tumor invasion through the basal membranes underlying epithelia and surrounding blood vessels. We also identified the interaction of podoplanin with CD44s, the standard isoform of CD44, which promotes the directional migration of SCC cells. CD44 is a glycoprotein involved in cell adhesion and motility, whose gene gives rise to CD44s and a wide variety of variable isoforms (CD44v) by alternative splicing.

During the last two years, we characterized CD44v3-10, CD44v6-10 and CD44v8-10 as the main variable isoforms expressed in human and murine SCC cell lines. CD44s and these CD44v isoforms co-localize and physically interact with podoplanin on the cell surface and in the adhesion ring of invadopodia. The interaction PDPN-CD44 occurs through the transmembrane domains and is regulated positively by the cytoplasmic tails and negatively by glycosylation of the extracellular domains. Furthermore, we identify CD44v10 as the only variable isoform expressed together with CD44s in highly aggressive spindle carcinoma cells, a fact apparently associated with an epithelial-mesenchymal transition. The selective silencing of podoplanin and CD44 (as well as expression of single CD44 isoforms) in SCC cells indicate that both glycoproteins are involved in invadopodia-mediated ECM degradation, but they exerts different functions. Podoplanin promotes invadopodia stability and a focal, efficient, degradation of the ECM, as mentioned above. In contrast, CD44 does not affect invadopodia stability, but is needed for the spatial control of proteolytic activity, being CD44s the main isoform involved in this function. Preliminary experiments of ex vivo invasion, using a native basal membrane, suggest that both podoplanin and CD44 are essential for SCC cell invasion through the basal membrane. Currently, we are investigating the relevance of PDPN-CD44 interaction on the expression and subcellular localization of invadopodia typical metalloproteases, such as MMP14 (MT1-MMP), MMP9 and MMP2.

Finally, collateral results of these studies suggest an involvement of PDPN-CD44 interaction in cell division, likely in the control of cytokinesis.

Role of podoplanin in epidermal homeostasis, regeneration and carcinogenesis

Podoplanin is absent from normal epidermis in adult mice, but it is expressed in basal keratinocytes during wound healing, after a pro-inflammatory stimulus, and in skin tumors.
In order to study the role of podoplanin in the epidermis during physiopathological conditions, we generated three models of mutant mice: K5Cre.PDPN, K14Cre-ERT2.PDPN y K15Cre-PR1.PDPN. In the two first models, the Pdpn gene is deleted in the basal layer of interfollicular epidermis and the outer root sheet of hair follicles, either in a constitutive manner (K5Cre.PDPN) or induced by Tamoxifen (K14Cre-ERT2.PDPN); whereas in K15Cre-PR1.PDPN mice, silencing of podoplanin is induced by RU486 (a synthetic analogue of progesterone) in the bulge region of hair follicles, where a population of epidermal stem cells involved in the growth and regeneration of hair follicles as well as wound healing resides.

At present, our studies have been focused in the K5Cre.PDPN model (K14Cre-ERT2.PDPN mice showed high morbidity after experimental manipulation). Podoplanin silencing in the basal layer induces an acceleration of keratinocyte differentiation, which enhances after a pro-inflammatory stimulus with the phorbol ester TPA, as shown by the aberrant expression and localization of differentiation markers: E-cadherin, citokeratins 10 and 6, involucrin, loricrin and filaggrin. These changes result in a disorganized epidermal architecture and an abnormal orientation basal keratinocyte nuclei, which may affect cell division (whether symmetric or asymmetric). In addition, preliminary experiments show alterations in the hair growth of mutant mice after activation of the anagen phase of the cycle by shaving. In addition, we have observed a lengthening in the latency of tumor appearance after mutant mice were subjected to chemical carcinogenesis with dymethylbenzanthracene (DMBA) and TPA, and changes in the histology of tumors in mutant with respect to normal mice were also observed.

Currently, we are analyzing the effect of podoplanin silencing in epidermal and hair follicle organogenesis during embryonic development, as well as defects in the epidermal barrier in young and adult mice. In addition, we are studying the effect of podoplanin silencing in the apico-basal polarity of basal keratinocytes, and the frequency of symmetric and asymmetric divisions during epidermal morphogenesis and regeneration. Another important goal of the group in the near future is the study of the role of podoplanin in the proliferative capacity and mobilization of epidermal stem cells, by using the K15Cre-PR1.PDPN mouse model.

Endoglin protein interactome

Endoglin is a transmembrane glycoprotein, which acts as an auxiliary co-receptor of TGF-beta and has been involved in cardiovascular diseases and cancer. Previous studies from our laboratory and other groups have shown that endoglin behaves as a tumor suppressor in skin carcinogenesis and, in general, in SCC formation. Endoglin is shed from the plasma membrane releasing a circulating form, called soluble endoglin (sEng), comprising almost all the extracellular domain. Increased levels of sEng have been related to vascular diseases, such as preeclampsia, and some cancer types, such as leukemias as well as breast and colorectal cancer. sEng interacts with TGF-beta receptors, MMP14 (the main metalloprotease involved in endoglin shedding) and Met, the tyrosine kinase receptor of HGF. In collaboration with Dr. Carmelo Bernabéu laboratory (Center for Biological Research, CIB, CSIC, Madrid), we have performed a microarray screen with more than 9.000 unique human proteins, using recombinant sEng as bait. By this approach, we identified 22 new putative ligands that bind sEng with high affinity. From these proteins, we identified the specific interaction of endoglin with galectin-3, a secreted member of the lectin family that is involved in angiogenesis, metastasis and immune surveillance; and with TRIM21, an E3-ubiquitin-protein ligase that behaves as a tumor suppressor in breast cancer.

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Lucía Montero Montero

"La interacción podoplanina-CD44 modula la degradación de la matriz extracelular asociada a invadopodios y la transmigración de células de carcinoma escamoso a través de la membrana basal".

Universidad Autónoma de Madrid. Medicina.
2019. Director/es: Miguel Quintanilla.
Calificación: Sobresaliente cum laude

Funding

"Función de podoplanina en la progresión maligna del cáncer epitelial. Relevancia de la interacción podoplanina-CD44."
Financiado por: Ministerio de Ciencia, Innovación y Universidades. Año 2018-2021

Cancer Stem Cells and Tumor Microenvironment

Principal Investigator
Sainz Anding, Bruno

Investigators under contract
Alcalá Sánchez, Sonia
Sánchez Tomero, Patricia
López Gil, Juan Carlos

Palencia Campos, Adrián
Ruiz Cañas, Laura
Yuste Pérez, Mª Lourdes

Predoctoral
Martín Hijano, Laura
Valle Rodríguez, Sandra

Cordero Barreal, Alfonso

Support Personnel
Navarro Vera, Diego

Keywords: Pancreatic ductal adenocarcinoma, cancer stem cells, cancer cell metabolism, patient-derived xenografts, tumor microenvironment, tumor-associated macrophages.

Research Lines

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 my 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. Specifically, the avenues of research that my laboratory is pursuing are:

1) The identification and characterization of new biomarkers for the detection of CSCs from different solid tumors.

By definition, CSCs are the sole source of tumor initiation, metastasis and cellular heterogeneity, giving rise to intermediate progenitors and terminally-differentiated bulk tumor cells. However, whether the CSC is a hardwired entity or a state has been a point of debate for the past 5 years. We now know that CSC-ness is not an intrinsic feature of a subpopulation of cells, but rather, CSC-ness is a state governed and driven by temporal and spatial characteristics, and strongly influenced by the tumor microenvironment (TME). Thus, only by targeting the CSC, the non-CSC transient cells and the TME will we succeed in curing cancer.

In order to study these cells, CSCs need to be identified and isolated via cell surface or internal markers that are over-expressed in and/or on these cells. We have identified and continue to discover new CSC markers that enable us to quantify the number of CSCs in patient biopsies as well as separate these cells away from the bulk tumor population in order to interrogate them on a single-cell level. While no one marker or combination of markers can identify all of the CSC populations present within a tumor at a given time, we hope that by identifying new markers, we can fine tune our ability to identify and isolate these cells.

Our laboratory is work towards this goal by using new approaches to scan the surface of CSCs in search of new CSC markers.

2) 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.

At the "omic" level, CSCs are different than their non-CSC counterparts. These differences are believed to be epigenetically driven as well as a consequence of post translational modifications. We have dissected CSCs and discovered what makes them tick, and learned how to genetically and pharmacologically target these weaknesses. Using different approaches, including high-throughput methylation analysis, mircoRNA arrays, and RNAseq, we are closer to understating what makes a CSC a CSC.

For example, we recently 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. Specifically, ISG15 is necessary for the recycling of aged or damaged mitochondria. Since pancreatic CSCs use oxidative phosphorylation (OXPHOS) to meet their energy requirements, the mitochondria of CSCs need to be recycled via a process known as mitophagy. ISG15 and ISGylation is necessary for this process as inhibition of ISG15 renders pancreatic CSCs less tumorigenic.

Our laboratory continues to work towards better understanding the "omic" landscape of CSCs and non-CSCs in order to design and develop new therapeutics to specifically target CSCs.

3) 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 in response to cues from the tumor and how these proteins alter the function of the CSCs at the level of EMT and chemoresistance.

The use of animal models of PDAC and patient-derived xenografts are essential for understanding the tumor microenvironment. Via collaborations with national and international hospitals, we are creating one of the largest Biobanks of pancreatic patient-derived xenografts (PDXs) in Spain. PDXs are generated by implanting resected tumor pieces into immune compromised mice. The resulting "avatar" mice can serve as pre-clinical models to assess the effect of CSC inhibitors on PDAC tumor formation. Likewise, we have at our disposal genetically-engineered mouse models of PDAC, that faithfully mimic important phenotypes of human PDAC in a mouse setting. These models allow us to study all aspects of the disease, from onset to metastasis, as well as dissect and analyze the components of the the tumor microenvironment that are necessary for all of these processes, in an immune competent setting.

Publications

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Doctoral theses and other works

Sandra Valle Rodríguez

"Explotación de la fosforilación oxidativa para promover las propiedades madre e inmunoevasivas de las células madre del cáncer de páncreas".

Universidad Autonoma de Madrid. Medicina. 2020. Director/es: Bruno Sainz. Calificación: Sobresaliente

Funding

"Design and development of peptide inhibitors of SARS CoV2 virus entry for human use." Financiado por: Spanish Ministry Science, Innovation & Universities, ISCIII – Fondo-COVID19. Año 2020-2021

"AptaBreast: Desarrollo Preclínico de un Aptámero para el Tratamiento del Cáncer." Financiado por: Convocatoria Retos-Colaboración 2019, Spanish Ministry Science, Innovation & Universities. Año 2020-2022

"RuCSC- targeting cancer stem cells using ruthenium compounds." Financiado por: Program IGNICIA proof of concept, an initiative of the Agencia Gallega de Innovación (GAIN). Año 2019-2020

"Combating Pancreatic Cancer by Identifying Those Genes Essential for Cancer Stem Cell-Mediated Tumorigenicity." Financiado por: Fundación FERO. Año 2019-2020

"Photoactivable nanoparticles to immunostimulate the tumour microenvironment in pancreatic cancer (PANIPAC)." Financiado por: Spanish Ministry Science, Innovation & Universities. Año 2019-2021

"The basal subtype of pancreatic cancer as a new tool towards personalized medicine: cellular and molecular characterization for the development of new therapies." Financiado por: Spanish Ministry Science, Innovation & Universities, ISCIII. Año 2019-2021

"Identifying pancreatic cancer stem cell immune escape receptors." Financiado por: Asociación Cáncer de Páncreas. Año 2017-2020

"A multi-faceted approach to treating pancreatic cancer (Grupos Coordinados Estables 2016)." Financiado por: Asociación Española Contra el Cáncer (AECC). Año 2016-2021

"Development of a liquid biopsy assay to isolate circulating cancer stem cells in the blood for their characterization and validation as a biomarker for early detection of pancreatic cancer." Financiado por: Spanish Ministry of Innovation & Competiveness, ISCIII. Año 2016-2019

"Targeting mitochondrial respiration, an Achilles' heel of cancer stem cells." Financiado por: CONquer CanCER Now Award, Concern Foundation. Año 2016-2019

Integrative molecular analysis for the study of thyroid differentiation in development and cancer

Principal Investigator
Santisteban Sanz, Pilar

Associate Investigator
Riesco Eizaguirre, Garcilaso

Investigator under contract
Acuña Ruiz, Adrián
Morillo Bernal, Jesús

Contract Doctor
Zaballos Sánchez, Miguel Angel

Predoctoral
Fernández Méndez, Celia
Ramírez Moya, Julia A.
Carrasco López, Carlos

Support Personnel
Martínez Cano, Andrea
Makiadi Alvarado, Jennifer

Undergraduate Students
Aramendía Cotillas, Elena
Galache Poveda, Jaime

Collaborations
Castro Calvo, Alejandro
Martín Duque, Mª Pilar
Vieja Escolar, Antonio de la
Mielu-, Lidia Mirela

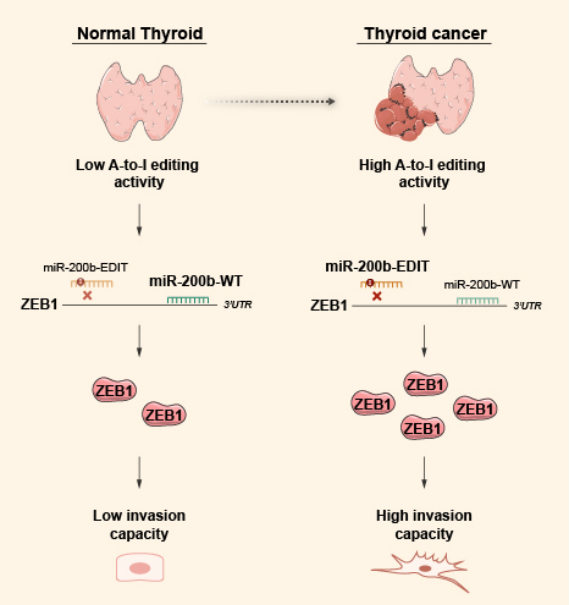
Keywords: Signaling, transcription, proliferation, differentiation, miRNAs, iPSC, mESC, development, cancer, thyroid.

Research Lines

We continue working on unraveling the mechanisms involved in thyroid differentiation in development and cancer. Our model system is the thyroid gland, which is an organ derived from the endoderm and that is able to originate the most frequent endocrine tumor.

Regarding development our work, in collaboration with Dr. Zaret from Penn University, have described the interaction between the forkhead factor FOXA1 with core histones, demonstrating a chromatin open activity for this factor. This activity, demonstrated in vitro for the pioneers' transcription factors. is functional in mouse embryogenesis. Furthermore, we have advanced in the regulation of other forkhead factor FOXE1, by hormone and growth factors in the process of thyroid differentiation. Besides, we have demonstrated that in thyroid cancer FOXE1 regulates migration and invasion. Using mESC and iPS we have described that an excess of iodide impairs development and differentiation by a mechanism involving epigenetic modifications.

Our work on the topic of thyroid cancer, have focused on two main aspects (i) Study the role of microRNA and RNA editing



and (ii) Deepen the role of the ERK pathway. (i) After RNA-Seq thyroid tumors, we have identified the miRnome and transcriptome demonstrating that miRNA expression patterns define clinically-relevant subclasses and may contribute to loss of differentiation and tumor progression. Among them, the miR-146b represses DICER1 expression driving cancer cell aggressiveness. Furthermore, global upregulation of miRNAs by enoxacin administration, to mice with thyroid tumors, suppress tumor growth.

The silencing of ADAR1, an enzyme involved in RNA editing, suppresses thyroid tumorigenesis both in vivo and in vitro. The image represents our results about the mechanism involved in ADAR1 action in thyroid cancer: The ADAR1 dependent A-to-I editing is low in normal thyroid and consequently miR-200b is in its wt form. In this way this miRNA inhibits ZEB and the cells has low invasion capacity. In thyroid cancer, in which there is high ADAR1 editing activity, more mir-200b is edited with impaired ability to inhibit ZEB and therefore increasing invasion and tumor aggressiveness

ii) We are characterizing the Ras-ERK signaling pathway and its interaction with other pathways in differentiated thyroid and tumor cells. In addition, we are performing functional study of ERK dimerization and its inhibition in tumor cells. For this purpose, we using DEL22379, an ERK inhibitor, and we have observed that this compound inhibits thyroid tumor growth.

Other proteins and signaling pathways are being studied in our work. These include the TGFβ/Smads, the PI3K and the Hippo pathways, the scaffolds proteins IQGAP1 and 2, as well as the transcription factor Six1 Our work expands to translational studies in human thyroid cancer, due to an extensive network of national and international collaborations.

Publications

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Doctoral theses and other works

Adrián Acuña Ruiz

"La dimerización y fosforilación de ERK y la expresión de Six1 como diana moleculares para el tratamiento del cáncer anaplásico de tiroides".

Universidad Autónoma de Madrid (UAM). Medicina. 2020. Director/es: Pilar Santisteban. Calificación: Sobresaliente Cum Laudem

“Genes y vías de señalización emergentes en el desarrollo y el cáncer de tiroides.”
Financiado por: Agencia estatal de Investigacion, MINECO. Año 2017-2020

“Fisiopatología tiroidea: Mecanismos implicados en cáncer, autoinmunidad y mecanismo de acción de hormonas tiroideas2018.”
Financiado por: Programa de Biomedicina Comunidad de Madrid. Año 2018-2021

“Nuevas dianas moleculares en la ruta RAS-ERK: Potencial terapéutico en el cáncer de tiroides.”
Financiado por: Asociación Española contra el cancer (AECC). Año 2014-2021

“Análisis Molecular Integral para el estudio de la diferenciación en el cáncer de Tiroides.”
Financiado por: Agencia Estatal de Investigación (MICIN). Año 2020-2023

2 Endocrine and Nervous System Physiopathology

2019-2020

2 Endocrine and Nervous System Physiopathology

Department of Endocrine and Nervous System Physiopathology

The research carried out by the Department of Endocrine and Nervous Systems Pathophysiology focuses on the study of these two key systems which control a large number of key processes and that will very often act together to regulate animal physiology.

To study how these systems operate in physiological as well as in pathological conditions, its research groups apply multidisciplinary research from molecules and cells to whole organisms providing the right expertise needed for both fundamental and translational research. During this period two groups working on biomedical magnetic resonance joined the department, which provided an outstanding addition to the know-how and expertise of its research groups. The extensive scientific collaborations with clinical investigators and other national and international research centers allows for the sharing of the knowledge generated and its translation into the clinical setting.

The following specific research topics are covered:

- Thyroid hormones in the central nervous system during development and adult life;
- Pathophysiology of the thyroid gland and nuclear receptors;
- Regulation of the development, differentiation and function of pancreatic islets;
- Signaling cascades modulated by neurotrophins and myelin formation,
- Molecular basis of hearing function and the pathophysiology of hearing loss;
- Mechanisms of neurodegeneration and neuroprotection taking place in the nervous system including the identification of diagnostic markers and target molecules that will help developing new and efficient therapeutic drugs.

These topics are extremely relevant from a biomedical point of view as they deal with fundamental physiological processes and highly prevalent pathologies with a strong social and health impact.

2019-2020

Role of the Map3k8 and the thyroid hormones in survival and immune response to malaria

PRINCIPAL INVESTIGATOR
Aranda Iriarte, Ana

Keywords: Malaria, Map3K8, thyroid hormones, Plasmodium-infection.

Research Lines

About 200 millions people per year are infected with malaria and, despite efforts of the international scientific community, an effective vaccine is not yet available Therefore, **there is an urgent need for new therapies to fight malaria.**

Recent work of our research team suggests that **both Map3k8 and the thyroid hormones are good candidates to orchestrate the immune response to Plasmodium-infection.**

Map3k8, with capacity to modulate the generation of inflammatory mediators, such as cytokines and chemokines, is required for mounting an effective response to infection of different types of pathogens including other parasites. Map3k8 function has been best studied in the intracellular signaling of the different TLRs, mediating Erk1/2 phosphorylation and fine-controlling the activation state of Akt and JNK. However, Map3k8 also participates in the intracellular signaling of other type of immune receptors.

On the other hand, we have recently demonstrated that the **thyroid hormones alter the sensibility to sepsis and the development of fibrosis** through their capacity to control the activation state of key elements involved in different immune intracellular pathways, such as Erk1/2 and Stat3 phosphorylation following IL6 activation or TGFbeta-induced activation of Smad transcription factors. Moreover, **decreased thyroid hormones levels have been reported in Plasmodium-infected patients**, although it is still uncertain if this represents a defense mechanism or is detrimental for the patients.

In this project **we propose to study how Map3k8 and the thyroid hormones affect the outcome of Plasmodium-infection in experimental malaria in mice, including cerebral malaria, the most severe pathology of this disease.**

We will analyze the survival of Wt, Map3k8-/- mice and mice with different circulating levels of thyroid hormones following infection with P.berghei, which is the lethal murine infective-species that most faithfully reproduces the neurological symptoms observed in humans with cerebral malaria. Brain damage and quantification of brain-infiltrated immune cells will be also evalauted in these animals. We will also examine the role of Map3k8 and the thyroid hormones in the severity of anemia, a hallmark of the disease, and in vivo and in vitro erythropoeisis following infection with P.yoelli, which causes a non-lethal form of malaria infection in mice. Anemia is accompanied by an expansion of myeloid cell populations. Thus, the generation, activation state and function of the different types of blood, bone marrow and splenic immune cells, including monocytes, dendritic cells, macrophages, natural killer cells and T- and B-lymphocytes will be also analyzed in the different groups of P.yoelli-infected animals. The correlation of the affected populations with changes in the expression levels of cytokines and chemokines, which play a key role in the inmune response to malaria will be also examined. We also propose to carry on a variety of in vitro experiments in Wt, Map3k8-/- macrophages treated or not with T3, with the purpose of analyzing the underlying cellular and molecular mechanisms by which Map3k8 and the thyroid hormones could modulate the development and outcome of malaria infection.

Hopefully, we will generate new knowledge that will be useful to develop novel therapeutic strategies to fight malaria.

Publications

Prieto, I, Zambrano, A, Laso, J, Aranda, A, Samper, E, Monsalve, M. (2019). *Early induction of senescence and immortalization in PGC-1α-deficient mouse embryonic fibroblasts.* Free Radic. Biol. Med. 138: 23-32 (PMID: 31029787)

Thyroid Hormone Action in the Brain

Principal Investigator
Bernal Carrasco, Juan

Co-Principal Investigator
Guadaño Ferraz, Ana

Associate Investigator
Morte Molina, Beatriz

Investigator under contract
Salas Lucía, Federico

Keywords: Brain development, thyroid hormones, membrane transporters, neonatal hypotonia, hypothyroidism, gene expression, fetal human development, radial glia, interneurons, Deiodinases, thyroid hormone receptors, single cell genomics.

Research Lines

Relative role of thyroid hormone transporters and deiodinases in the brain action of thyroid hormones

Thyroid hormones cross the blood-brain barrier through integral membrane transporter proteins, the monocarboxylate 8 (MCT8) transporter and the organic anion transporter polypeptide 1C1 (OATP1C1). MCT8 is encoded by the SLC16A2 gene located in the X crhomosome. It is specific for T3, T4, and other thyroid hormone derivatives. Disruption of the gene causes an X-linked syndrome with altered thyroid hormone metabolism and action, and profound neuromotor and cognitive impairement (Allan-Herndon-Dudley syndrome, or AHDS). It is thought that the syndrome is due to deficient transport of T4 and T3 through the blood-brain barrier from fetal stages. OATP1C1 is encoded by the SLCO1C1 gene, located in chromosome 12, with high affinity for T4 but very low for T3. A neurodegenerative syndrome has recently been described caused by mutation in this gene, and the relationship with lack of T4 transport is suspected but not demonstrated.

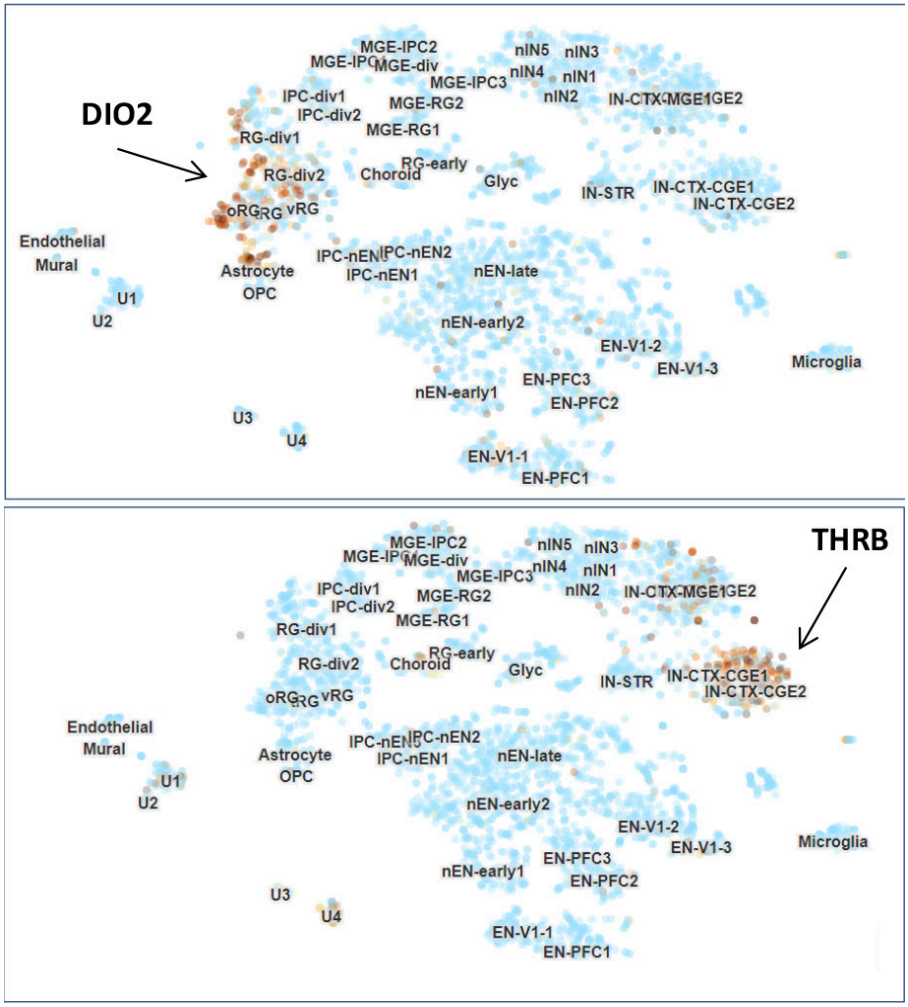
The active thyroid hormone is T3, which binds to nuclear receptors and regulates gene expression. T4, the main hormone produced by the thyroid gland, is a prohormone which generates T3 in tissues by the action of deiodinases. These are selenoenzymes which remove iodine atoms from the iodothyronine molecule generating the active product T3, from T4 (types 1 and 3 deiodinases, or DIO1 and DIO2), or inactive metabolites from T4 or T3 (Type 3 dedinase, or DIO3). Brain T3 derives in part from the circulation, and in part from DIO2-catalyzed, T4 deiodination in the astrocytes. During early development, most brain T3 is derived from T4 which, as stated above, enters the brain through MCT8 and OATP1C1. OATP1C1 is expressed at very low levels in the human blood-brain barrier, and therefore the human brain is strictly dependent on MCT8. In contrast, the rodent brain expresses similarly MCT8 and OATP1C1 so that MCT8 knock out mice do not have neurological phenotype, because lack of MCT8 is compensated by OATP1C1.

Alternative mdels of AHDS have been generated. One, the MCT8 and DIO2 double KO (dKO), in which T3 transport and T3 generation from T4 are compromised. Another, the MCT8 and OATP1C1 dKO, in which T4 and T3 transport is abolished. In either case similar T3 deficiency and brain hypothyroidism should occur. However, a series of theoretical and experimental considerations cast doubts on this model. We compared the effects of the dKO models, and systemic hypothyroidism on gene expression in the cerebral cortex and the striatum using RNA-Seq. We analyzed the following groups: wild type mice, systemic hypothyroid mice after chemical thyroidectomy, MCT8 and DIO2 dKO, and MCT8

and OATP1C1 dKO. The two dKO cause hypothyrodism limited to the cerebrum, whereas systemic hypothyroidism affects all tissues.The results show that the impact of systemic hypothyroidism is much more profound in terms of gene expression than cerebral hypothyroidism. In addition, there is only a partial overlap between the two models of cerebral hypothyroidism. The results obtained by us strongly question current hypothesis on the mechanisms of disease in AHDS.

Analysis of the cellular coexpression of thyroid hormone transporters, deiodinases and receptors in single cells of the human brain

Our research interest is the mechanisms of action of thyroid hormones in the human brain during development. Along this line we are interested in the identification of the thyroid hormone sensitive cells during cortex development. Recently, the accumulation of transcriptomics datasets on single cells isolated from human samples makes it possible to perform in silico studies to analyze the co-expression of thyroid hormone transporters, thyroid hormone receptors, and thyroid hormone activating and inactivating enzymes in single cells, as a mean to identify the sensitive cells. One of these databases was generated recently by Tomasz J. Nowakowski and coworkers (Nowakowski et al, Spatiotemporal gene expression trajectories reveal developmental hierarchies of the human cortex, Science 358: 1318-1323, 2017). This database contains transcriptomics data of 4260 single cortical cells from 73 human fetal subjects, and is deposited in the database on Genotypes and Phenotypes (dbGaP) (https://dbgap.ncbi.nlm.nih.gov/gap/) under the label "STUDIES OF HUMAN DEVELOPMENTAL NEUROGENESIS phs000989.v3.p1."



DIO2 and THRB expression (brown color) in single cells of the developing human cerebral cortex. DIO2 is the enzyme that generates the active thyroid hormone T3 from the T4 precursor by deiodination. It is expressed in clusters of cells with the identitity of radial glia cells and astrocytes. THRB is the thyroid hormone nuclear receptor beta subtype, and is specifically expressed in a cluster of cells with the identity of interneurons. The data were extracted from the databases generated by Novakowski et al. (Science 358, 1318–1323, 2017). The cell clusters were generated by unbiased clustering and weighted gene coexpression network analysis after RNA-Seq of 4261 individual cells. Our analysis indicates that the radial glia is a local source of the active thyroid hormone, and that the thyroid hornone receptor beta subtype is involved in differentiation of interneurons. (RG: Radial glia; IN, Interneurons; NE, excitatory neurons).

We are performing in silico analysis of this dataset. Clustering and differential expression analysis will enable to identify groups of cells showing evidence of regulation by thyroid hormones. For this we will focus on genes involved in thyroid hormone production and transport, their nuclear receptors and genes known to be regulated by thyroid hormones in humans and mice. Some preliminary data indicate that DIO2 is present in radial glia cells that also express OATP1C1. The MCT8 transporter is associated with the thyroid hormone receptor alpha and with many of the T3 target genes. Interestingly the TRbeta is present in a cluster containing interneurons (Fig 1). The data will shed light as to the specific role of thyroid hormones on cortical development and the mechanisms involved.

Publications

López-Espíndola, D, García-Aldea, Á, Gómez de la Riva, I, Rodríguez-García, AM, Salvatore, D, Visser, TJ, Bernal, J, Guadaño-Ferraz, A. (2019). *Thyroid hormone availability in the human fetal brain: novel entry pathways and role of radial glia.* Brain Struct Funct. 224(6): 2103-2119 (PMID: 31165302)

Advances in Biomedical Magnetic Resonance Imaging and Spectroscopy

Principal Investigator
Cerdán García-Esteller, Sebastián

Associate Investigator
Lado Touriño, Isabel

Investigators under contract
Calle Hernández, Daniel
Negri-, Viviana
García Álvarez, Isabel
Lage Negro, Eduardo
Lizarbe Serra, Blanca
Pacheco Torres, Jesús

Predoctoral
Benitez Sánchez del Campo, Ania
Gandía González, Maria Luisa

Support Personnel
Guillén Gómez, María José

Keywords: Magnetic Resonance Imaging, Magnetic Resonance Spectroscopy, Positron Emission Tomography, Contrast Agents, Obesity, Glioma.

Research Lines

Artifical Intelligence Analysis from Magnetic Resonance Images of Hunger Stimulation

We have **designed, implemented and validated a novel image processing strategy to obtain in vivo maps of hunger stimulation in the brain of mice, rats and humans**, combining Diffusion Weighted Magnetic Resonance Imaging (DWI) datasets from fed and fasted subjects.Hunger maps were obtained from axial/coronal (rodents/humans) brain sections containing the hypothalamus and coplanar cortico-limbic structures using Fisher's Discriminant Analysis of the combined voxel ensembles from both feeding situations. These maps were validated against those provided by the classical mono-exponential diffusion model as applied over the same subjects and conditions. Mono-exponential fittings revealed significant Apparent Diffusion Coefficient (ADC)decreases through the brain regions stimulated by hunger, but rigorous parameter estimations imposed the rejection of considerable number of pixels. Theproposed approachavoided pixel rejections and provided a representation of the combined DWI dataset as a pixel map of the "Hunger Index" (HI), a parameter revealing the hunger score of every pixel. **The new methodology proved to be robust** both, by yielding consistent results with classical ADC maps and, by reproducing very similar HI maps when applied to newly acquired rodent datasets. ADC and HI maps demonstrated similar patterns of activation by hunger in hypothalamic and cortico-limbic structures of the brain of rodents and humans, albeit with different relative intensities, rodents showing more intense activations by hunger than humans, for similar fasting periods. **The proposed methodology may be easily extended to other feeding paradigms or even to alternative imaging methods.**

Correlations between Magic Angle Spinning Magnetic Resonance Spectra and Genomic Expression

We used ^{1}H, ^{13}C HRMAS and genomic analysis to investigate regionally the transition from oxidative to glycolytic phenotype and its relationship with altered gene expression in adjacent biopsies through the brain of rats bearing C6 gliomas. Tumor-bearing animals were anesthetized and infused with a solution of [1-^{13}C]-glucose, and small adjacent biopsies were obtained spanning transversally from the contralateral hemisphere (regions I and II), the right and left peritumoral areas (regions III and V, respectively), and the tumor core (region IV). These biopsies were analyzed by ^{1}H, ^{13}C HRMAS and by quantitative gene expression techniques. Glycolytic metabolism, as reflected by the [3-^{13}C]-

lactate content, increased clearly from regions I to IV, recovering partially to physiological levels in region V. In contrast, oxidative metabolism, as reflected by the [4-^{13}C]-glutamate labeling, decreased in regions I-IV, recovering partially in region V. This metabolic shift from normal to malignant metabolic phenotype paralleled changes in the expression of HIF1α, HIF2α, HIF3α genes, downstream transporters, and regulatory glycolytic, oxidative, and anaplerotic genes in the same regions. Together, **our results indicate that genetic and metabolic alterations occurring in the brain of rats bearing C6 gliomas colocalize in situ and the profile of genetic alterations in every region can be inferred from the metabolomic profiles observed in situ by multinuclear HRMAS.**

Advances Contrast Agents for Multimodal Biomedical Imaging based in Nanotechnology

Clinical imaging modalities have reached a prominent role in medical diagnosis and patient management in the last decades. Different image methodologies as **Positron Emission Tomography, Single Photon Emission Tomography, X-Rays**, or **Magnetic Resonance Imaging** are in continuous evolution to satisfy the increasing demands of current medical diagnosis. **Progress in these methodologies has been favored by the parallel development of increasingly more powerful contrast agents.** These are molecules that enhance the intrinsic contrast of the images in the tissues where they accumulate, revealing noninvasively the presence of characteristic molecular targets or differential physiopathological microenvironments. The contrast agent field is currently moving to improve the performance of these molecules by incorporating the advantages that modern nanotechnology offers. These include, mainly, the possibilities to combine imaging and therapeutic capabilities over the same theranostic platform or improve the targeting efficiency in vivo by molecular engineering of the nanostructures. In this review, we provide an introduction to multimodal imaging methods in biomedicine, the sub-nanometric imaging agents previously used and the development of advanced multimodal and theranostic imaging agents based in nanotechnology. We conclude providing some illustrative examples from our own laboratories, including recent progress in theranostic formulations of magnetoliposomes containing ω-3 poly-unsaturated fatty acids to treat inflammatory diseases, or the use of stealth liposomes engineered with a pH-sensitive nanovalve to release their cargo specifically in the acidic extracellular pH microenvironment of tumors.

Biomedical MRI applications of functionalized multi-walled carbon nanotube suspensions

We investigated the magnetic properties of stable suspensions from oxidized Multiwalled Carbon Nanotubes (MWCNT) functionalized with aminopyrene (AP). MWCNT form π–π stacking adducts with AP (AP-MWCNT), originating homogenous, stable, suspensions in N,N-dimethylformamide (DMF) or melted agarose.First, we investigated the magneto-optical properties of these adducts. When applying series of pulsed magnetic fields to nanotube suspensions in DMF, the pattern of light dispersed increased during the magnetic pulse and decreased in the intervals, a behavior consistent with magnetic field induced orientation of the adducts. When adducts were suspended in a melted agarose gel under an external magnetic field, the extinction coefficient of polarized light through the gel, was larger when the polarization plane was parallel to the magnetic field direction. Based on the magneto-optical responses observed, we further investigated the magnetic properties of AP-MWCNT implementing measurements with Superconducting Quantum Interference Device, Zero Field Cooling and Field Cooling, Thermogravimetric and Differential Scanning Calorimetry.Pre-oriented AP-MWCNT suspensions depicted a clear superparamagnetic character with hysteresis loops revealing larger magnetic susceptibility values along their longitudinal axis. In summary, magneto-optical and SQUID measurements revealed that nanotube adducts in suspension, behave as nanoscale compass needles aligning their long axis parallel to externally applied magnetic fields.

Carbonic anhydrase IX is a pH-stat that sets an acidic tumour extracellular pH in vivo.

We used ^{1}H magnetic resonance spectroscopic imaging (MRSI) of the extracellular pH probe imidazolyl succinic acid (ISUCA) to measure and spatially map extracellular pH in HCT116 tumours transfected to express CAIX and empty vector controls in SCID mice. We also measured intracellular pH in situ with ^{31}P MRS and measured lactate in freeze-clamped tumours. CAIX-expressing tumours had 0.15 pH-unit lower median extracellular pH than control tumours (pH 6.71 tumour vs pH 6.86 cont rol, P=0.01). Importantly, CAIX expression imposed an upper limit for tumour extracellular pH at 6.93. Despite the increased lactate concentration in CAIX-expressing tumours, ^{31}P MRS showed no difference in intracellular pH, suggesting that CAIX acidifies only the tumour extracellular space. CAIX acidifies the tumour microenvironment, and also provides an extracellular pH control mechanism. We propose that CAIX thus acts as an extracellular pH-stat, maintaining an acidic tumour extracellular pH that is tolerated by cancer cells and favours invasion and metastasis.

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Physiology of neurotrophins and nuclear receptors in the nervous system

Principal Investigator
Cosgaya Manrique, José Miguel

Co-Principal Investigator
Jiménez Lara, Ana María

Support Personnel
Martínez Crespo, Ana

Undergraduate Students
López Domínguez, David
Juberías Fernández, Lorena

Collaborations
Latasa Sada, María Jesús

Keywords: Neurotrophins, retinoic acid, Innate immune system, Gillain-Barré Syndrome

Research Lines

Regulation of the peripheral myelination process by neurotrophic factors.

Besides neurotrophins, the main group of neurotrophic factors capable of affecting sensory neurons is constituted by GFLs, growth factors originally identified as trophic factors involved in the survival and morphological differentiation of dopaminergic neurons, of which four members have been described to date: the glia-derived neurotrophic factor (GDNF), neurturin (NRTN), artemin (ARTN) and persephin (PSPN).

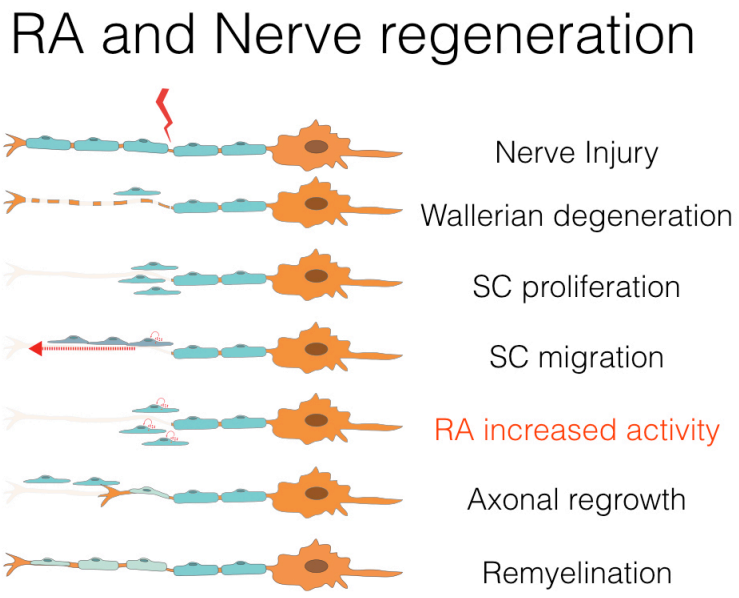
GFLs act through specific binding to the family of glycosylphosphatidylinositol receptors (GFR) α1-4. All of them interact with RET, a tyrosine kinase receptor that is the mediator of transmembrane signaling and is only activated if the ligand is previously attached to its corresponding GFRα co-receptor. Furthermore, GFLs can also use NCAM as a signaling receptor, an adhesion molecule that can act as a receptor activating different signal transduction pathways, such as Fyn kinase, or indirectly through the transactivation of the FGF receptor.

Previous laboratory data confirmed in the literature indicate that GDNF acts on the peripheral myelination process by stimulating myelin formation. Our work has allowed us to extend these studies to other members of this family of neurotrophic factors, as well as to identify the molecular mechanisms by which this effect occurs.

In addition, we have also studied the effect of this family of neurotrophic factors on Schwann cell migration, identifying the receptors and signal transduction pathways involved in the regulation of this process.

Regulation of the peripheral myelination process by retinoic acid.

Several evidences indicate that hormonal agents that act through the superfamily of nuclear receptors are implicated in the regulation of the myelinization process. In the Peripheral Nervous System, studies have so far focused mainly on the effect of steroid hormones, although there are different indications that members of the thyroid hormone/retinoid/vitamin D3 receptor family may also be involved in the myelination process.



Our studies have focused on the regulation of peripheral myelin formation by retinoic acid (RA). Using both in vitro models of Schwann cell cocultures with dorsal ganglion root neurons and in vivo during sciatic nerve development, we have been able to demonstrate a regulatory effect of retinoic acid on peripheral myelination by acting through a double mechanism. On the one hand RA inhibits the expression of MAG, a minority myelin protein for which a signaling role in the interaction between neurons and Schwann cells has been postulated. On the other hand RA is able to produce an increase in the levels of Krox20, a transcription factor that plays a central role in the development and functionality of the Schwann cell. This increase in Krox20 levels results in an increase in the accumulation of myelin proteins such as MBP or P0. As is the case in major human peripheral myelinopathies, this disproportionate increase in the expression of various myelin proteins produces a saturation of the system resulting in a blockage in normal myelin formation. As a side effect, this increase in the accumulation of myelin proteins is accompanied by reticulum stress.

We have continued the studies about the actions of RA focusing in other aspects of the myelination process, namely SC migration and differentiation.

During nerve regeneration, there is an increase in endogenous RA production and signaling. RA produces an up-regulation of NEDD9, a member of the CAS family of scaffold proteins previously implicated in migratory and invasive behavior in gliomas, melanomas and the neural crest cells from which Schwann cells derive. This RA-induced NEDD9 accumulation is due to augmented mRNA levels, as well as an increase of NEDD9 protein stability. Although all NEDD9 phospho-isoforms present in Schwann cells are induced by the retinoid, the hormone also changes its phosphorylation status, thus altering the ratio between the different isoforms.

As a consequence, RA increases Schwann cell migration, while silencing NEDD9 had no effect on basal migratory ability, but completely abrogated RA-induced enhanced migration. Collectively, our results indicate that RA could be a major regulator of Schwann cell migration after nerve injury, thus offering a new insight into peripheral nerve repair.

Currently, we are interesting in elucidating other actions of RA on the physiology of the Schwann cell, basically:
* Schwann cell de-differentiation and re-differentiation during nerve repair.
* Interaction of RA with the Notch signaling pathway in Schwann cells.
* RA-dependent miRNA-mediated regulation of gene expression.

Peripheral neuropathies: Innate immune system and the acute inflammatory demyelinating polyneuropathy (AIDP) Gillain-Barré Syndrome (GBS) variant.

The importance of myelin is showed by the harmful effects that its loss or failure has on the correct function of the nervous system. Examples of diseases that affect proper myelin function in the PNS are Guillain-Barré syndrome (GBS), with one-two cases every 100,000 people or the much more common Charcot-Marie-Tooth disease (CMT) that affects one in every 3,500 people, and multiple sclerosis that is suffered by approximately one in a thousand people in the CNS.

Since the virtually disappearance of poliomyelitis, GBS is the main cause of acute disabling paralysis. GBS is a severe neurological disorder characterized by inflammatory demyelination of peripheral nerves. Patients with GBS develop a rapidly ascending neuromuscular paralysis followed by a loss in sensitivity and pain perception. Many patients have a good recovery, but in severe cases patients need months of intensive care and are left with severe weakness, sensory disorder and pain. Moreover, 5% of patients die due to complications, making GBS a medical emergency with a high morbidity and significant mortality.

Acute inflammatory demyelinating polyneuropathy (AIDP), acute motor axonal neuropathy (AMAN), and acute motor sensory axonal neuropathy (AMSAN) are clinical variants of GBS, principally defined through electrophysiological studies and supported by pathological findings.

While AMAN and ASMAN variants are clearly linked to a loss of immunological tolerance to self-antigens and probably constitute the best example of antigen mimicry with the occurrence of anti-ganglioside antibodies able to bind to some constituents of the outer myelin membrane or the nodes of Ranvier, the same does not hold true for AIDP.

Most of AIDP cases are preceded by viral infection, such as CMV or EBV and, more recently, it was also described an increase of GBS cases after ZIKV outbreaks in French Polynesia, Brazil and Colombia.

We are currently interested in analyzing the implication of the intrinsic Schwann cell antiviral response mediated by the innate immune system in AIDP etiology hoping that this knowledge could provide a starting point for therapeutic intervention in AIDP.

Neuroprotective strategies for neurodegenerative diseases

PRINCIPAL INVESTIGATOR
Cuadrado Pastor, Antonio

ASSOCIATE INVESTIGATOR
Rojo Sanchís, Ana Isabel

POSTDOCTORAL
Escoll Guerrero, María Isabel
García Yagüe, Ángel Juan
Pajares Cabetas, Marta

PREDOCTORAL
Blanco García, Ruth
Fernández Ginés, Raquel
Lastra Martínez, Diego
Robledinos Antón, Natalia

UNDERGRADUATE STUDENTS
Chamoso Sánchez, David
Stokes-, Caroline

MASTER STUDENTS
González Díaz, Alicia

Keywords: NRF2, KEAP1, TAZ, Hippo, WNT, Hedgehog, primary cilia, neural stem cells, gliomas, oxidative stress, neuroinflammation, Alzheimer, Parkinson, neurodegenerative diseases, cancer, Covid-19, diabetes

Research Lines

Aging is the main factor contributing towards both **Parkinson's** (PD) and **Alzheimer's** (AD) diseases. These chronic diseases are incurable and their disabling effects may continue for years or even decades. Studies on animal models of AD and PD and on human postmortem brain tissues, indicate that many pathological changes in the brain derive from a network of local stresses, like **oxidative stress**, tightly connected to **inflammatory and proteotoxic stresses**. Local stressful conditions are probably challenged by pathologically modified proteins, and, through a vicious cycle, may further trigger alteration of key molecules. Our team has been studying **protective mechanisms** used to maintain homeostatic respones and how these mechanims coudl be **targeted pharmacologically** to provide superior defense.

We are currently studying the role of **transcription factor NRF2** in protection against stimuli that induce neurodegeneration. NRF2 is a protein that regulates the expression of about 250 genes. These genes possess the antioxidant response element (ARE) in their promoters. The genes participate in **adaptive responses to oxidative, inflammatory and proteotoxic stress** and in the regulation of enzymes involved in biotransformation and glutathione metabolism.

Using **genetically modified rodent models as well as pharmacological approaches**, we are studying the contribution of this transcription factor to the protection against oxidative damage and neuroinflammation in toxic (MPTP and 6-OHDA) and genetic (**alpha-synuclein**) models of Parkinson's disease and in transgenic mice possessing **amyloidopathy** (APPV717I) and **tauopathy** (TauP301L), which are characteristic of Alzheimer's disease. Objectives:

• **Generation of knowledge:** Understanding the mechanisms that regulate NRF2 is fundamental to determine its physiological role and its pathological alterations as well as to design new pharmacological strategies. We are currently studying the regulation of NRF2 by signaling pathways. We have already described its regulation by the GSK-3/beta-TrCP pathway. We are now analyzing its participation in cell signaling by primary cilium and proliferative stimuli.

• **Low-grade chronic inflammation** is a key element of neurodegenerative diseases. We are studying the crosstalk between **NF-kB and NRF2**, key elements in the pro and anti-inflammatory phenotypes of microglia.

• **Applicability:** in collaboration with several companies, we are looking for novel mechanisms of regulation of NRF2 in the brain that could serve to reinforce its activity against neurotoxic stimuli. In preclinical models of Parkinson's disease, we are focusing on **repurposing of dimethyl fumarate**, a compound already used in clinical practice for multiple sclerosis.

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Neuroprotective peptides in excitotoxicity and stroke

Principal Investigator
Díaz-Guerra González, Margarita

Associate Investigator
Rodríguez Peña, María Angeles

Investigators under contract
Esteban Ortega, Gema María
San Antonio Sánchez, María Esther

Predoctoral
Marqués Pascual, Carlos

Undergraduate Student
Ugalde Triviño, Lola

Master Students
Castillo Ransanz, Lucía
del Río Astorga, Raquel

Collaborations
Iglesias Vacas, Teresa
Massieu, Lourdes
Fahnestock, Margaret
Burlina, Fabianne
Gascón Jiménez, Sergio
Pons, Miquel
Varela Nieto, Isabel
Dreyfus, Cheryl

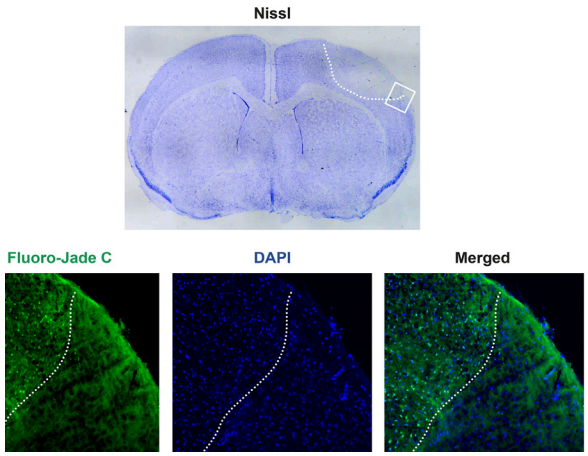
Keywords: Neuroprotection, repair, stroke, excitotoxicity, cell-penetrating peptides, multiple sclerosis, glutamate/NMDAR, BDNF/TrkB, calpain, CREB/MEF2, PSD-95.

Research Lines

Neuroprotective strategies to pharmacologically treat stroke, a prominent cause of death, disability and dementia, have remained elusive. A new approach is restriction of excitotoxic neuronal death in the infarct penumbra through promotion of survival pathways initiated by brain-derived neurotrophic factor (BDNF).

However, boosting of neurotrophic signaling after ischemia is challenged by downregulation of BDNF high-affinity receptor, full-length tropomyosin-related kinase B (TrkB-FL), due to calpain-degradation and, secondarily, regulated intramembrane proteolysis (RIP). Additional mechanisms contribute to aberrant signaling, such as RIP of TrkB-T1, a major mechanism for this receptor isoform producing a BDNF-scavenger, or CREB inactivation.

We have designed several blood–brain barrier permeable cell-penetrating peptide (CPP) able to preserve BDNF/TrkB/CREB signaling. Thus, peptide TFL_{457} contains TrkB-FL sequences that prevent receptor endocytosis and, secondarily, interfere TrkB-FL cleavage. By preserving downstream CREB and MEF2 activities, TFL_{457} initiates a feedback mechanism favoring increased levels of critical prosurvival proteins in excitotoxic neurons. This peptide could be very relevant for stroke therapy since, in a severe model of ischemia, it counteracts TrkB-FL

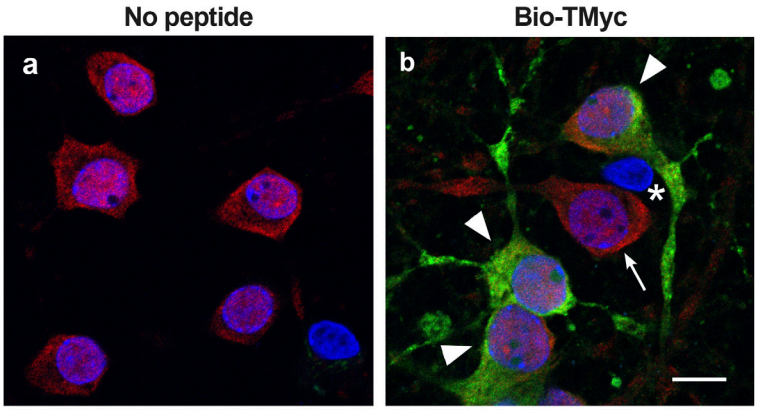


Degenerating neurons identified by Fluoro-Jade C in ischemic mice are located in the infarcted cortex, defined as a hypochromatic area after Nissl staining

downregulation, decreases infarct volume and improves neurological outcome. We are currently developing other CPPs targeted to TrkB-T1 and CREB.

In addition to neuronal death within the gray matter (GM), white matter (WM) injury also takes place in most strokes. Ideal therapies should promote WM repair while neuroprotecting the GM. After injury, BDNF enhances oligodendrocyte differentiation to myelin protein-expressing cells, mediated by TrkB receptors. We want to investigate if, in addition to neuroprotection, TFL457 might also promote oligodendrocyte maturation and reduce WM damage.

We will also challenge the designed CPPs in other diseases associated to reduced BDNF signaling, or cell reprogramming. For example, we will study the effects of combined TFL457 treatment with enhanced BDNF secretion by astrocytes on myelin protein synthesis using models of multiple sclerosis (MS) where the mechanisms of myelin injury is similar to that occurring in stroke.



Fluorescein Avidin/NeuN/DAPI

Entry of a biotin-labeled CPP, detected by Fluorescein Avidin D, into most neurons (arrowheads) present in a primary culture of neurons (labeled with neuronal-specific antibody NeuN) and glial cells (asterik)

Publications

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Funding

"Optimización de péptidos neuroprotectores frente al ictus que atenúan la inactivación en excitotoxicidad de las vías de supervivencia del NMDAR y TrkB." Financiado por: Ministerio de Economía y Competiitividad. Año 2016-2020

"Ayudas Extraordinarias para la preparación de proyectos a realizar en el marco del Plan estatal de I+D+i." Financiado por: Agencia Estatal de Investigación. Año 2020-2020

"Estrategias neuroprotectoras y reparadoras con péptidos que promueven la vía de señalización BDNF/TrkB/CREB." Financiado por: Agencia Estatal de Investigación. Año 2020-2023

Thyroid hormones and central nervous system

Principal Investigator
Guadaño Ferraz, Ana

Co-Principal Investigator
Bernal Carrasco, Juan

Associate Investigators
Bárez López, Soledad
Rausell Tamayo, Estrella
Ausó Monreal, Eva

Investigators under contract
Grijota Martínez, María del Carmen
García Aldea, Angel
Guillén Yunta, Marina

POSTDOCTORAL
Montero Pedrazuela, Ana

Predoctoral
García Aldea, Angel
Valcárcel Hernández, Víctor
Guillén Yunta, Marina
Wang-, Ting
Wang-, Yu

Undergraduate Students
Rojo Pardillo, María
López de Toledo Soler, Inés
Pascual Herrero, Julia

Master Students
Guillén Yunta, Marina
Hidalgo Álvarez
Senovilla Ganzo, Rodrigo
López Carrobles, Nerea

Collaborations
Refetoff, Samuel
Scanlan, Thomas S.
García Verdugo, José Manuel

Keywords: Thyroid hormones, brain, thyroid hormone transport, Allan-Herndon-Dudley Syndrome, MCT8, deiodinase 2, hypothyroidism, blood-brain barrier, brain barriers, thyromimetics.

Research Lines

Our research focuses on:

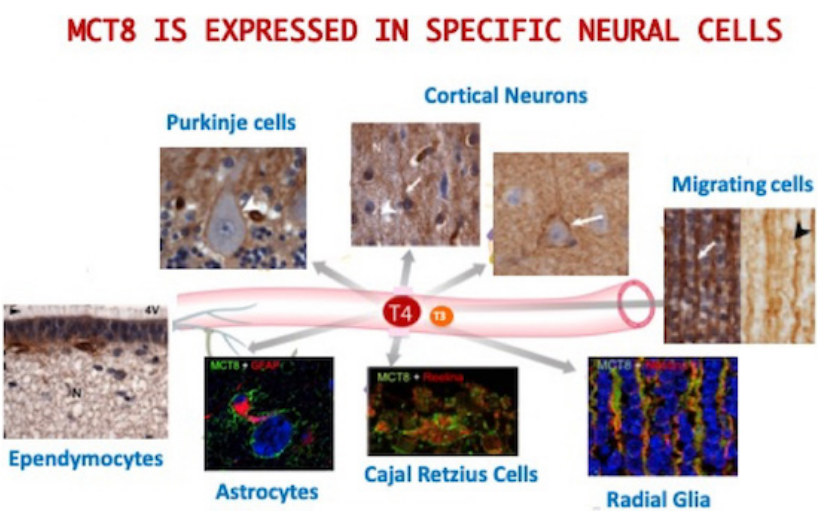
- Understanding the role of thyroid hormones (T4 or thyroxine and T3 or triiodothyronine) in the CNS, both during development and adulthood.
- Characterizatione of the pathophysiology associated with defects in the availability and/or signaling of thyroid hormones in the CNS, with a special interest in the study of rare diseases due to these abnormalities.
    - Specifically, we study the physiopathology of Allan-Herndon-Dudley syndrome associated to mutations in the transmembrane transporter of thyroid hormones known as the Monocarboxylate transporter 8 MCT8 (SLC16A2 gene)
    - The physiopathology of congenital hypothyroidism due to dysfunction of the thyroid gland.
- Finally, we seek the development of possible therapies to alleviate the structural and/or functional alterations in the CNS associated with these rare diseases.

To achieve our goals:

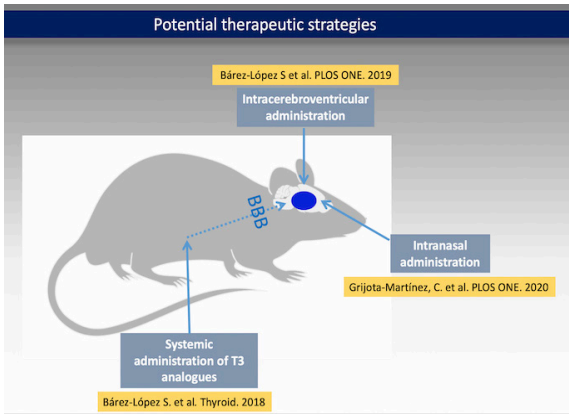
· We analyze the phenotype of several animal models, some of which have been developed in our own laboratory. These models are animals with congenital hypothyroidism, animals deficient in MCT8, the main transporter of thyroid hormones in brain barriers and neural cells, and animals deficient in proteins 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 Allan-Herndon-Dudley syndrome to test the action of different thyroid hormone analogues on thyroid hormone target neural cells under MCT8-deficient conditions.

· We analyze the histopathology of human autopsy brain tissue from patients with genetic diagnosis of Allan-Herndon-Dudley syndrome.



With these studies we want to increase our knowledge of the physiopathology and disease mechanisms in the CNS associated with rare diseases due to defects in thyroid hormone signaling. Our studies aim to characterize possible therapeutic targets and new approaches that favor the development of therapeutic strategies in these diseases. Our research will also contribute to a better understanding of the role of thyroid hormones in brain activity and plasticity.



Research lines

· Allan-Herndon-Dudley Syndrome:
Physiopathology of the Allan-Herndon-Dudley Syndrome (SLC16A2 mutations) and the consequences of thyroid hormone transport deficiency using transporter deficient mice. Development of new therapeutic approaches based on thyroid hormone analogues with alternative transport. Histopathological studies from MCT8-deficient human brains.

· Congenital hypothyroidism and maternal hypothyroxinemia:
Physiopathology of neural alterations due to thyroid hormone deprivation during the fetal and neonatal periods. Influence of maternal thyroid hormones and consequences of maternal hypothyroxinemia on fetal brain development.

· Mechanisms of thyroid hormone availability and action in the brain:
Physiopathology of animal models deficient in important proteins for the neural availability of thyroid hormones. Understanding the modulation of the expression pattern of these proteins at the regional and cellular level during development and at adult stages in the human brain.

Publications

Escalona, C., Vázquez, P., Mera, P., Zagmutt, S., García-Casarrubios, E., Montero-Pedrazuela, A, Rey-Stolle, F., Guadaño-Ferraz, A, Rupérez, FJ., Serra, D., Herrero, L., Obregón, MJ., Valverde, A.M. (2020). *Moderate SIRT1 overexpression protects against inflammation of brown adipose tissue.* Mol. Metab. 101097.

Grijota, MDC., Bárez, S., Ausó, E., Refetoff, S., Frey, WH., Guadaño-Ferraz, A (2020). *Intranasal delivery of Thyroid hormones in MCT8 deficiency.* PLOS ONE. 15(7): e0236113.

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Ordás, P., Hernández-Ortego, P., Vara, H., Fernández-Peña, C., Reimúndez, A.,

Morenilla-Palao, C., Guadaño-Ferraz, A, Gomis, A., Hoon, M., Viana, F., Señarís, R. (2019). *Expression of the cold thermoreceptor TRPM8 in rodent brain thermoregulatory circuits.* J. Comp. Neurol.

Doctoral theses and other works

Angel García Aldea

"Tesis Doctoral: *Pathophysiology of MCT8 deficiency: studies in humans and mice".* Universidad Autónoma de Madrid. Medicina. 2020. Supervisor/s: Ana Guadaño. Calificación: Sobresaliente Cum Laude

Marina Guillén Yunta

"Trabajo Fin de Máster: *Alteraciones de la población glial en el Síndrome de Allan-Herndon-Dudley".* Universidad Autónoma de Madrid. Medicina. 2019. Supervisor/s: Ana Guadaño. Calificación: Matrícula de Honor

Jorge Hidalgo Álvarez

"Trabajo fin de Máster: *Administración intracerebroventricular de TRIAC en ratones Mct8/Dio2 KO como modelo animal del síndrome de Allan-Herndon-Dudley".* Universidad Complutense de Madrid. Ciencias Biológicas. 2020. Supervisor/s: Ana Guadaño. Calificación: 9.7/10.

Funding

"Mecanismos patogénicos en la deficiencia de MCT8: un enfoque multidisciplinar hacia tratamientos basados en el conocimiento." Financiado por: Ministerio de Economía, Industria y Competitividad. Year 2018-2021

"Therapeutics for the Allan-Herndon-Dudley: Assessing new treatment delivery pathways." Financiado por: Sherman Foundation. Year 2018-2020

"Terapias para el síndrome de Allan-Herndon-Dudley: Evaluación de la fisiopatología y nuevos tratamientos." Financiado por: Asociación Corriendo con el Corazón por Hugo. Year 2020-2021

Novel Targets in Neurodegeneration and Neuroprotection

Principal Investigator
Iglesias Vacas, Teresa

Postdoctoral
Sebastián Serrano, Álvaro

Predoctoral
Pose Utrilla, Julia

Simón García, Ana
Sánchez-Miranda Pajuelo, Luis
Clares Pedrero, Irene

Support Personnel
Prudencio Sánchez-Carralero, Marina
González Martín, Ainhoa

López Menéndez, Celia

Undergraduate Student
Sánchez Romero, Javier

Keywords: Protein Kinase D1 (PKD1), Kidins220, Oxidative Stress, Excitotoxicity, Neurodegeneration, Neuroprotection, Stroke, Alzheimer, Huntington.

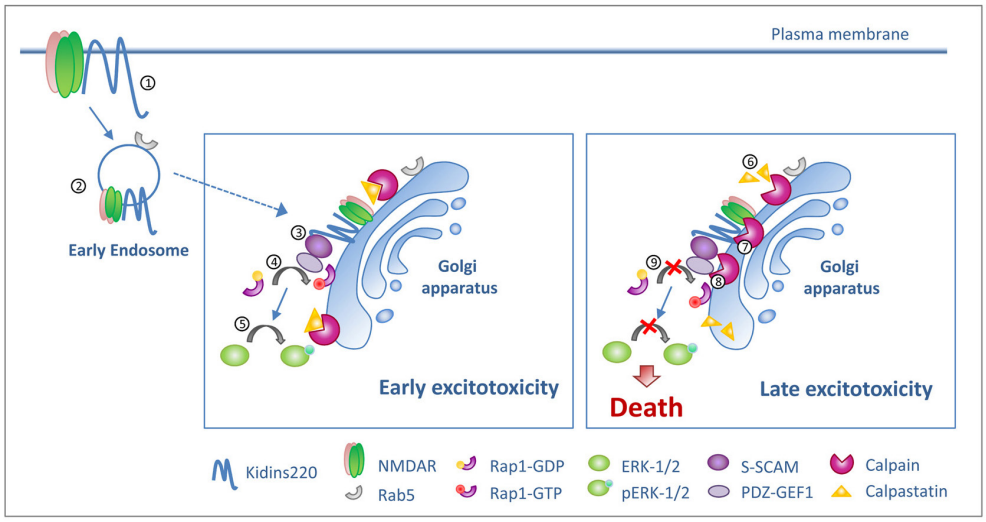
Research Lines

- In our research group, we are investigating the molecular mechanisms involved in neuronal survival. In multiple neuropathologies alterations in the nervous system homeostasis lead to a type of neuronal death known as excitotoxicity, triggered by an excess of the excitatory neurotransmitter glutamate. Excitotoxic neuronal death occurs in many chronic neurodegenerative diseases (such as amyotrophic lateral sclerosis, Parkinson´s disease, Huntington disease and Alzheimer´s disease) and in diseases where damage to the nervous tissue is acute (such as that taking place after stroke or traumatic brain injury). Therefore, knowing the molecules and processes that participate in neuronal survival and how they are altered in excitotoxic conditions could help us to design neuroprotection strategies for a wide variety of neurodegenerative diseases.

- So far, our research has allowed us to identify several neuroprotective pathways that are downregulated by excitotoxicity. One of them is controlled by PKD1 (Protein kinase D1, or PRKD1) and promotes the elimination of mitochondrial free radicals in healthy neurons, thus reducing the risk of neuronal loss by oxidative stress damage (Pose-Utrilla & García-Guerra et al, Nat Commun, 2017). We have developed viral vectors for the expression of a constitutively active mutant of this kinase that greatly enhance neuronal survival in highly excitotoxic environments. To examine whether the neuroprotective potential of these viral vectors is universal, we are using them in preclinical studies with different models of neurodegenerative diseases.

- Another pathway that plays a crucial role in neuronal viability is regulated by Kidins220 (Kinase D interacting substrate of 220 kDa), a PKD1 substrate whose degradation contributes to excitotoxic death. Recently we have been able to establish the cellular processes that precede Kidins220 excitotoxic proteolytic processing and downregulation of this prosurvival pathway, also determining the subcellular compartments and macromolecular complexes involved (López-Menéndez et al, Cell Death Dis, 2019).

- Studying Huntington's disease mouse models and tissue from patients, we have discovered a differential regulation of Kidins220 isoforms in neurons and astrocytes that occurs from early presymptomatic stages of the disease, identifying some of the molecular mechanisms responsible for these pathological changes (Sebastián-Serrano et al, Brain Pathol, 2019). We will continue our research to determine the contribution of the observed modifications to the etiopathology of Huntington's disease, and to design neuroprotective approaches.



Excitotoxicity induces Kidins220 recruitment to the Golgi apparatus and the inactivation of Rap1/ERK complexes compromising this way neuronal survival

- We have generated conditional Prkd1 and Kidins220 deficient mice in different cell lineages and are analysing their phenotype in several neuropathological situations that cause neuronal death.

Publications

Sebastián, Á., Simón, A., Belmonte, A., Pose, J., Santos-Galindo, M., del Puerto, AM., García, L., Hernández, IH., Schiavo, G., Campanero, M., Lucas, JJ., Iglesias, T. (2020). *Differential regulation of Kidins220 isoforms in Huntington's disease.* Brain pathology (Zurich, Switzerland). 30(1): 120-136.

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Martín, C., Chiodo, Y., Jiménez, R., Bernabé, M., Cayuela, ML., Iglesias, T., Campanero, M. (2019). *CDCA7 finely tunes cytoskeleton dynamics to promote lymphoma migration and invasion.* Haematologica.

Gamir, A., Sacristán, S., Medina, M., Iglesias, T. (2019). *Effects of Thioflavin T and GSK-3 Inhibition on Lifespan and Motility in a Caenorhabditis elegans Model of Tauopathy.* J Alzheimers Dis Rep. 3(1): 47-57.

Funding

"Mecanismos moleculares implicados en daño cerebral y neurodegeneración causados por deficiencias en Kidins220 o por eliminación selectiva de PKD1 en neuronas y astrocitos." Financiado por: MINECO. Year 2018-2021

"Targeting CPEB-dependent impaired mitochondrial metabolism and synaptic and stem cell function in Huntington's disease." Financiado por: CIBERNED. Year 2019-2021

"Metabolic basis of Neurodegeneration." Financiado por: COMUNIDAD DE MADRID. Year 2018-2021

Retinoids signaling in cancer

PRINCIPAL INVESTIGATOR
Jiménez Lara, Ana María

MASTER STUDENTS
Franco Caspueñas, Sandra
Menéndez Cámara, Álvaro

COLLABORATIONS
Cosgaya Manrique, José Miguel

Keywords: Retinoids, innate immune signaling

Research Lines

Retinoids and innate immune signaling in cancer

The search for therapies to manage breast cancer constitutes an area of intensive research. Due to its ability to regulate the growth, differentiation and apoptosis of cancer cells, retinoic acid (RA) is considered a signaling molecule with promising therapeutic potential in oncology. Transcriptome analysis using microarrays from breast cancer cell lines treated with RA has revealed a striking regulation of different transcription programs. Among them, Toll-like Receptor 3 (TLR3) arises as a interesting target for RA. We found that RA is able to induce the intrinsic ability of breast cancer cells to recognize double-stranded RNA (dsRNA) through the upregulation of TLR3 expression. RA, co-administered with the dsRNA mimicker polyinosinic-polycytidylic acid (poly(I:C)), synergizes to mount a specific response program able to sense dsRNA through the concurrent upregulation of TLR3, the dsRNA helicases Melanoma Differentiation-Associated Antigen-5 (MDA-5) and Retinoic acid-Inducible Gene-1 (RIG-1), and the dsRNA-activated Protein Kinase (PKR) expression, driving ultimately breast cancer cells to die by a TRAIL (Tumor-Necrosis-Factor Related Apoptosis-Inducing Ligand)- dependent apoptotic program (Cell Death Dis. 2013 Jan 31;4:e479. doi: 10.1038/cddis.2013.5. PMID: 23370279).

In addition, we also found that RA/poly(I:C) co-treatment, synergically, induce the activation of Interferon Regulatory Factor-3 (IRF3) in breast cancer cells. IRF3 activation is mediated by TLR3, since its depletion abrogates IRF3 activation by RA/poly(I:C) co-treatment. Besides induction of TRAIL, apoptosis induced by RA/poly(I:C) correlates with the increased expression of pro-apoptotic TRAIL receptors,

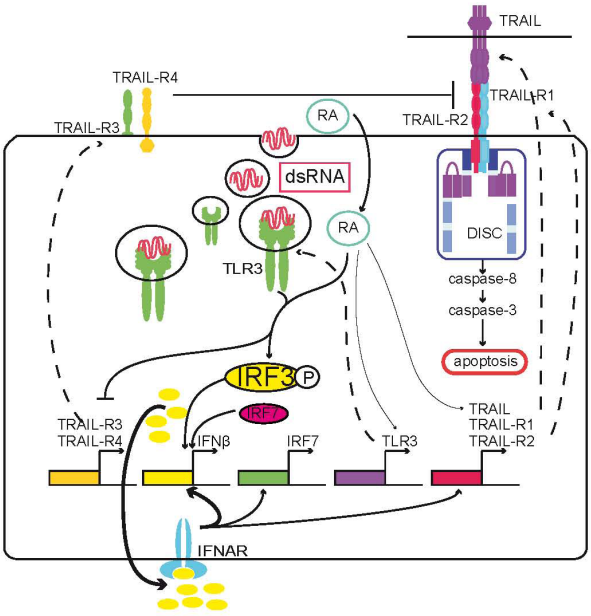

Figure 1. Schematic representation of a proposed model for RA/dsRNA-induced cell death. RA and dsRNA synergize to induce the activation of IRF3, which drives IFNβ expression upregulation. IFNβ triggers autocrine an paracrine circuitries that result in TRAIL death pathways upregulation and cell death

TRAIL-R1/2, and the inhibition of the antagonistic receptors TRAIL-R3/4. IRF3 plays an important role in RA/poly(I:C)-induced apoptosis since IRF3 depletion suppresses caspase-8 and caspase-3 activation, TRAIL expression upregulation and apoptosis. Interestingly, RA/poly(I:C) combination synergizes to induce a bioactive autocrine/paracrine loop of type-I Interferons (IFNs) which is ultimately responsible for TRAIL and TRAIL-R1/2 expression upregulation, while inhibition of TRAIL-R3/4 expression is type-I IFN-independent (Apoptosis. 2017 Jul;22(7):920-932. doi: 10.1007/s10495-017-1377-z. PMID: 28409399). Our findings reveal important links among RA, TLR3, IRF3, type-I IFNs and TRAIL and highlight the combined use of RA and poly(I:C) as a potential effective tumor therapy by improving the apoptotic response of cancer cells with low sensitivity to the action of synthetic dsRNA.

Molecular mechanisms of DUSP1 phosphatase in prostate cancer

PRINCIPAL INVESTIGATOR
Lasa Benito, Marina

INVESTIGATOR UNDER CONTRACT
Cilleros Rodríguez, Darío

PREDOCTORAL
Martínez Martínez, Desireé

Keywords: Prostate cancer, Phosphatase, DUSP1, Metastasis, Tumor biomarker

Research Lines

Prostate cancer is considered the fifth most common type of cancer worldwide. The formation and progression of these tumors is caused by the combination of several events, culminating in the acquisition of an androgen-resistant phenotype, which makes their treatment difficult. Thus, advanced tumors must be treated with chemotherapeutic agents, although, in many cases, they develop resistance to these treatments and become tumors with a poor prognosis. At the molecular level, it is known that prostate tumors present modifications in the signaling pathways that lead to the activation of androgen receptors in the absence of ligand. Alternatively, the progression of this type of tumors is also explained through the regulation of alternative pathways that activate target genes independently of androgens.

The dual specificity phosphatase DUSP1 is an inducible MAPK phosphatase that plays an important role in the formation and progression of different tumors, acting as an anti- or pro-tumor molecule, depending on the tumor etiology. For example, our group, in collaboration with Dr. Aranda´s group (IIBM), has previously shown that DUSP1 inhibits NF-kB and p38MAPK signaling pathways and induces apoptosis in a pituitary tumor cell model. Furthermore, this same interconnection has been demonstrated in collaboration with the group of Dr. Iglesias (IIBM) in a neurotoxicity model. Focusing on prostate cancer, we have previously shown that DUSP1 inhibits NF-kB and p38MAPK signaling pathways and induces apoptosis in prostate cancer cells. In addition, in collaboration with Dr. Angulo (Head of the Urology Service of Getafe University Hospital) and with the research groups of Dr. Toledo, Dr. Ropero and Dr. Chiloeches (University of Alcalá), we have demonstrated that the expression levels of DUSP1 decrease as the degree of malignancy increases in human samples of prostate tumors, in which a negative correlation of the expression levels of DUSP1 with the levels of p65/NF-kB as well as with the activation of p38MAPK is shown.

Considering that a high percentage of advanced prostate tumors develop resistance to treatment, during this period we have focused on studying the implication of DUSP1 in the development of more effective therapies than traditional chemotherapy, based on the combination of the phytoestrogen resveratrol with the chemotherapeutic agent cisplatin. Our data in androgen-independent prostate cancer cells have shown that resveratrol induces the expression of DUSP1, which in turn participates in the inhibition of both the NF-κB pathway and the expression of Cox-2, as well as in the induction of the apoptosis by this phytoestrogen. One of the most important findings of this stage is the demonstration that resveratrol cooperates with cisplatin, both in the positive regulation of DUSP1 levels, and in the promotion of apoptosis, which suggests that this phosphatase is an important determinant of cisplatin sensitivity to apoptosis. These results reveal a novel molecular mechanism, by which resveratrol induces apoptosis in prostate cancer cells, and highlight the importance of DUSP1 in future therapeutic approaches based on the use of this polyphenol and cisplatin

.

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Doctoral theses and other works

Desireé Martínez Martínez

"Nuevos mecanismos moleculares de la fosfatasa DUSP1 como supresor tumoral en cáncer de próstata". Universidad de Alcalá. Medicina. 2020. Director/es: Marina Lasa. Calificación: Sobresaliente Cum laude.

New therapeutic strategies in neurodegenerative diseases: Parkinson's disease, tauopathies and amyotrophic lateral sclerosis

Principal Investigator
Lastres Becker, Isabel

Predoctoral
Castro Sánchez, Sara

Master Students
Galán Ganga, Marcos

Investigator under contract
Arribas Blázquez, Marina

Undergraduate Student
López García, Darío

Keywords: TAU, tauopathies, Parkinson's disease, ALS, mitophagy, oxidative stress, inflammation, proteostasis.

Research Lines

New therapeutic strategies in neurodegenerative diseases: Parkinson's disease, tauopathies and amyotrophic lateral sclerosis

The aging of the population poses a growing burden in society. This is associated with an increase in disability and diseases that have a high impact on health care, on patients and their families. Also, aging is associated with the emergence of different neurodegenerative diseases among which include Alzheimer's disease (AD), Parkinson's disease (PD) and amyotrophic lateral sclerosis (ALS). Therefore, the development of advanced biological markers, new drugs and appropriate technology is the key to establishing a treatment for these diseases, which is currently an important social challenge.

In our laboratory we study the molecular basis of neurodegeneration. The research projects we develop have a multidisciplinary approach that combines basic and translational research, using cell culture techniques, murine models and postmortem samples from patients with AD, PD and ALS.

Currently, **our research is focused on addressing three key aspects of neurodegeneration:**

1) **Proteinopathy:** the accumulation of beta-amyloid plaques and neurofibrillary tangles of TAU protein, involved in neurodegeneration processes, appear in AD. In the case of PD, the alpha-synuclein protein plays a key role in the degeneration of dopaminergic neurons, forming part of the Lewy bodies. And in ALS there is alteration of RNA metabolism and homeostasis. Recent work that connects TDP-43 and FUS to stress granules has suggested how this cellular pathway, which involves the aggregation of proteins as part of their normal function, is altered in ALS. We are interested in determining the role of these proteins in the neurodegeration process.
2) **Inflammation:** it is a process that appears in the first stages of the disease and aggravates neurodegeneration. Alzheimer's and Parkinson's diseases are characterized by what is called chronic low-grade inflammation, so we want to determine what causes this inflammation and how it can be prevented or stopped.
3) **Oxidative stress:** it is imbalance between the production of reactive oxygen species and the ability of a biological system to quickly decode the intermediate reagents or repair the resulting damage. It has been

observed that this imbalance is present in these neurodegenerative diseases, so we want to study what it is that causes it and how to reverse it.

Publications

Castro-Sánchez, S, Zaldivar-Diez, J, Luengo, E, López, MG, Gil, C, Martínez, A, Lastres-Becker, I. (2020). *Cognitive enhancement, TAU phosphorylation reduction, and neuronal protection by the treatment of an LRRK2 inhibitor in a tauopathy mouse model.* Neurobiol Aging. 96: 148-154 (PMID: 33007689).

Martin-Hurtado, A, Lastres-Becker, I, Cuadrado, A, Garcia-Gonzalo, FR. (2020). *NRF2 and Primary Cilia: An Emerging Partnership.* Antioxidants (Basel). 9(6)(PMID: 32498260).

Martin-Hurtado, A, Martin-Morales, R, Robledinos-Antón, N, Blanco, R, Palacios-Blanco, I, Lastres-Becker, I, Cuadrado, A, Garcia-Gonzalo, FR. (2019). *NRF2-dependent gene expression promotes ciliogenesis and Hedgehog signaling.* Sci Rep. 9(1): 13896 (PMID: 31554934).

Galán-Ganga, M, Del Río, R, Jiménez-Moreno, N, Díaz-Guerra, M, Lastres-Becker, I. (2019). *Cannabinoid CB2 Receptor Modulation by the Transcription Factor NRF2 is Specific in Microglial Cells.* Cell. Mol. Neurobiol. (PMID: 31385133).

Galán-Ganga, M, García-Yagüe, ÁJ, Lastres-Becker, I. (2019). *Role of MSK1 in the Induction of NF-κB by the Chemokine CX3CL1 in Microglial Cells.* Cell. Mol. Neurobiol. 39(3): 331-340 (PMID: 30830503).

Castro-Sánchez, S, García-Yagüe, ÁJ, Kügler, S, Lastres-Becker, I. (2019). *CX3CR1-deficient microglia shows impaired signalling of the transcription factor NRF2: Implications in tauopathies.* Redox Biol. 22: 101118 (PMID: 30769286).

Magnetic resonance in the study of the physiopathology of the central nervous system

Principal Investigator
López Larrubia, Pilar

Investigators under contract
Arias Ramos, Nurial

Predoctoral
Guadilla Gómez, Irene

Support Personnel
Arcos Hódar, Javier
Alcalde Gómez, Juan

Undergraduate Students
Campillo Mareen, Basilio Willem
Yagüe Jiménez, Balbino
de Galdo López, Angela
Sánchez Lardín, Celia

Master Students
Veríssimo Cabete, Inês Joao

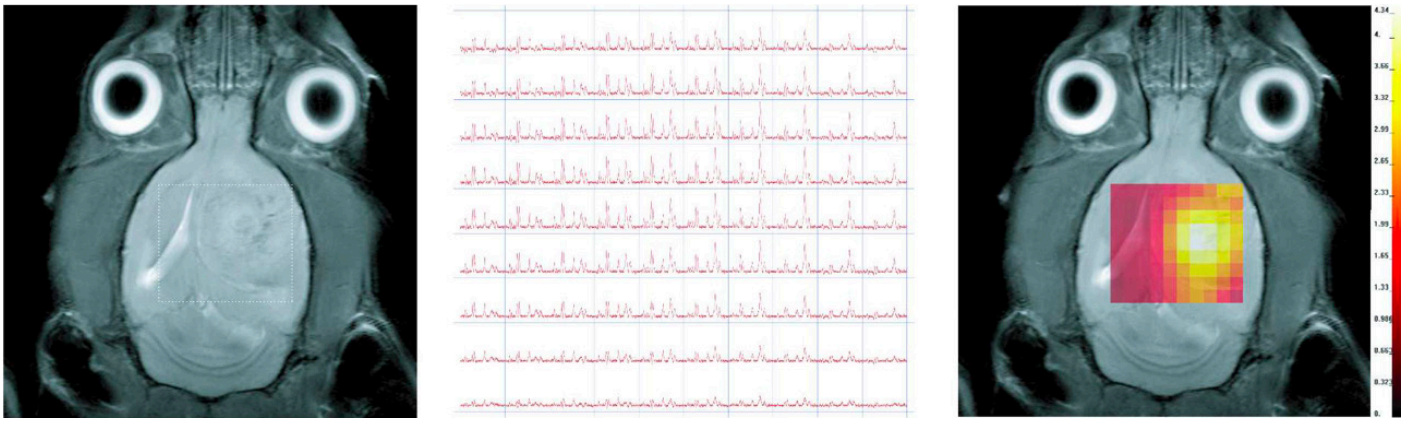
Visiting researchers
Ibarra-, Luis Exequiel
Da Costa Pereira, José Tiago

Keywords: Preclinical Imaging, magnetic resonance imaging, magnetic resonance spectroscopy, central nervous system, inflammation, theranosis.

Research Lines

Multiparametric magnetic resonance characterization of the tumor microenvironment

This line is focused in the **non-invasive characterization** of the main features that define the tumoral physiopathology in brain tumor animal models. The obtained results can be direct and easily translate to the clinical setup to improve



^{1}H-Magnetic resonance spectroscopic imaging of cerebral lactate distribution in the C6 glioma model

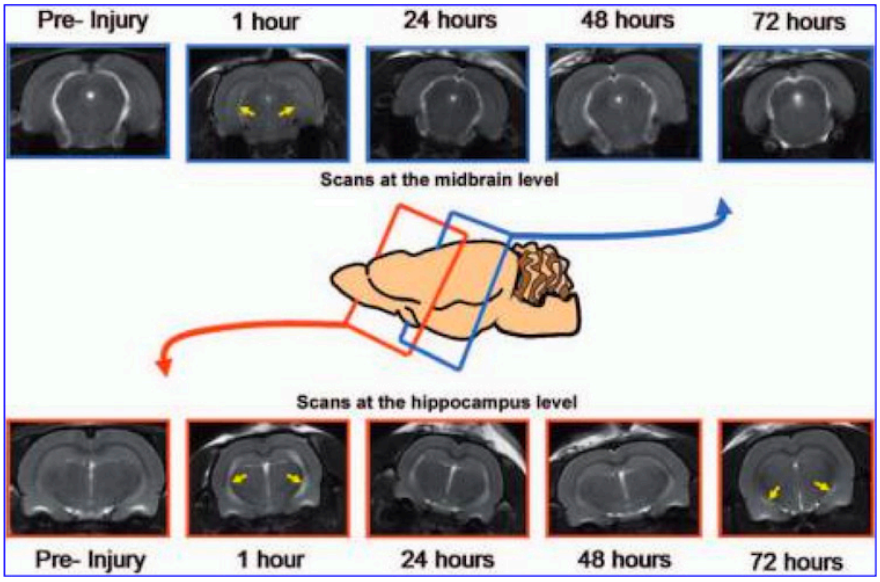
the diagnosis, prognosis and therapy validation of similar pathologies in human beings. The studies will develop a **multiparametric evaluation** supported in **magnetic resonance imaging (MRI) and spectroscopy (MRS)** methodologies to characterize the tumoral microenvironment, validating the results with histologic and genomic evaluation. The last aim

is to identify the **radiomic-histologic-genomic** interaction of high- and low-grade brain tumors orthotopically growing in rats and mice.

Our group also will assess some functional parameters in tumors like capillary permeability, extracellular pH (pHe), oxygen tension (pO_2) and metabolomic profile. The obtained results will be correlated with the expression of genes related to vascularization (VGEF, VGEFR…), hypoxia (HIF1), monocarboxylate and glucose metabolisms (SLC12, SLC16…) and tumoral transformation (TP53, PTEN…) in brain tumors. Finally, a linear discriminant analysis (LDA) will evaluate all the variables to hierarchically select those variables that better discriminate between different tumors types and grades, significantly improving the clinical management of these pathologies.

Identification of biomarkers of brain inflammation

Iammation is the intricate process within the body in charge of overcoming damaging conditions caused by virtually any organism or injury from any traumatic event. Acute inflammation is the body's healthy reaction to traumatized tissues discharging histamine, which in turn causes capillaries to expand and even rupture, maximizing the flow of blood within the affected area and adjoining damaged tissues. However, the **mechanisms of inflammatory responses** in the induction of a wide range of inflammatory diseases or cancer that are manifested in tissues as site-specific conditions are **not well understood**.



Early anatomical ventricles evolution in a traumatic brain injury animal model

Magnetic resonance imaging (MRI) and spectroscopy (MRS) acquisitions are nowdays routinely used for clinical diagnosis and assessment of different diseases like cancer (breast, brain, liver, …), brain injury, rheumatoid arthritis, crown disease, inflammatory muscle diseases, multiple sclerosis, and so on. But the contribution of inflammatory processes to those MR data is not clear. Inflammation can induce a range of different processes all of which are potentially identifiable using magnetic resonance, including alterations in tissue perfusion, changes in tissue water diffusion related to energetic compromise, edema formation and demyelination prodecces, water distribution between different pools, modifications of relaxation parameters, and more recently, the development of molecular imaging techniques that can accurately identify markers of early inflammation by using new contrast agents.

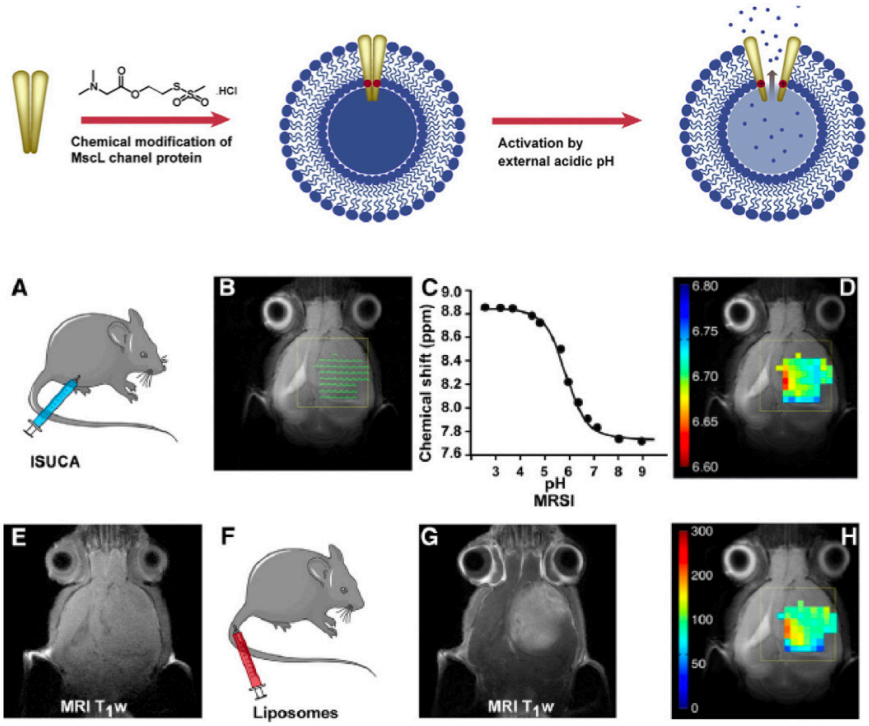
On these grounds, in our groups are working with all these ***in vivo* and *ex vivo* MR** tools to signal the main **inflammation biomarkers** and contribute to a better understanding of the inflammatory response in the body. So, to identify the role played by inflammation either in the development or the outcome of the pathology, we are performing in vivo MRI/MRS and ex vivo MRS studies of different animal models of brain diseases like: **cancer** (glioblastoma), **depression** (chronic mild stress), traumatic brain injury (TBI) or acute brain inflammation induced with lipopolysaccharide (LPS) intracranially injected.

Liposomes as multitarget theragnostic compounds for glioma treatment

Gliomas represent the most common brain tumor with the **glioblastoma multiforme** (GBM) as the most aggressive manifestation. Currently, there is not any curative treatment but palliative strategies that mainly involve the maximal surgical resection, radiotherapy and co-adjuvant chemotherapy, with an overall survival expectation of 12-14 months. The treatments employed usually failed because of the unavoidable adverse drug effects, including tumor resistance and recurrence. In this line we aim to improve both circumstances by using **multitarget strategies** with lower drug doses, effectively targeted to the tumor and non-invasively visualized with magnetic resonance imaging (MRI). For that, we are developing **liposomes containing the therapeutic** of interest (temozolamide, irinotecan, bevacizumab, genitibib…), an MRI detectable **contrast agent** and a structure that enables the theranostic liposome to target and bound the cancer cells.

On these grounds, we want to develop and validate theragnostic multitarget liposomes to be used in the GBM treatment. We work with orthotopic animal models (rats and mice) of high-grade glioma, employing different tumoral cell lines, that are characterize and monitor with in vivo and ex vivo MR methodologies identifying biomarker of the pathology to be used in the therapy validation. As antitumoral multitarget formulation we propose to use liposomes a generic platform including several drugs like alkylating agents, topoisomerase inhibitors or tyrosine kinases.

On the other side, the genomic profile of gliomas has been described recently. Between the most relevant findings, the **IDH1 mutations** linked to a higher survival are specially highlighted, but the relationship of this mutation with the tumoral response to antineoplastic compounds remains without clarify yet. The new **metabolomic tools** accessible by **^{1}H HRMAS** (proton high resolution magic angle spinning) can contribute to a better understanding of this evidence. In order to assess the role of the IDH1 mutation in the response to antineoplastic multitarget treatments, we are working with human biopsies from patients diagnosed with high grade glioma (provided by the Neurosurgery Department, Hospital Universitario La Paz). Our objective is to generate **neutrospheres** from biopsies **IDH1+** and **IDH1-** to be injected in the caudate nucleus of NOD-SCID mice, and perform the same characterization and biomarkers identification previously carried out in animals with glioma murine cells. Also, the multitarget liposomes will be tested in these avatars of human glioma.



Design of liposomes as theranostic platforms in glioma management

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Metelo, Ana M.; Arias-Ramos, Nuria; López-Larrubia, Pilar; Castro, M. Margarida C. A. (2019). *Metabolic effects of VO(dmpp)2 – an ex vivo 1H-HRMAS NMR study to unveil its pharmacological properties.* New J. Chem.,. 43: 17841.

Doctoral theses and other works

Inês Joao Veríssimo Cabete

TFG"Validación de la eficacia de fármacos anticancerígenos: estudio en modelo de glioma en Wistar por MRI". Universidad de Coimbra. Facultad de Ciencias y Tecnología. 2019. Director/es: Pilar López. Calificación: Sobresaliente.

Celia Sánchez Lardín

TFG. "Biomarcadores tempranos del desarrollo de la obesidad en el cerebro del ratón" Universidad Autónoma de Madrid. 2020. Directoras: Pilar López Larrubia y Blanca Lizarbe Serra. Calificación: Matrícula de Honor

Balbino Yagüe Jiménez

TFG. "Magnetic resonance Imaging approaches to follow-up the outcome of a glioblastoma rat model with anti-inflammatory-NSAIDs treatment" Universidad Autónoma de Madrid. 2020. Directoras: Pilar López Larrubia y Nuria Arias Ramos. Calificación: Sobresaliente.

Ángela De Galdo Casado

TFG. "Modelo experimental de glioblastoma: monitorización de terapia Antiinflamatoria por resonancia magnética" Universidad Alfonso X El Sabio, 2019. Directora: Pilar López Larrubia. Calificación: Sobresaliente.

Basilio Willen Campillo

TFG. "Early MRI and MRS biomarkers in mice obesity development" Universidad Autónoma de Madrid. 2019. Directoras: Pilar López Larrubia y Blanca Lizarbe Serra. Calificación: Sobresaliente.

"Imagen Multimodal de la respuesta terapéutica a estrategias multidiana en enfermedades neurológicas. Subproyecto: "Terapias multidiana guiadas por imagen contra el glioblastoma"." Financiado por: Comunidad de Madrid. Año 2018-2021

"Papel de los centros de recompensa cerebral en la regulación del apetito detectada mediante resonancia magnética nuclear ponderada en difusión." Financiado por: Ministerio de Economía y Competitividad. Año 2018-2021

Analysis of human adipose tissue mitochondrial proteome in obesity and type 2 diabetes

Principal Investigator
Peral Fuentes, María Belén

Co-Principal Investigators
Monsalve Pérez, María.
Martínez Valverde, Ángela María

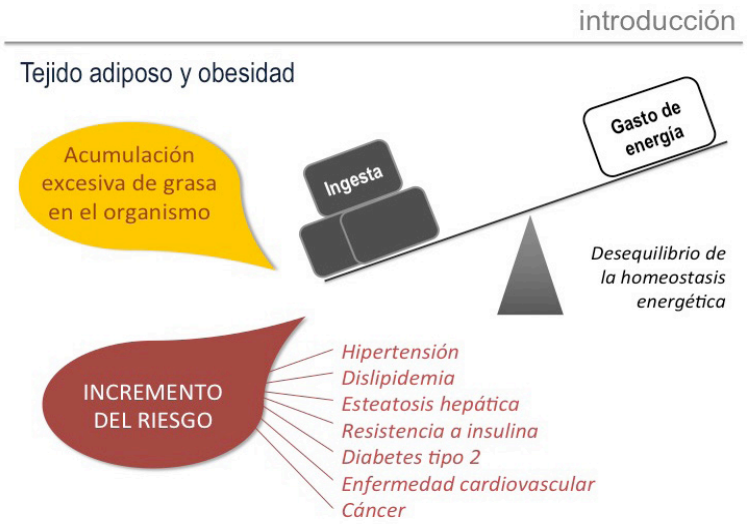
Support Personnel
Urgel Couso, Tamara

Collaborations
Camafeita, Emilio
López, Juan Antonio
Vázquez, Jesús
Rubio, Miguel Ángel
Sánchez-Pernaute, Andrés
Torres, Antonio
Bretón, Irene
Lago, Jesús
Sastre Belloch, Juan

Visiting investigator
Guerra, Liliana

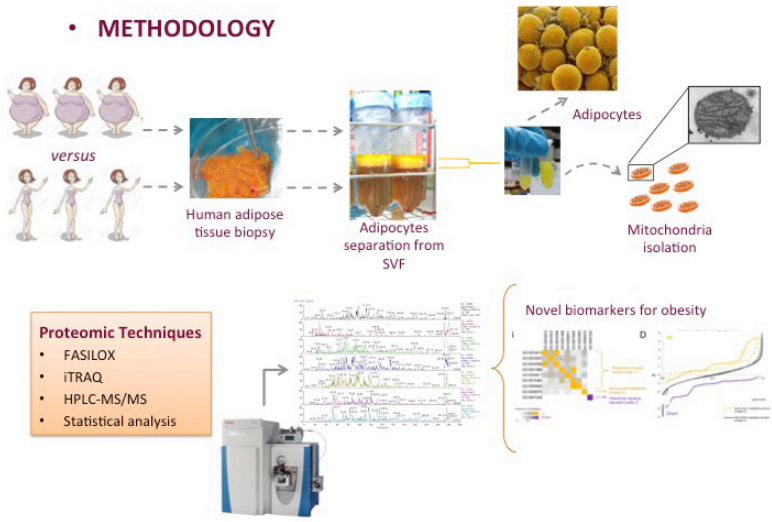
Keywords: Obesity, type 2 diabetes, proteomics, human adipose tissue, mitochondria, oxidative stress, redox signaling.

Research Lines



Main Objective:

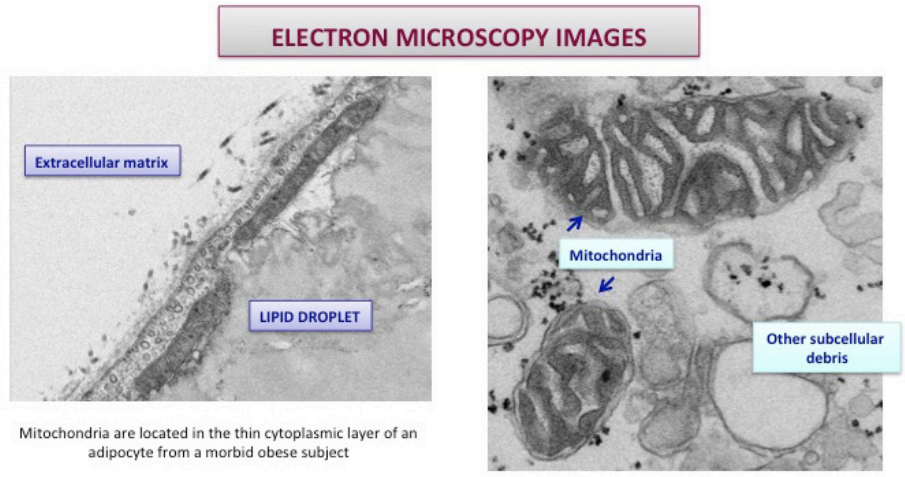
Identify potential therapeutic targets to develop advanced integrative models of proteome regulation and redox signalling pathways, providing a basis for improved redox-based therapeutics in obese patients.



Specific objectives:

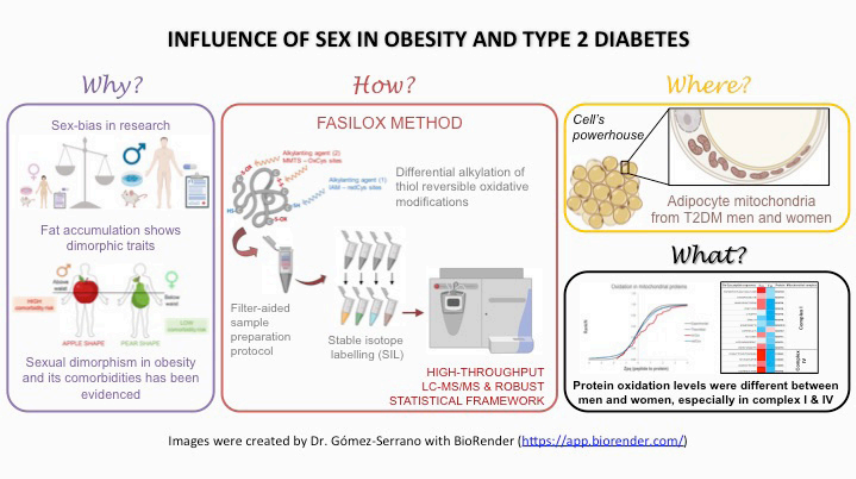
· To investigate the role for adipose tissue mitochondria in obesity and its comorbidities

· To assess the role of oxidative stress in healthy obesity and in obesity linked to severe metabolic and cardiovascular diseases



· To evaluate the effects of sex and aging in adipocyte mitochondrial proteome and redoxome from obese subjects

· To uncover if oxidative phosphorylation (OXPHOS) system could be impaired in morbid obesity



Publications

Bonzon-Kulichenko E, Camafeita E, López JA, Gómez-Serrano M, Jorge I, Calvo E, Núñez E, Trevisan-Herraz M, Bagwan N, Bárcena JA, Peral B, Vázquez J. (2020). *Improved integrative analysis of the thiol redox proteome using filter-aided sample preparation.* J Proteomics. 1;214: 1-6.

Ortega FJ, Moreno-Navarrete JM, Mercader JM, Gómez-Serrano M, García-Santos E, Latorre J, Lluch A, Sabater M, Caballano-Infantes E, Guzmán R, Macías-González M, Buxo M, Gironés J, Vilallonga R, Naon D, Botas P, Delgado E, Corella D, Burcelin R, Frühbeck G, Ricart W, Simó R, Castrillon-Rodríguez I, Tinahones FJ, Bosch F, Vidal-Puig A, Malagón MM, Peral B, Zorzano A, Fernández-Real JM. (2019). *Cytoskeletal transgelin 2 contributes to gender-dependent adipose tissue expandability and immune function.* FASEB J. 33(8): 9656-9671.

Funding

María Monsalve Pérez

"Aplicación de la Medida de la Plasticidad Metabólica al Diagnóstico y Seguimiento de la Respuesta al Tratamiento en Enfermedades Crónicas." Financiado por: MINISTERIO DE CIENCIA, INNOVACIÓN Y UNIVERSIDADES. Año 2019-2021

María Belén Peral Fuentes

"A mi pueblo también vienen las científicas." Financiado por: FUNDACION GENERAL DEL CSIC. Año 2020-2021

Juan Sastre Belloch

"BIOLOGÍA Y MEDICINA REDOX." Financiado por: MINISTERIO DE CIENCIA, INNOVACIÓN Y UNIVERSIDADES. Año 2020-2021

Ángela María Martínez Valverde

"Identificación de biomarcadores metabólicos para enfermedades crónicas y sus tratamientos." Financiado por: Acciones de Dinamización "Europa Investigación 2020" MINISTERIO DE CIENCIA E INNOVACION. Año 2020-2020

Neuroprotective peptides in excitotoxicity and stroke

PRINCIPAL INVESTIGATOR
Rodríguez Peña, María Angeles

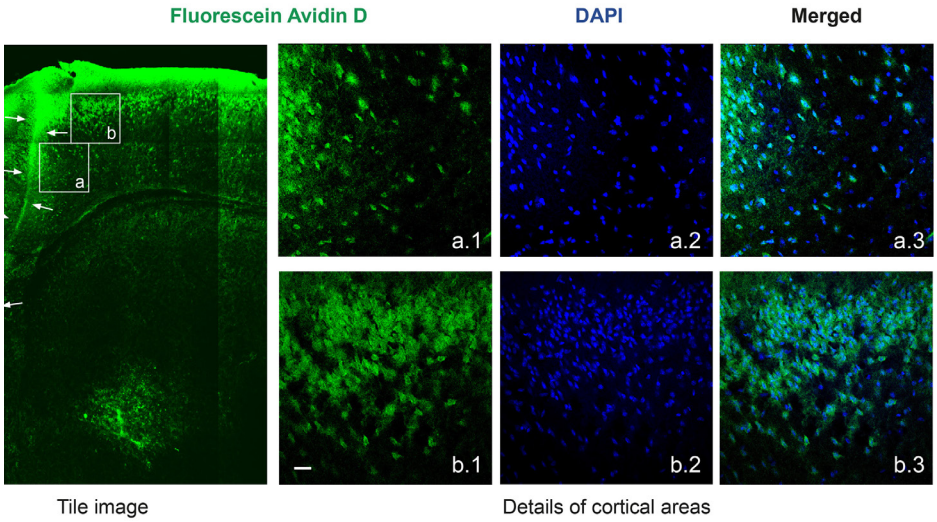
ASSOCIATE INVESTIGATOR
Díaz-Guerra González, Margarita

Keywords: Neuroprotection, repair, stroke, excitotoxicity, cell-penetrating peptides, multiple sclerosis, glutamate/NMDAR, BDNF/TrkB, calpain, CREB/MEF2, PSD-95.

Research Lines

Stroke is the second cause of death worldwide and leading cause of adult disability and dementia. Pharmacological therapies for ischemic stroke (85% of cases) are still limited to thrombolytic drugs, which can be only administered to very few patients.

Cerebrovascular accidents are unpredictable and, therefore, primary death of neurons in the ischemic core cannot be avoided. However, secondary neuronal death progressively affecting the ischemic penumbra might be potentially prevented to reduce brain damage. In order to develop neuroprotective drugs for stroke therapy, we propose:



Tile image of mice cortical and sub-cortical areas showing entry of an intravenously injected biotinylated-CPP into brain

· A comprehensive **characterization of the pathological processes induced by excitotoxicity**, main mechanism of the secondary neuronal death, which subvert pro-survival pathways such as those regulated by neurotrophins and neurotrasmitters.

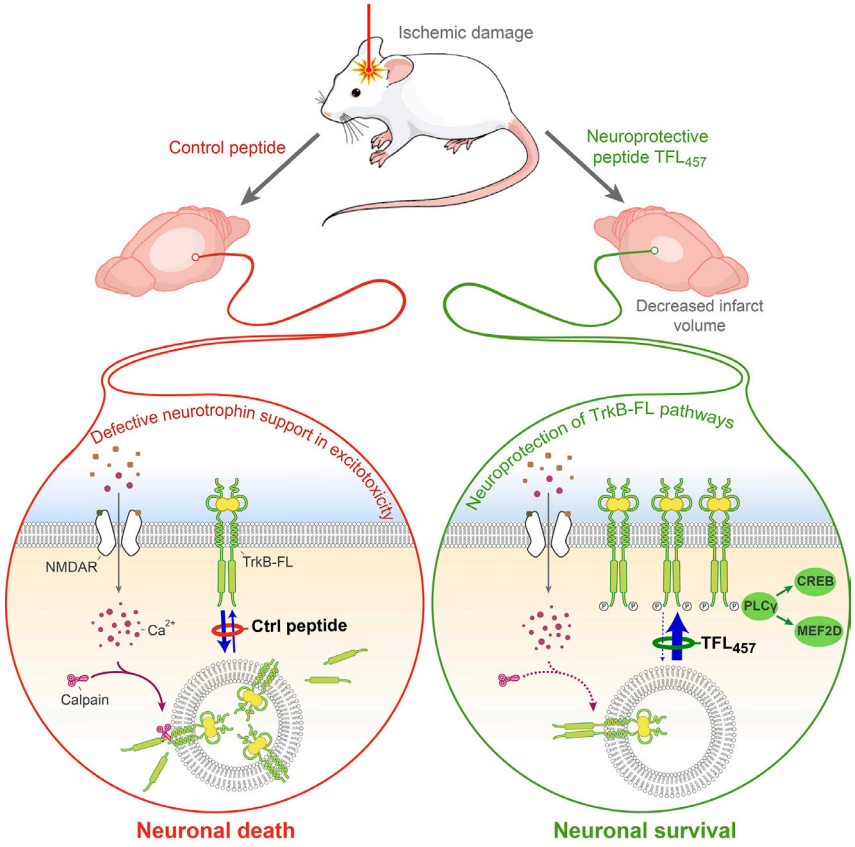
· Selection of rational **targets for stroke neuroprotection** such as increased endocytosis/processing of BDNF receptor TrkB or dephosphorylation/calpain processing of CREB transcription factor, both induced in excitotoxicity.

· **Development and refinement of cell-penetrating peptides (CPPs) able to cross the blood-brain barrier** and preserve functioning of those critical survival pathways in pathological conditions.

· In depth characterization of these **CPPs' mechanisms of action in neurons and/or astrocytes**.

· **Test of these neuroprotective peptides in pre-clinical models of stroke and neurodegenerative diseases** associated to excitotoxicity. The complexity of these pathologies instructs us to combine peptides having different targets or those CPPs with BDNF, TrkB agonists or certain NMDAR antagonists

To accomplish these objectives, we are using primary cultures of cortical neurons and/or astrocytes, animal models of brain ischemia and human samples from stroke patients (plasma and brain necropsias).



Model explaining the mechanism of action of neuroprotective peptide TFL_{457} which prevents excitotoxicity-induced processing of BDNF receptor TrkB-FL

Role of aurora kinase B in epigenetic regulation induced by the hepatitis C virus

Principal Investigator
Sánchez Pacheco, Aurora

Predoctoral
Francisco Recuero, Irene
López López, Ana

Undergraduate Students
Camblor Murube, Marina

Master Students
Ramos Luzardo, Álvaro

Collaborations
Gil García, Ana Isabel
Samaniego Rey, Javier
Perales, Celia
Domingo, Esteban
Robledo, Pedro

Keywords: Aurora Kinase B, fibrosis, cirrosis, hepatocelular carcinome, microbiota, immunotherapy.

Research Lines

MOLECULAR MECHANISMS INVOLVED IN HEPATOCELULAR CARCINOME DEVELOPMENT

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 codified for a threonine residue that contributes to the kinase activity of AURKB, essential in the phosphorylation of P53 and the CHMP4C protein. Thus, the presence of these SNPs could contribute to the development of precancerous lesions in the liver through defects in the cell cycle progression and in the chromosomal segregation and cytokinesis.
Genetic studies performed in our laboratory by next-generation sequencing assays of cell populations in which these variants are expressed, 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

One of the main goals in our laboratory is related to the effects of immunotherapy on the intestinal microbiota population. Biological therapy is a new therapy against cancer which blocks specific targets of tumor cells, among which are immune ckeckpoint 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 and frequently associated with cancer is malnutrition. Recent research seems to indicate that a significant proportion of patients subjected to this type of therapy undergoes a modification in the composition of the intestinal microbiota, associated to biological therapy. The microbiota is essential for proper body growth, with essential functions such as metabolism, the regulation of immunity, as well as 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 the development of side effects at the level of digestive

function. Therefore, in our laboratory we are analyzing by third generation massive sequencing the composition and evolution of the microbiota in cancer patients. These studies are focus to anticipate both the appearance of nutritional disorders that compromise adherence to treatment, and to identify 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 the microbiota, but also how the variation of populations affects the progression of liver disease. The phenomenon of 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 the design of innovative strategies for the treatment of chronic liver disease including HCC.

Doctoral theses and other works

Guillermo Calvo

TFM: Efectos epigenéticos después de la extinción del Virus de la Hepatitis C con antivirales de acción directa en cultivo celular. UCM (2019)

Alvaro Ramos Luzarno

TFM: Identificación de polimormismos (SNPs) de Aurora quinasa B asociados con el desarrollo de cirrosis hepática y carcinoma hepatocelular. UAM (2019)

Ana María Montes

TFG: Análisis de metagenoma en pacientes oncológicos tratados con terapia biológica. Grado Bioquímica UAM (2020)

Ana Marchena Pasero

TFG: Asociación de polimorfismos de Aurora Quinasa B con el desarrollo de carcinoma hepatocelular en pacientes curados de infección por virus de la hepatitis C. Grado Bioquímica UAM (2020)

Funding

"Desarrollo de Kits de detección de miRNAs del epitelio de la mucosa intestinal relacionados con alteraciones en el balance de la microbiota intestinal en pacientes oncológicos en tratamiento". IND2018:BMD9499 Comunidad Autónoma de Madrid (10 Enero 2019- 10 Enero 2023)

Transcriptional control of metabolic homeostasis

Principal Investigator
Vallejo Fdez. de la Reguera, Mario

Predoctoral
Pereira Bouzas, Paula
Pérez Taboada, Iara

Investigators under contract
Mirasierra Cuevas, Mercedes

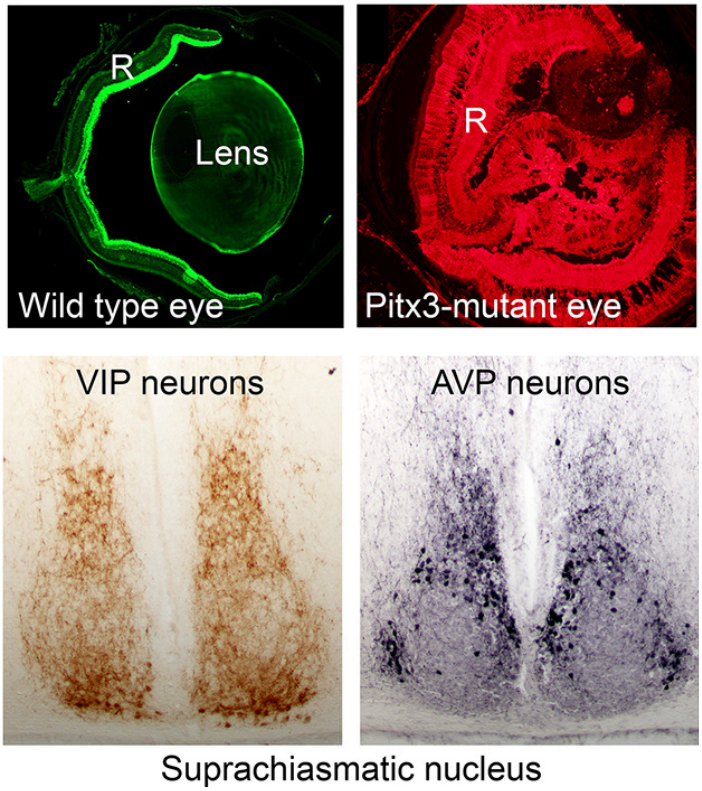
Undergraduate Student
Nuevo Gutiérrez, Paula Virginia

Keywords: Metabolic homeostasis, Diabetes, Pancreatic islets, Arcuate nucleus, Energy expenditure

Research Lines

Our group is interested in the study of the mechanisms that regulate the maintenance of metabolic homeostasis, both peripherally and centrally.

Our studies have shown the importance of the transcription factor Alx3in the regulation of glycemic homeostasis. We discovered that Alx3 is expressed in the pancreatic islets of Langerhans, where it participates in the transcriptional regulation of the insulin gene in beta cells. In subsequent studies we documented that Alx3 deficiency compromises cell survival in pancreatic islets, leading to hyperglycemia and glucose intolerance. We also documented an important regulatory role of Alx3 in alpha cells that express glucagon. In this case Alx3 acts as a sensor of glucose levels in a bimodal mode. When blood glucose levels decrease, Alx3 promotes glucagon gene expression acting on its promoter. On the contrary, when glucose levels raise, Alx3 interacts with the transcription factor Pax6 and inhibits the expression of glucagon. These findings identify mechanisms with a possible etiopathogenic significance in the development of diabetes mellitus. We are pursuing further studies to investigate the mechanisms by which Alx3 coordinately regulates pancreatic islet function to maintain glucose homeostasis.



In the central nervous system, we have investigated the mechanisms by which diabetes affects the function of particularly vulnerable neurons in the brain, increasing the risk of neurodegeneration as indicated by several epidemiological studies. We have focused on Parkinson´s disease using two different mouse models of diabetes corresponding to type 1 and obesity-related type 2 diabetes. Using these models, we have discovered that diabetes induces changes in dopaminergic neurons that translate into altered neurotransmission in the striatum. These changes affect the levels of proteins that regulate stimulus-dependent dopamine release and reuptake, and are associated with increased oxidative stress in the brain. In addition, we demonstrated that dopaminergic neurons in diabetic mice are more vulnerable to neurodegeneration leading to motor impairment.

We are also interested in the study of the mechanisms by which different hypothalamic nuclei regulate the metabolic homeostasis of the organism via the coordinated control of processes such as food intake, energy expenditure, fat distribution and body composition. Initially we focused on the importance of the central regulation of circadian rhythms for the maintenance of peripheral metabolic homeostasis. We used a mouse model in which a spontaneous mutation of the gene encoding the transcription factor Pitx3 caused a congenital eye defect that prevents the innervation of the suprachiasmatic nucleus, the central circadian pacemaker, with retinal axons. As a consequence, cyclic behavioral (including feeding patterns), metabolic and endocrine outputs oscillate out of phase. Importantly, despite synchronization of the endogenous molecular clocks of metabolic organs such as liver and brown adipose tissue, energy expenditure, locomotor activity and corticosterone secretion are resistant to metabolic entrainment by time-restricted feeding. Also at the hypothalamic level, we are pursuing studies to investigate the role of Alx3 expressed in the arcuate nucleus in the regulation of food intake and energy expenditure via modulation of the proopiomelanocortin system.

Publications

Pérez-Taboada I, Alberquilla S, Martín ED, Anand R, Vietti-Michelina S, Tebeka NN, Cantley J, Cragg SJ, Moratalla, R. Vallejo, M. (2020). *Diabetes Causes Dysfunctional Dopamine Neurotransmission Favoring Nigrostriatal Degeneration in Mice*. Movement Disorders 35: 1638-1648.

Ràfols, P., Heijs, B., Del Castillo, E., Yanes, O., McDonnell, L.A., Brezmes, J., Pérez-Taboada, I., Vallejo, M., García-Altares, M., Correig, X. (2020). *rMSIproc: an R package for mass spectrometry imaging data processing.* Bioinformatics. 36: 3618-3619.

Castaño C, Mirasierra M, Vallejo M, Novials A, Párrizas M. (2020). *Delivery of muscle-derived exosomal miRNAs induced by HIIT improves insulin sensitivity through down-regulation of hepatic FoxO1 in mice.* Proceedings of the National Academy of Sciences USA 117: 30335-30343.

Murillo-Cuesta S, Artuch R, Asensio F, de la Villa P, Dierssen M, Enríquez JA, Fillat C, Fourcade S, Ibáñez B, Montoliu L, Oliver E, Pujol A, Salido E, Vallejo M, Varela-Nieto I. (2020). *The Value of Mouse Models of Rare Diseases: A Spanish Experience*. Frontiers in Genetics 36: 3618-3619.

Del Río-Martín, A., Pérez-Taboada, I., Fernández-Pérez, A, Moratalla, R., De la Villa, P., Vallejo, M. (2019). *Hypomorphic Expression of Pitx3 Disrupts Circadian Clocks and Prevents Metabolic Entrainment of Energy Expenditure*. Cell Reports 29: 3678-3692.

Lizarbe, B., Fernández-Pérez, A., Caz, V., Largo, C., Vallejo, M., López-Larrubia, P. Cerdán, S. (2019). *Systemic Glucose Administration Alters Water Diffusion and Microvascular Blood Flow in Mouse Hypothalamic Nuclei- An fMRI Study*. Frontiers in Neuroscience 13: 921.

Doctoral theses and other works

Iara Pérez Taboada

"Neuronal vulnerability of the dopaminergic nigrostriatal system in the presence of metabolic alterations associated with diabetes".
Universidad Autónoma de Madrid. Medicina. 2019. Director/es: Mario Vallejo. Calificación: Sobr.cum laude, mención internacional

Funding

"Mecanismos de control de la homeostasis metabólica y consecuencias de su desajuste."
Financiado por: Ministerio de Economía, Industria y Competitividad. Año 2018-2021

Neurobiology of Hearing

Principal Investigator
Varela Nieto, Isabel

Associate Investigators
Cediel Algovia, Rafael
Contreras Rodríguez, Julio
Magariños Sánchez, Marta
León Alvarez, Yolanda
Lassaletta Atienza, Luis
Zubeldia Ortuño, Jose Manuel

Investigators under contract
Rodriguez de la Rosa, Lourdes

Postdoctoral
López Guerrero, Aida Mª

Predoctoral
Bermúdez Muñoz, José María
García Mato, Ángela
Pulido Sánchez, Sara

Support Personnel
Jareño Flores, Tania
Martín Bernardo, Belen

Undergraduate Students
Sanz Sánchez- Roldán, Almudena
Cuchet Oliver, Elena
López Incera, Marina
de Andrés Álvarez, Javier

Master Students
Anguiano Batanero, Esther
Lara Astiaso, Ester

Undergraduate Students
Mertens-, Melanie

Visiting researchers
Giraldez Orgaz, Fernando

Keywords: Hearing, hearing loss, vestibular schawnnoma, IGFs, cellular senescence.

Research Lines

1. Genetic and molecular basis of hearing loss of different etiology.

1.1 IGF-1 deficiency: a rare syndromic human deafness. Pathophysiology of deficit and haploinsufficiency in IGF-1. Animal and cell models. Response networks to IGF-1. Neuroinflammatory signature and redox balance.

1.2 Genetic predisposition to hearing damage. Genome-environment interaction in animal models of hereditary hearing loss subjected to environmental stress: ototoxic, noise and nutritional deficit. Mitochondrial damage. Cellular senescence during the development of the inner ear and in the progression of auditory pathology.

1.3 Head and neck tumors: vestibular schwannoma.

1.4 Deafblind. Retinal degeneration associated with deficit in the IGF system and changes in its intracellular targets.

2. Identification of new therapeutic targets and biomarkers of progression of hearing loss.

2.1 Regulators of pro-inflammatory kinases p38 MAPK / JNK and their phosphatases.

2.2 Regulators of the gene function of the autophagy catabolic pathways.

2.3 Micronutrients and homocysteine metabolism (hyperhomocysteinemias).

3. Pre-clinical trial of new therapies with small molecules in animal

and cellular models of sensorineural deafness. Co-therapies for the cochlear implant.

3.1 Inhibitors of apoptosis.

3.2 Facilitators of cell survival. Antioxidants

3.3 Development of routes of administration, biomedical imaging and testing of biocompatible vehicles.

3.4 Application and development of the research lines described above to improve the diagnosis and use of cochlear implants.

Publications

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3 Metabolism and Cell Signaling

2019-2020

3 Metabolism and Cell Signaling

Department of Metabolism and Cell Signaling

The Metabolism and Cell Signaling Program integrates studies on how organs and cells adapt and respond to biological and environmental stressors and the diseases arising from the dysregulation of these responses. Cell stressors include pathogenic insults, inflammation, ischemia, nutrient deficiency, genotoxic agents and autoantigens. Our Department focuses on both basic and translational research aiming to: a) understand how cells translate the environmental into biochemical events that trigger specific signaling and metabolic pathways, control gene activity, and modify cell behavior; b) identify potential biomarkers and molecular targets for diagnosis, prevention and therapeutic intervention of human diseases; c) identify and assess at the preclinical level new therapies against different diseases, and d) develop new yeast mutants with industrial interest based on metabolic adaptations.

The Metabolism and Cell Signaling Department brings together investigators with complementary expertise in biochemistry, biotechnology, cellular and molecular biology, immunology, physiology, nanobiology and comparative medicine. Our multidisciplinary research involves the study of diseases ranging from diabetes mellitus, metabolic syndrome, heart failure and Parkinson's disease to autoimmunity, inflammation-driven diseases and cancer. Our goal is to provide new insights and therapeutic avenues for combating these major human diseases.

The department of Metabolism and Cell Signaling has a strong translational vocation, which is reflected in patent applications and multiple collaborations with biotechnology and biomedical companies.

The department is structured into three major research areas embracing different laboratories with complementary goals, aiming to promote collaborations and to maximize resources. These areas are:

Physiopathology of the immune system: Susana Alemany, Lisardo Bosca, Victor Calvo, Antonio Castrillo, Alicia González Martín, Manuel Izquierdo and Juan Manuel Zapata

Complex diseases: Juan Jose Aragon, Carmen Delgado, Jose Gonzalez Castaño, Paloma Martin Sanz, Oscar Martinez Costa, Angela Martinez Valverde and Maria Monsalve.

Metabolism of yeasts: Carlos Gancedo

A brief description of the research performed by the members of the Department in 2019 and 2020 is provided in the following pages.

Impact of the host metabolic state on the inmune response

Principal Investigator
Alemany de la Peña, Susana

Co-Principal Investigator
Aranda Iriarte, Ana

Postdoctoral
Sánchez Sánchez, Angela Mª

Predoctoral
Rodríguez Muñoz, Diego

Keywords: Infection, immune response, metabolic status.

Research Lines

Role of Map3k8 and thyroid hormones in survival and immune response to malaria

Map3k8 regulates the production of inflammatory mediators such as cytokines and chemokines and is required for generating an effective response to different types of pathogens, including parasites. Map3k8 is involved in phosphorylation of Erk1/2 upon activation of different TLRS and modulates the activation state of Akt and JNK, and is also involved in intracellular signalling. We have also shown that thyroid hormones alter susceptibility to sepsis and the development of fibrosis by their ability to control the activation state of crucial elements involved in different intracellular signalling pathways in immune cells, such as Erk1/2 and Stat3 following activation by IL6 or the activation of Smad transcription factors by TGFbeta. In addition, Plasmodium-infected patients show decreased thyroid function, although this is not known whether this is a defense mechanism or detrimental to the patients.

Role of Map3k8 and thyroid hormones on Plasmodium infection in a malaria model

We have analyzed the survival of Wt, Map3k8-/- and thyroid hormone-deficient mice after infection with *Plasmodium berghei*, which is the species that most closely reproduces the neurological symptoms observed in humans with cerebral malaria. In these groups of mice, we have also examined brain damage and immune cell infiltration. We have also examined the role of Map3k8 and thyroid hormones in the severity of anemia, a hallmark of the disease, as well as in erythropoiesis in vivo, following infection with *Plasmodium yoelli*, which causes a non-lethal form of malaria in mice. Since the anemia caused by malaria is accompanied by an expansion of myeloid cells and both these and cells of the lymphoid system play an essential role in the response to malaria, we propose to study the populations of different immune cells including the populations of different immune cells including monocytes, dendritic cells, macrophages, T and B lymphocytes, and NK cells in infected animals.

Based on all these data performed we have concluded that MAP3K8 does not have a significative role in the progression and outcome of cerebral experimental malaria. However, hypothyroidism confers protection to experimental cerebral malaria by a disease tolerance mechanism. Hypothyroid mice display increased survival after infection with *Plasmodium berghei* ANKA, diminishing brain damage, without altering pathogen burden, blood brain barrier disruption or immune cells infiltration. This protection is reversed by treatment with a Sirtuin 1 inhibitor, while treatment of euthyroid mice with a Sirtuin 1 activator induces tolerance and reduces lethality. This indicates that thyroid hormones and Sirtuin 1 are novel targets for cerebral malaria treatment, a major killer of children in endemic malaria areas.

Impact of the metabolic status in the outcome of experimental malaria

Thyroid hormones regulate metabolic state and we have observed that thyroid hormones by altering metabolic state aregulate survival in experimental cerebral malaria, the most severe form of malaria, which is induced in mice by P.berghei infection. Hypothyroidism increases survival in experimental cerebral malaria by regulating the activation state of key metabolic enzymes. Hypothyroid mice do not show significant alterations in motility and respiratory capacity during the course of the disease. At the cerebral level, hypothyroidism protects against inflammation and compression of cerebral arteries and significantly reduces the generation of haemorrhages, key processes involved in death by cerebral malaria. Thyroid hormones, however, do not influence the survival of mice in severe non-cerebral malaria. We are currently studying how thyroid status regulates moderate non-cerebral malaria.

Thyroid hormones as biomarkers in COVID-19

Patients with severe infections present the “euthyroid sick syndrome”, a drop of up to 90% in the levels of circulating thyroid hormones, T3 and T4. It is not clear whether this decrease of first T3 and then T4 in the blood is a defense mechanism of the host or whether it aggravates the patient’s situation even more. Recently, it has been proposed that depending on the type of pathogen causing the infection, euthyroid syndrome may aggravate or worsen the course of the disease. We are currently evaluating parameters of severity, cytokine production, and metabolomic markers in serum from sars-cov-2 infected euthyroid patients and in sars-cov-2 infected patients with an inability to generate thyroid hormones and thereby received T4, which has been maintained during the course of the infection.

Publications

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Funding

“Thyroid hormones as biomarkers in COVID-19.” Financiado por: csic. Año 2020-2021

Macrophage physiopathology and cardiovascular diseases

Principal Investigator
Boscá Gomar, Lisardo

Investigators under contract
Fernández García, Bárbara Victoria
González Ramos, Silvia
Iñigo Jaén, Rafael
Mojena Sánchez, Marina
Paz García, Marta
Povo Retana, Adrián
Prieto Chinchilla, Patricia
Rosales Mendoza, César Eduardo

Support Personnel
Canales Bueno, Natalia
Chaves Coira, Irene
Fernández García, Bárbara Victoria
Gómez Sáez, Ana
Terrón Arcos, Verónica

Master Students
Muñíz Molina, Alejandro
Sánchez García, Sergio

Visiting Investigators
Acosta Medina, Emilio F.
Merino Vázquez, Luis Javier

Keywords: Macrophage, inflammation, atherogenesis, NLRs, mTOR, Immunometabolism.

Research Lines

Crossroad between bioactive lipids and purinergic signalling in macrophages

Cardiovascular diseases (CVD) originated from atheromatous lesions and subsequent plaque rupture is one of the major causes of morbidity and mortality around the World. Macrophages are key components and players in atherogenesis initiation and progression and, even today, at least half of the 'Major Adverse CV Events (MACE)' cannot be ascribed to specific causes (non-culprit MACE). Our aim it to understand the metabolic phenotype of these macrophages to develop new therapeutic strategies to avoid atherothrombotic events. In this line, we envision to integrate the metabolic role of the mammalian target of rapamycin (mTOR), that plays a crucial role in the metabolism of macrophages, with the sensing of 'danger signals' associated to the necrotic environment of the atheroma, in particular the role of the P2Y2/4/6 and P2X7 purinergic receptors, and the fate of the active lipids that accumulate in the course of inflammation. By studying this crosstalk we can establish how macrophage metabolism is controlled by these regulatory nodes, and provide a better understanding on how its manipulation can stabilize the plaque. The ultimate aim is to metabolically re-program macrophages to choose the right phenotype that preserves plaque integrity and stability, attenuating the extension of the lesion and, if possible, favoring regression.

Immunometabolic regulation of macrophage fate: Interaction with graphene layers

The development of new materials such as graphene, with unique properties in its conductivity, allow designing new biomaterials whose expectations for biotechnology, therapeutic and diagnostic use are poorly defined and in full expansion. Our aim is to design graphene:macrophage structures of different natures. The capacity of these biomaterials to integrate efficient remodeling of the extracellular matrix through regulating the functional macrophage polarization based on the control of its electrophysiological properties are a goal that few laboratories have the appropriate tools and

experience for its characterization. We are characterizing the mechanical, physical, chemical and biological properties of graphene: macrophage dual systems.

We also propose to determine its functional response, ability to phagocytose, to remodel the extracellular matrix and to produce bioactive molecules in the cardiovascular field, such as the stabilization of atherogenic lesions, the prevention of cardiac fibrosis and the promotion of heart endo-regeneration. These studies will be performed both in murine and human macrophages, in which we have achieved conditions that maintain a high viability of these cells. These new composite biomaterials will allow a functionalization of the macrophage contributing to the resolution of the inflammation characteristic of the processes in which these cells participate, accelerating the production of the molecules responsible for tissue homeostasis.

The development of new materials such as graphene, with unique properties in its conductivity, allow designing new biomaterials whose expectations for biotechnology, therapeutic and diagnostic use are poorly defined and in full expansion. Our aim is to design graphene:macrophage structures of different natures. The capacity of these biomaterials to integrate efficient remodeling of the extracellular matrix through regulating the functional macrophage polarization based on the control of its electrophysiological properties are a goal that few laboratories have the appropriate tools and experience for its characterization. We are characterizing the mechanical, physical, chemical and biological properties of graphene: macrophage dual systems. We also propose to determine its functional response, ability to phagocytose, to remodel the extracellular matrix and to produce bioactive molecules in the cardiovascular field, such as the stabilization of atherogenic lesions, the prevention of cardiac fibrosis and the promotion of heart endo-regeneration. These studies will be performed both in murine and human macrophages, in which we have achieved conditions that maintain a high viability of these cells. These new composite biomaterials will allow a functionalization of the macrophage contributing to the resolution of the inflammation characteristic of the processes in which these cells participate, accelerating the production of the molecules responsible for tissue homeostasis.

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Prieto, P., Iñigo, R., Calle, D., Gómez, M., Núñez, E., Fernández, M., Martín, P., Alonso, S., Vázquez, J., Cerdán, S., Peinado, MÁ., Boscá, L. (2019). *Interplay between post-translational cyclooxygenase-2 modifications and the metabolic and proteomic profile in a colorectal cancer cohort.* World J. Gastroenterol. 25(4): 433-446.

Patents

"Treatment Of Tlr-4 Mediated Diseases And Conditions With Aptamers Targeting Tlr-4." Año 2019

"Treatment of ischemic stroke with aptamers targeting tlr-4." Año 2019

Study of the mechanisms involved in the polarised traffic of MVBs/lytic granules and their role in the secretion of exosomes by T lymphocytes during the processes of cytotoxicity and activation-induced cell death

PRINCIPAL INVESTIGATOR
Calvo López, Víctor

Keywords: Immune synapse, actin, formins.

Research Lines

Exosomes are small membrane vesicles (nanovesicles) formed by inward budding of the limiting membrane of late endosomes into their lumen. The exosomes accumulate as intraluminal vesicles into secretory vesicles/multivesicular bodies (MVBs). The stimulation of cells from diverse lineages induces the fusion of the limiting membrane of the MVBs with the plasma membrane and the secretion of exosomes (Fig. 1). Several evidences support the hypothesis that exosomes represent a novel modality of intercellular communication, particullarly in the immune system. In the immune system, T lymphocyte activation with antigen through the T-cell receptor (TCR) induces the acquisition of essential effector functions and controls the activation, proliferation and apoptosis of T lymphocytes. In some of these biological responses, which include the cytotoxic activity exerted by cytotoxic T lymphocytes (CTLs), T lymphocyte activation and activation-induced cell death (AICD) processes, the exosomes appear to play an important role. The MVBs from cytotoxic T lymphocytes (CTLs) are called lytic granules (Fig. 1). Upon challenge with antigen, CTLs develop different mechanisms to induce the apoptosis of target cells. Included among these strategies, the inducible expression of Fas ligand (FasL) in lytic granules and its polarized secretion at the immune synapse are thought to be important mediators of CTL-mediated killing. In addition, FasL contributes to the homeostatic control of the T lymphoid compartment that occurs through activation-induced cell death (AICD). Regarding FasL function, it has been shown that the secretion of bioactive (apoptosis-inducer) FasL into exosomes constitutes an important mechanism controlling FasL activity.

Therefore, our aims are:

1) To gain insights into the mechanisms by which T lymphocytes control the polarised traffic of MVBs/lytic granules and regulate the secretion of exosomes.
2) To establish the role of pro-apoptotic exosomes in the cytotoxicity mediated by CTLs and homeostatic AICD. With this knowledge in hand, it will be eventually possible to modulate crucial immune functions such as CTL-mediated apoptosis and the homeostatic control of the T lymphocyte compartment.

Publications

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Funding

"Estudio de las bases moleculares del trafico de los cuerpos multivesiculares y secrecion de exosomas por los linfocitos t t:funcion en la apoptosis y en migracion linfocitarias."

Financiado por: Ministerio de Economía y Competitividad (Madrid). Año 2016-2020

Transcriptional Regulation of macrophage functions by LXR Nuclear Receptors

Principal Investigator
Castrillo Viguera, Antonio

Associate Investigators
Alemany de la Peña, Susana
Sánchez Sánchez, Angela M.
Boscá Gomar, Lisardo

Investigators under contract
Ramón Vázquez, Ana

Predoctoral
Ramón Vázquez, Ana
Celorio Orizaola, Marta
Rodríguez Muñoz, Diego

Keywords: Macrophage, inflammation, gene expression, nuclear receptors, innate immunity.

Research Lines

Macrophages are professional phagocytic cells that play crucial roles in immune processes, but they also perform other important functions in the regulation of metabolism and maintenance of tissue homeostasis. A simple definition of the term “macrophage” is currently a challenge due to the continuous advances in the field, with the classification of their multiple origins, the study of their reprogramming capacities during homeostasis and disease. Indeed, exciting research findings have emerged during the last several years, in which macrophage heterogeneity is now believed to be determined by a combination of signals governed by the cellular origin and others coming from the environment. Our group has studied the transcriptional regulatory circuits that control macrophage behaviour in response to different physiological or pathological situations. A fraction of the transcriptional control of macrophage functions is achieved by the Liver X receptors, LXRαand LXRβ, which are transcription factors that belong to the nuclear hormone receptor superfamily. LXRs are involved in the regulation of cholesterol, fatty acid and phospholipid metabolism. In addition to their role in sterol metabolism, LXRs are important for the immune response against microbial pathogens. LXRα and LXRβ are highly similar in sequence and most of their reported functions are substantially overlapping. During this period of 2017-18, we have expanded our understanding of LXR biology. With a combination of genetically engineered mice, functional assays, expression profiling and ChIP-sequencing data, we paved the path to understand LXR´s cell-specific targets and the specific actions of LXRα and LXRβ in macrophage immune responses. Collectively, our work aims to understand the role of LXR transcription factors in immune processes and their impact in macrophage biological processes.

Publications

Sánchez, AM., Celorio, M., Rodríguez, D., Aranda, A., Castrillo, A., Alemany, S. (2020). *Stress erythropoiesis in atherogenic mice.* Sci Rep. 10(1): 18469.

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Doctoral theses and other works

Marta Celorio Orizaola

"LXR transcription factors in the specialization on tissue resident macrophages and their role in iron homeostasis". Universidad Autónoma de Madrid. Facultad de Medicina. 2020. Director/es: Antonio Castrillo. Calificación: Sobresaliente cum laude unanimidad

Funding

"REGULACION TRANSCRIPCIONAL DEL Metabolism DEL HIERRO, HEMOFAGOCITOSIS Y ERITROPOYESIS POR RECEPTORES NUCLEARES LXR EN MACROFAGOS RESIDENTES EN TEJIDOS."

Financiado por: Ministerio de Ciencia, Investigación y Universidades, y Fondo Europeo de Desarrollo Regional (FEDER). Año 2020-2023

Molecular and cellular regulation of cardiac hypertrophy and heart failure

Principal Investigator
Delgado Canencia, Carmen

Associate Investigators
García-Miguel Piedras, Mª José
Fernández Velasco, María
Gómez Hurtado, Mª Nieves

Predoctoral
Tamayo García, María

Support Personnel
Martín Nunes, Laura

Undergraduate Student
Rios Méndez, Sara

Visiting researchers
Bucchi-, Annalisa
Bertoli-, Giorgia

Keywords: Cardiac hypertrophy, heart failure, ionic channels, patch-clamp technique, vitamin D, calcitriol, paricalcitol, Aril hydrocarbon receptor (AhR), kynurenines.

Research Lines

Myocardial hypertrophy in response to pathological stimuli has traditionally been seen as an adaptive response of cardiac muscle to the altered conditions of haemodynamic load, during which the increase in wall thickness fulfils the function of regaining normal wall stress. However, in the long term, myocardial hypertrophy predisposes individuals to heart failure, arrhythmia and sudden death. This clinical evidence suggests that the hypertrophic process cannot be entirely beneficial. We are actively engaged in attempting to understand the cellular and molecular events that underlie the hypertrophic response in the adult heart in response to pathological stimuli such as pressure overload (hypertension), ischemic injury, obesity or inflammatory mediators.

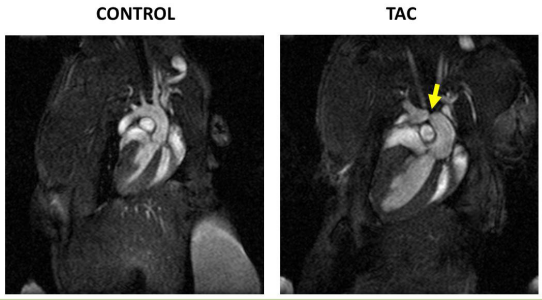
Last stage of cardiac pathologies, heart failure (HF) is a major cause of morbility and mortality. Despite therapeutic improve HF patients 's prognostic is very poor and more than 50% of those patients with severe HF are likely to die within one year. Sudden arrhythmogenic cardiac death is the major cause of mortality of these patients. We are working on elucidate the pathological mechanisms involved in contractile dysfunction and arrthythmogenesis, in order to find new therapeutic targets and develop efficient pharmacology.

Our Research Group has dilated experience and expertise on cellular cardiac electrophysiological techniques (patch-clamp) to study the cardiac channel remodeling that play an important role in the higher risk for cardiac arrhythmias associated with hypertrophied and failing hearts.

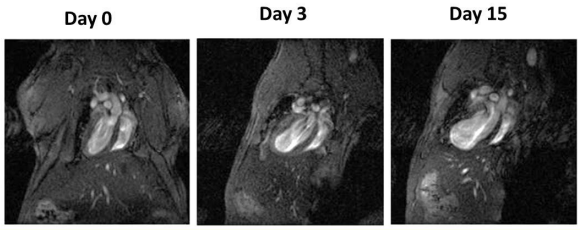
Patch-clamp technique used to record L-type Ca^{2+} current in Ventricular cardiomyocytes isolated from a mouse heart

Cellular and molecular mechanisms underlying the cardioprotective effects of vitamin D

Calcitriol, the bioactive metabolite of vitamin D, exerts its effects through interaction with the nuclear vitamin D receptor (VDR) to induce genomic responses. Calcitriol may also induce rapid responses via plasma membrane-associated VDR, involving the activation of second messengers and modulation of voltage dependent channels. We have investigate the effects of calcitriol on L-type Ca2+ channels, K+channels and intracelular calcium handling (Cai2+) in ventricular myocytes. Furthermore, we have used a model of HF induced by pressure overload to test the hypothesis that the treatment with paricalcitol (vitamin D analogue), prevents the progression of the disease and has cardioprotective effects.



Cardiac magnetic resonance images obtained in one control mouse and in one mouse with transverse aortic constriction (TAC)



Cardiac magnetic resonance images obtained in the same mouse 0, 3 and 15 days after ligation of left anterior descending coronary artery (LAD). The progression of cardiac dilation is evident

Aryl hdrocarbon receptor (AhR) in myocardial infarction

Aril hydrocarbon receptor (AhR) is a ligand activated transcription factor that mediates the toxicity of environmental pollutants. In addition, there is growing evidence suggesting that AhR has normal physiological functions and that it likely has endogenous ligands. In recent years, the AhR endogenous ligand L-kynurenine has been recognized as a biomarker that could influence cardiovascular diseases. In the present project we propose to integrate both the biology of kynurenines and AhR to gain insight on new mechanisms that can be involved on adverse ventricular remodeling after experimental myocardial infarction with the kynurenine pathway emerging as a new target for drug development.

Publications

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Doctoral theses and other works

María Tamayo García

"Mecanismos implicados en el efecto cardioprotector de la vitamina D". Universidad Autónoma de Madrid. Facultad de Medicina. 2020. Directoras: Carmen Delgado, María Fernández-Velasco. Calificación: Sobresaliente Cum Laude.

Funding

"Efectos del Paricalcitol, un activador del receptor de vitamina D, sobre el acoplamiento excitación-contracción cardiaco y el remodelado eléctrico deletéreo arritmogénico en un modelo experimental de insuficiencia cardiaca en el ratón." Financiado por: Sociedad Española de Cardiología. Año 2018-2022

"Papel del receptor de hidrocarburos de arilos (AhR) en el infarto agudo de miocardio." Financiado por: MINECO. Año 2018-2021

N-acetylglucosamine kinase from Yarrowia lipolytica as a moonlighting protein

PRINCIPAL INVESTIGATOR
Gancedo Rodríguez, Carlos

POSTDOCTORAL
Flores Mauriz, Carmen Lisset

UNDERGRADUATE STUDENT
Pérez Arroyo, Irina
San Martín Álamo, Clara Isabel
Bori Handschuh, Tania
Bravo Pareja, Nicolás
Chavez Izasa, Stephania

Keywords: Moonlighting, N-acetylglucosamine, Yarrowia.

Research Lines

Moonlighting proteins are an outstanding subgroup of the multifunctional proteins family. They have become an important object of study as their properties impinge on several areas of biology. For example, it has been estimated that up to 78% of candidate moonlighting proteins are associated to a human disease, in contrast to a ca.18% disease association for proteins in general. The mechanisms by which moonlighting proteins exhibit multiple functions differ from case to case.

We are studying the moonlighting role of the N-acetylglucosamine (NAGA) kinase from Yarrrowia lipolytica in the regulation of the transcriptionof the genes encoding enzymes of the N-acetyl glucosamine catabolic pathway. Besides measuring the effects of the expression of heterologous NAGA kinases from different organisms (Homo sapiens, Candida albicans, Magnaporthe grisea and Arabidopsis thaliana)on the transcription of those genes we have initiated a study using aleatory mutations in the gene encoding the protein in Y. lipolytica. The strategic goal is to separate the catalytic and the regulatory functions of the protein. The tactic is the following one: to construct a strain with a fusion of the promoter of YlNAG5, the gene encoding the kinase, fused to lacZ inserted in the genome of aYlnag5 mutant. This strain was transformed with a library of randomly mutated YlNAG5 and subjected to screening in different media using the lacZ gene as a reporter. To this practical end, an improved, rapid permeabilization method for Y. lipolytica was developped.

In several independent regulatory mutants so far characterized in a first screening, mutations in similar positions have been found, e.g F320S, N69Y, etc., pointing to a role of these amino acids in the moonlighting function. The effects of single and multiple mutations are being studied. It ought to be noted that in some cases it has been reported that more than one amino acid substitution is required to eliminate the moonlighting function.

We have detected the appearance of an spontaneous mutation that interferes with the derepression of the NAG genes and are trying to clone the responsible gene.

In addition we have cloned some uncharacterized genes that might have a bearing in the regulation of the transcription of the genes encoding the enzymes of the N. acetyl glucosamine catabolic pathway.

Publications

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Funding

"Interacciones entre la proteina N-acetil glucosamina kinasa y diferentes circuitos genéticos reguladores en Yarrrowia lipolytica."
Financiado por: Fundación Ramón Areces.
Año 2017-2020

Proteostais and neurodegerenation

PRINCIPAL INVESTIGATOR
González Castaño, José

PREDOCTORAL
Sánchez Lanzas, Raúl

Keywords: Neurodegeneration, parkinson's disease, proteosatasis, protein degradation, ubiquitin-proteasome, autophagy, alpha-synuclein, PARK7/DJ-1.

Research Lines

Mechanism of degradation of SMN and SMN delta7 implicated in Spinal Muscular Atrophy

Spinal muscular atrophy (SMA) by loss of lower motor neurons and atrophy of muscle is the leading genetic cause of infant mortality. Spinal muscular atrophy is due to mutations affecting the SMN1 gene coding for the full-length protein (survival motor neuron; SMN) and the SMN2 gene that preferentially generates an exon 7-deleted protein (SMNΔ7) by alternative splicing. We have studied SMN and SMNΔ7 degradation in the cell,using tagged versions at the N- (Flag) or C-terminus (V5) of both proteins. Transfection of those constructs into HeLa cells and treatment with cycloheximide showed that those protein constructs were degraded. Proteasomal degradation usually requires prior lysine ubiquitylation. Surprisingly, lysine-less variants of both proteins tagged either at N- (Flag) or C-terminus (V5) were also degraded. The degradation of the endogenous SMN protein, and the protein constructs mentioned above, was mediated by the proteasome, as it was blocked by lactacystin, a specific and irreversible proteasomal inhibitor. We have also tried by cotransfection of wild-type or K0 SMN constructs with HA-ubiquitin to detect both canonical and non-canonical ubiquitylation sites like Ser/Thr/Tyr or Cys, but no ubiquitylation was detected even in the presence of proteasome inhibitors. These results indicated that degradation of SMN and SMNΔ7 does not absolutely require usual internal Lys or N-terminal ubiquitylation for degradation. Another critical point, overlooked previously, is that tagging at the N-terminus of a protein also blocks its N-terminal acetylation. In fact, the N-terminal of SMN (and likely of SMNΔ7) has been shown to be acetylated at two positions in acetylome studies (30). The N-terminal sequence of SMN is MAMSS (acetylated residues indicated with bold characters). The acetylation observed in SMN proteins by MS studies is either at the N-terminal Ala2 residue, exposed after removal of the N-terminal Met by methionine aminopeptidase or at Ser4. Ser4 acetylation is likely due to a translational initiation of SMN protein at Met3, leaving Ser4 at the N-terminus after Met3 removal by the aminopeptidase. Accordingly, SMN (and likely SMNΔ7) could be a substrate of the N-terminal acetylated N-rule pathway of degradation mediated by Doa10/March6 in a physiological context and tagging at the N-terminus prevents N-terminal acetylation. The use of the tagged of wild type and mutant proteins for the study of the physiological relevant mechanisms of the degradation of SMN and SMNΔ7 is inadequate, as those tagged constructs are unlikely to match all post-translational modifications and interactions of the natural cell endogenous SMN proteins. To untangle the problem of SMN turnover, deep proteomic techniques have to be developed for the study of the turnover of the components of SMN (orphan and bound) complex, in order to get the basic physiological mechanisms of degradation of steady state SMN protein and its regulation. Eventually, these studies will allow the development of strategies aimed to increase SMN expression levels by regulation of its degradation as a possible therapeutic intervention for SMA patients.

Role of lysosomal and chaperone mediated autophagy in the degradation of DJ-1/PARK7, a protein implicated in Parkinson's disease

We have shown previously, using a lymphoblastoid cell line from a Danon patient, that the steady-state mRNA and protein levels of alpha-synuclein, IKBα, Rcan1, and glyceraldehyde-3-phosphate dehydrogenase, four proteins reported to be selective substrates of the chaperone mediated autophagy (CMA) pathway, were similar in control and Lamp-2-deficient cells. Furthermore, inhibition of protein synthesis showed that the half-life of alpha-synuclein, IKBα, and Rcan1 was similar in control and Lamp-2-deficient cells and its degradation prevented by proteasome inhibitors. We have now extended those results with the study of the role of lysosomes and the CMA pathway in the degradation of DJ-1/PARK7, whose mutations are associated with familial autosomal recessive Parkinson disease, moved by a recent publication reporting that those pathways participate in tits degradation. Using several cell lines with disrupted LAMP2 gene expression and their respective control cells, we show that Interruption of LAMP-2 expression did not result in an increase of the steady-state protein levels of DJ-1 /PARK7, as it would have been expected. Furthermore, no change in DJ-1 /PARK7 protein levels were observed upon inhibition of lysosomal function with NH4Cl or NH4Cl plus leupeptin, or after activation of CMA by serum starvation for 24h. Accordingly, we have found no evidence that DJ-1 /PARK7, or alpha-synuclein, (both proteins are implicated in Parkinson's disease) protein levels are regulated via lysosomal degradation or the CMA pathway.

Doctoral theses and other works

Raúl Sánchez Lanzas

"Degradation pathways of α-synuclein and DJ-1 / PARK7 proteins involved in the pathogenesis of Parkinson's disease".
Facultad de Medicina. Universidad Autónoma de Madrid. 2019. Sobresaliente Cum Laude

Funding

José González Castaño
Competitive Project (Program: Autonomous Community of Madrid. Developed in: UAM. Ref: S2017 / BMD-3700) *Metabolic bases of Neurodegeneration.* Duration: 01-01-2017 - 12-30-2021.

José González Castaño y Ana Pérez Castillo
Proyecto Competitivo (Ministerio de Economía, Industria y Competitividad, SAF2017/ 88885-R). *CCAAT/Enhancer binding protein β (C/EBPβ) como modulador de la neuroinflamación. Una nueva diana terapéutica en la enfermedad de Parkinson.* Duración:01/2018 - 12/2020.

MicroRNA control of immune tolerance, autoimmunity and cancer

Principal Investigator
González Martín, Alicia

Postdoctoral
Arribas Blázquez, Marina

Predoctoral
Bartolomé Cabrero, Rocío
Gámez Reche, Laura

Undergraduate Students
Prieto Muñoz, Ana María
Sanz Gallardo, Javier

Master Students
Cooper-, Gillian
Lorenzo López-Cortón, Beatriz

Visiting researchers
Manzano Franco, Diana
Falco-, Amel

Keywords: Immune tolerance, miRNAs, autoimmunity, tumor immunology, cancer, genome engineering, chemokine receptors.

Research Lines

Our laboratory is interested in understanding the cellular and molecular mechanisms of immune tolerance, autoimmunity and cancer. Specifically, we focus on studying how microRNAs (miRNAs) and their target genes regulate immune tolerance, autoimmune diseases and antitumor immunity. In addition, we are actively developing innovative genome engineering strategies for therapeutic purposes.

MicroRNAs have recently emerged as important factors in the post-transcriptional control of protein concentrations in metazoan organisms. For the past few years, we studied the functions of miRNAs in the mammalian immune system. We identified the first miRNA that regulates B cell tolerance and established its causative role in the development of lethal autoimmunity (Gonzalez-Martin et al, Nature Immunology, 2016 Apr;17:433-40). We also discovered critical roles for other microRNAs in different immune tolerance mechanisms and autoimmune diseases (Gonzalez-Martin and Lai et al, Nature Communications, 2016 Aug 2;7:12207, Ichiyama et al, Immunity, 2016 Jun 21;44:1284-98 and Liu et al, Journal of Experimental Medicine, Aug 22;213:1901-19). In addition, we developed the first B cell receptor reprogramming strategy using the latest genome editing technologies (Elife, 2019 Jan 17;8). Previously, work on tumor immunology established an important role for the chemokine receptor CCR5 in T cell antitumor responses (Gonzalez-Martin et al, Cancer Research, 2011 Aug 15;71:5455-66). Overall, our studies have established miRNAs as critical regulators of immune tolerance and autoimmunity, and revealed new mechanisms controlling antitumor immunity.

Current research in the lab continues to identify and study the roles of miRNAs and their target genes in immune tolerance, autoimmunity and tumor immunology by combining genetic, genomic, biochemical, and functional screen approaches to understand the functions and molecular mechanisms of miRNA control at molecular, cellular, and system levels. The mechanisms identified might provide valuable biomarkers or therapeutic targets for the treatment of autoimmune diseases and for cancer immunotherapy.

Publications

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Funding

"Identifying novel targets for cancer immunotherapy." Financiado por: Fundación Científica de la Asociación Española Contra el Cáncer (FC AECC). Año 2020-2023

"Functional analysis of microRNAs in immune tolerance and autoimmunity." Financiado por: Ministerio de Ciencia, Innovación y Universidades. Año 2019-2021

"Achieving Long-Term Humoral Protection Against HIV and other Antibody Resistant Pathogens." Financiado por: Bill and Melinda Gates Foundation. Año 2019-2022

"Nuevos reguladores de la tolerancia inmunológica de linfocitos B." Financiado por: Comunidad de Madrid. Año 2020-2022

"XIII Edición Premios de Investigación L´Oréal-UNESCO." Financiado por: L´Oréal and UNESCO Foundations. Año 2018-2019

"I Ayuda para investigadores nóveles Apadrina la Ciencia-VWR." Financiado por: Fundación Apadrina la Ciencia-VWR. Año 2018-2019

"Ramon y Cajal National Program." Financiado por: Ministerio de Ciencia, Innovación y Universidades. Año 2018-2023

Patents

"B cell receptor modification in B cells. International Patent, WO2019/028417." Año 2019

Awards

"Premio de Investigación L´Oréal-UNESCO. Fundación UNESCO y Fundación L´Oréal, 2018/2019." Año 2019

"AAI Early Career Faculty Travel Grant. American Association of Immunologists (AAI)." Año 2019

Nanoimmunology of T Lymphocyte activation and apoptosis

Principal Investigator
Izquierdo Pastor, Manuel

Associate Investigator
Calvo López, Víctor

Predoctoral
Bello Gamboa, Ana
Herranz Gómez, Gonzalo

Support Personnel
Garrido Moreno, Alejandro

Undergraduate Students
Carmona Carrasco, Noelia
Fernández Hermira, Sara
Loyens-, Anaïs
Moreno Yanino, Solange Andrea
Velasco Santiago, Marta

Master Students
Bello Gamboa, Ana

Visiting researchers
Bello Gamboa, Ana
Ilie-, Roxana Costina
Huetos Pérez, Silvia
Alcocer Cruz, Sergio

Keywords: T lymphocytes, immunological synapse, exosomes, multivesicular bodies, actin cytoskeleton, protein kinase C delta, FMNL1.

Research Lines

Study of the mechanisms involved in the polarised traffic of MVBs/lytic granules and their role in the secretion of exosomes by T lymphocytes during the processes of cytotoxicity and activation-induced cell death.

Exosomes are small membrane vesicles (nanovesicles) formed by inward budding of the limiting membrane of late endosomes into their lumen. The exosomes accumulate as intraluminal vesicles into secretory vesicles/ multivesicular bodies (MVBs). The stimulation of cells from diverse lineages induces the fusion of the limiting membrane of the MVBs with the plasma membrane and the secretion of exosomes (Fig. 1). Several evidences support the hypothesis that exosomes represent a novel modality of intercellular communication, particullarly in the immune system. In the immune system, T lymphocyte activation with antigen through the T-cell receptor (TCR) induces the acquisition of essential effector functions and controls the activation, proliferation and apoptosis of T lymphocytes. In some of these biological responses, which include the cytotoxic activity exerted by cytotoxic T lymphocytes (CTLs), T lymphocyte activation and activation-induced cell death (AICD) processes, the exosomes appear to play an important role. The MVBs from cytotoxic T lymphocytes (CTLs) are called lytic granules (Fig. 1). Upon challenge with antigen, CTLs develop

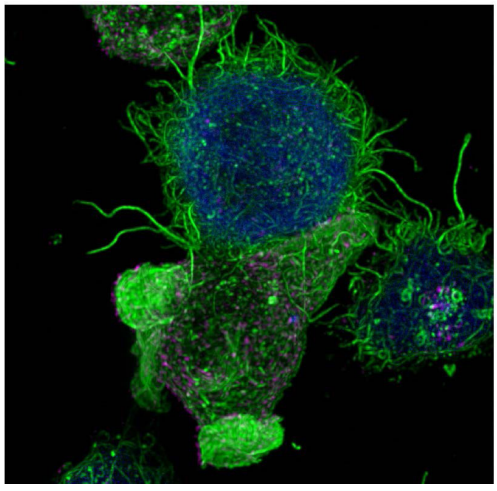

Figure 1. Fluorescence image showing a T lymphocyte (bottom), forming immune synapse (IS) - with a cell presenting antigen (top, blue). Filamentous actin in green color. The magenta-colored vesicles are the multivesicular bodies (MVBs), whose fusion in the synaptic membrane induces exosome secretion (Fig. 2).

different mechanisms to induce the apoptosis of target cells. Included among these strategies, the inducible expression of Fas ligand (FasL) in lytic granules and its polarized secretion at the immune synapse are thought to be important mediators of CTL-mediated killing. In addition, FasL contributes to the homeostatic control of the T lymphoid compartment that occurs through activation-induced cell death (AICD). Regarding FasL function, it has been shown that the secretion of bioactive (apoptosis-inducer) FasL into exosomes constitutes an important mechanism controlling FasL activity.

Therefore, our aims are:

1) To gain insights into the mechanisms by which T lymphocytes control the polarised traffic of MVBs/lytic granules and regulate the secretion of exosomes.

2) To establish the role of pro-apoptotic exosomes in the cytotoxicity mediated by CTLs and homeostatic AICD. With this knowledge in hand, it will be eventually possible to modulate crucial immune functions such as CTL-mediated apoptosis and the homeostatic control of the T lymphocyte compartment.

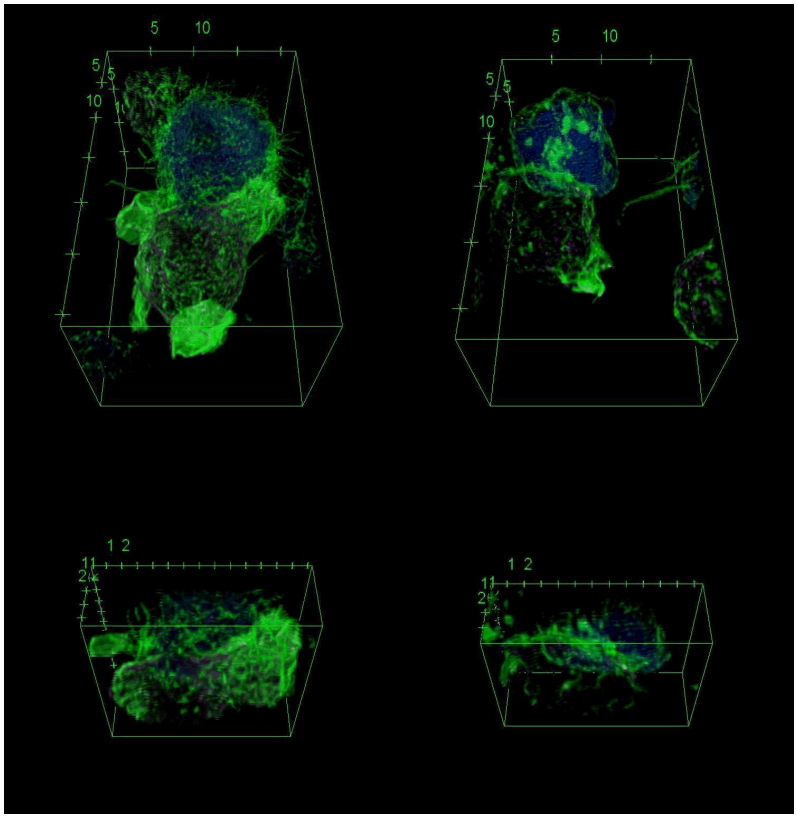

Fig. 3. Three-dimensional reconstruction of the synapse (Fig. 1). The synapse marked in white rectangle in the first frame. Filamentous actin in green color. The magenta-colored vesicles are the multivesicular bodies (MVBs), whose fusion in the synaptic membrane induces exosome secretion (Fig. 2).

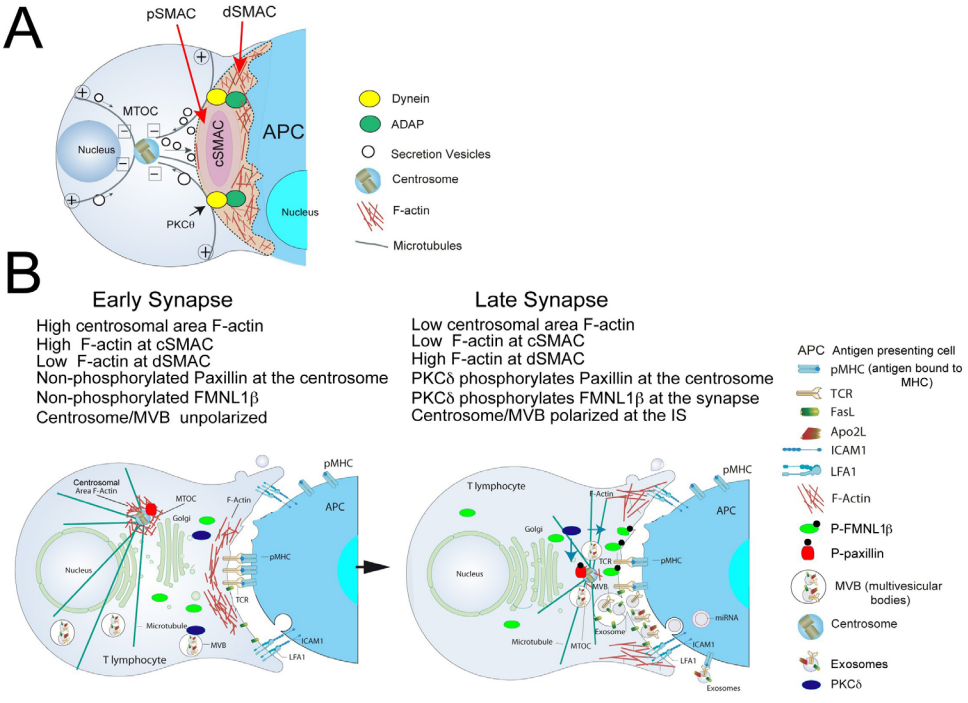

Figura 2. Cortical and centrosomal actin cytoskeleton reorganization and MTOC polarization. After the initial scanning contact of TCR with pMHC on the APC, both Th lymphocytes and effector CTL form mature IS with antigen-presenting cells (APC). Secretion vesicles (lytic granules in CTL and cytokine-containing vesicles in Th cells) are rapidly transported towards the MTOC (in the minus "-" direction) and, almost simultaneously, the MTOC polarizes towards the cSMAC of the IS, a F-actin poor area that constitutes a secretory domain. MTOC translocation to the IS appears to be dependent on PKCthetha-controlled dynein anchored to ADAP at the pSMAC, that pulls MTOC in the minus direction. In the IS, the initial F-actin reorganization in the cell-to-cell contact area, followed by a decrease in F-actin at the cSMAC and an accumulation of F-actin at the dSMAC appears to be involved in vesicle secretion. Panel B: Actin cytoskeleton reorganization events occurring at the CD4+ Jurkat T lymphocyte IS model: PKCdelta and MTOC/MVB polarization: both FMNL1β and paxillin are phosphorylated by PKCdelta. Before forming the IS, both FMNL1β (in the cytosol) and paxillin (located at the centrosome), proteins that regulate the assembly and disassembly of F-actin, are dephosphorylated, which keeps them inactive. Left panel: in an early IS there is an accumulation of F-actin in the central region of the IS, while the centrosome is surrounded by a dense F-actin network that keeps it retained near the nucleus and away from the IS. Right panel: after PKCδ is activated by TCR stimulation at the IS, FMNL1β is phosphorylated in the C-terminal, DAD autoinhibitory domain, and is located in the IS (P-FMNL1beta). In addition, paxillin is phosphorylated in Threonine 538 and remains located in the centrosome (P-paxillin). These events lead to F-actin reduction at the central region of the IS that corresponds to cSMAC, F-actin accumulation into the dSMAC and the depolymerization of F-actin surrounding the centrosome. All these processes, most probably acting in a coordinated manner, may facilitate the movement of the centrosome towards the IS and the convergence of MVB towards the F-actin depleted area in the cSMAC, which facilitates MVB fusion at the cSMAC and the subsequent secretion of exosomes in the synaptic cleft towards the APC. For more details please refer to group publications.

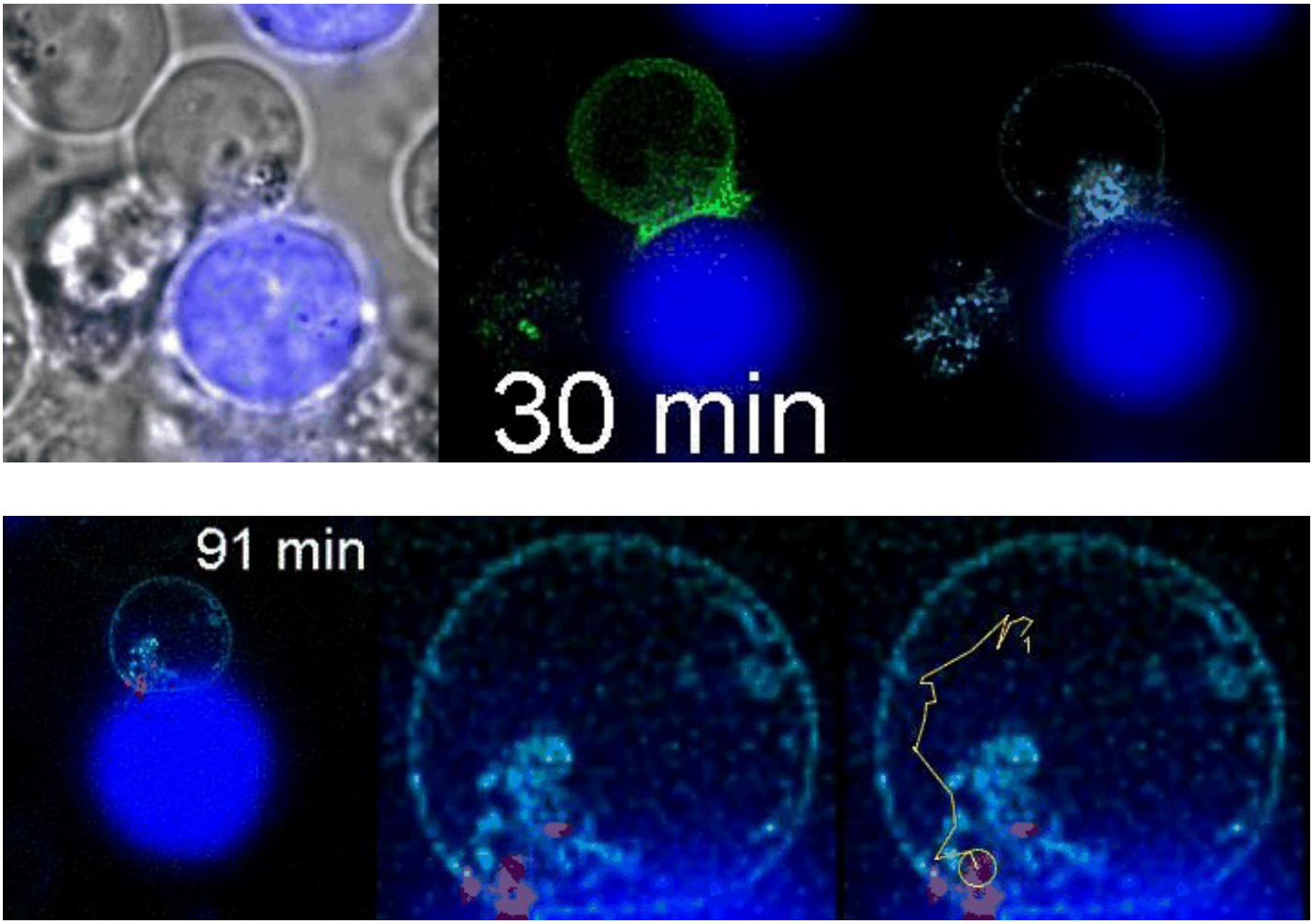

Fig. 4. Actin rearrangement (green) at the synapse and polarization of multivesicular bodies –MVBs- (cyan), microtubule organizing center-MTOC- (red). Antigen presenting cell is colored in blue.

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Funding

"Estudio de las bases moleculares del trafico de los cuerpos multivesiculares y secrecion de exosomas por los linfocitos t t:funcion en la apoptosis y en migracion linfocitarias."

Financiado por: Ministerio de Economía y Competitividad (Madrid). Año 2016-2020

COX-2 and liver physiopathology

Principal Investigator
Martín Sanz, Paloma

Associate Investigators
Boscá Gomar, Lisardo
Casado Pinna, Marta

Investigators under contract
Brea Contreras, Rocío

Predoctoral
Fuertes Agudo, Marina

Master Students
Casanova Melchor, Natalia

Undergraduate Student
Fraile Ramos, Juan

Keywords: COX-2, prostaglandins, liver, NAFLD, fibrosis, ischemia/reperfusion, miRNAs, nanoparticles, HLOs.

Research Lines

We study the relationship between COX-2 expression and liver pathology in experimental models and human biopsies and understanding the molecular mechanisms implicated in these processes. Cyclooxygenase (COX) is the enzyme that catalyzes the rate limiting step in the synthesis of prostanoids. Prostaglandins play an important role in many biological processes such as platelet aggregation, maintenance of the gastric mucosa, reproduction, etc. and also in pathological processes such as inflammation and cancer. Work of our research group have shown that the expression of COX-2 in hepatocytes protects against liver damage induced by hyperglycemia, the insulin resistance and obesity as well as against the steatohepatitis and fibrosis, suggesting improved mitochondrial function and oxidative phosphorylation. We are studying the role of COX-2 in liver ischemia/reperfusion injury and the effect on liver transplantation.

Research Lines

- **Dual Role of COX-2 in hepatic pathophysiology:** Given the protective role of COX-2 in many of the studied diseases but also taking into account its role as inflammatory agent; it is clear that COX-2 exerts various effects depending on the time and cell type that expresses. For this we have transgenic animals and cell models for COX-2.
- **Contribution of COX-2-dependent prostaglandins to the onset and progression of non-alcoholic fatty liver disease (NAFLD):** Our results have shown that COX-2 attenuates non-alcoholic steatohepatitis and liver fibrosis in mice. COX-2-dependent prostaglandins induce apoptosis of hepatic stellate cells and attenuate liver fibrosis by downregulating miRNAs. Our goal is to advance the study evaluating the role of COX-2 and associated miRNAs in the progression of the lesion, analyzing involved signaling pathways and molecular mechanisms.
- **Therapeutic options of COX-2 in NAFLD: approach to the manotechnoloy:** The main objective of this proyect is to analyze whetherthe induction of COX-2 plays a protective role as a physiologic response against different liver injuries and to explore the possible therapeutic use of COX-2 overexpression.To do this we will apply nanotechnology to overexpress COX-2/PGE_2 into hepatocytesthrough generating nanoparticles containing COX-2/PGE2. Nanoparticle toxicity, uptake and pharmacological activity will be analyze first in vitro in hepatic cell linesand in vivo after intravenous administration by means of Cy5-labelled nanosystems.We also plan to develop a human in vitro system for testing the nanoparticles, modeling liver NAFLD/NASH with pluripotent stem cell-derived organoids (HLOs).

- **COX-2 and mitochondrial function. Role in ischemia reperfusion (I/R) in the liver:** Our data support the view of a novel protective effect of COX-2 inductionin hepatic ischemia/reperfusion injury through a significant attenuation of the IRI-induced increase in oxidative stress and hepatic apoptosis, an increase in autophagic flux and a decrease in endoplasmic reticulum stress. Furthermore, measurement of PGE_2 levels in plasma from patients who underwent liver transplantation revealed a significantly positive correlation of PGE_2 levels and graft function, and an inverse correlation with the time of ischemia. These data suggest that the presence of prostaglandins in the graft may be a marker of prognosis in recovery of liver function in the transplanted organ.

Publications

González-Ramos S., Paz-García M., Fernández-García V., Portune K.J., Acosta-Medina E.F., Sanz Y., Castrillo A., Martín-Sanz P. Obregón M.J. & Boscá L (2020). *NOD1 deficiency promotes an imbalance of thyroid hormones and microbiota homeostasis in mice fed high fat diet.* Scientific Reports. doi: 10.1038/s41598-020-69295-2 32704052 10 (1):12317.

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González-Ramos S., Paz-García M., Rius C., del Monte-Monge A., Rodríguez C., Fernández-García V., Andrés V., Martínez-González J., Lasunción M.A., Martín-Sanz P., Soehnlein O. & Boscá L. (2019). *Endothelial NOD1 directs myeloid cell recruitment in atherosclerosis through VCAM-1.* FASEB J. 33: 3912-21.

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Funding

"Papel de la ciclooxigenasa-2 en el daño por isquemia/reperfusión en el hígado. Estudio de la función mitocondrial." Financiado por: MINECO. Año 2017-2019

"Consorcio para el estudio del fracaso renal agudo: Fisiopatología, nuevas terapias, biomarcadores y modelos experimentales." Financiado por: CAM. Año 2018-2021

"CIBER de Enfermedades Hepáticas y Digestivas." Financiado por: Instituto de Salud Carlos III. Año 2008-2021

"Opciones terapéuticas de la ciclooxigenasa-2 en la enfermedad del hígado graso no alcohólico: una aproximación a la nanotecnología." Financiado por: MICIU. Año 2020-2022

Molecular mechanisms of type 2 diabetes mellitus and metabolic associated diseases

Principal Investigator
Martínez Valverde, Ángela

Associate Investigators
Pescador Sánchez, Nuria
Medina Gómez, Mª Gema

Investigators under contract
Valdecantos Jiménez de Andrade, Pilar
Vázquez Pérez, Patricia
García Martínez, Irma
Rada Llano, Patricia

Predoctoral
Alén Alonso, Rosa María
Barahona Sanz, Inés
Grajales Abellán, Diana
Da Silva Ferreira, Vitor Manuel
Escalona Garrido, Carmen
Rubio Caballero, Carmen

Support Personnel
Montes San Lorenzo, Ángela
Hitos Prados, Ana Belén

Undergraduate Students
Rejas González, Raquel
Esquinas Román, Eva María

Master Students
Chamoso Sánchez, David
Higuera García, Marina

Visiting researchers
Forno-, Francesca
Cruz Oliveira Pinho, Aryane
Navarro Villarán, Elena

Keywords: Non-alcoholic fatty liver disease, inflammation, insulin resistance, obesity, progenitor liver cells, exosomes, non-alcoholic Steatohepatitis, GLP-1 receptor, glucagon receptor, liver regeneration, brown adipocytes, Thermogenesis, Insulin signaling, Sirtuin 1, Metformin, Hif1a, diabetic retinopathy, microglia, somatostatin, second generation antipsychotics, Insulin secretion, Type 2 Diabetes Mellitus.

Research Lines

Molecular mechanisms associated to the progression of non-alcoholic fatty liver disease: emerging role of the progenitor liver cells

Researchers involved: Pilar Valdecantos, Ines Barahona, Silvia Calero, Patricia Rada, Angela M Valverde.

We found that protein tyrosine phosphatase 1B modulates the progression of non-alcoholic faNAFLD to non-alcoholic steatohepatitis (NASH). PTP1B-deficient mice showed an accelerated progression of NASH manifested by higher infiltration of immune cells in the liver parenchyma. On the other hand, PTP1B deficiency led to an improvement in the reversion of the disease partly due to the increased sensitivity of PTP1B-deficient oval liver cells (progenitor liver cells) to HGF/Met signaling (Mol Metab. 2018 Jan; 7:132-146).

We are currently investigating the sensitivity os the oval cells of the lipotoxic effect of palmitic acid. Our data revealed that PTP1B-deficient oval cells are resistant to palmitic acid-induced lipotoxicity due to their ability to form lipid driplets (Figure). The main findings are:

Background and Aims: Oval cells are progenitor cells with an emerging role in liver regenerative responses by their

differentiation capacity. However, the characterization of their susceptibility to lipotoxicity in the context of obesity-associated non-alcoholic fatty liver disease is unknown. Inhibition of protein tyrosine phosphatase 1B (PTP1B) is a promising pharmacological strategy against insulin resistance and obesity. Also, PTP1B deficiency protects against acute and chronic hepatocyte damage. On that basis, we analyzed the susceptibility of oval cells with or without PTP1B to lipotoxicity and the molecular mechanisms involved.

Methods: Oval cells from PTP1B+/+ and PTP1B-/- mice were isolated, cultured and characterized. For lipotoxicity studies, oval cells wereResults: Treatment with PA for 24 h induced apoptotic cell death in PTP1B+/+ oval cells in parallel to a blockade of the autophagy flux. This lipotoxic effect was absent in PTP1B-/- cells that accumulated lipid droplets upon PA treatment and presented elevated levels of UPR-sensitive mediators, AMPK phosphorylation and sirtuin 1. These effects were also found in PTP1B silenced wild-type oval cells. Moreover, PA-treated PTP1B-/- oval cells showed an enhanced anti-oxidant response including nuclear factor erythroid 2-related factor nuclear translocation. Lipidomics revealed that upon PA treatment PTP1B-/-oval cells present elevations in stearoyl CoA desaturase 1 (Scd1) mRNA and higher unsaturated/saturated fatty acids after PA stimulation either free or incorporated into triacylglicerides, diacylglicerides and phospholipids. Blockade of autophagy flux in PTP1B-/- cells inhibited lipid droplet formation and restored lipoapoptosis.

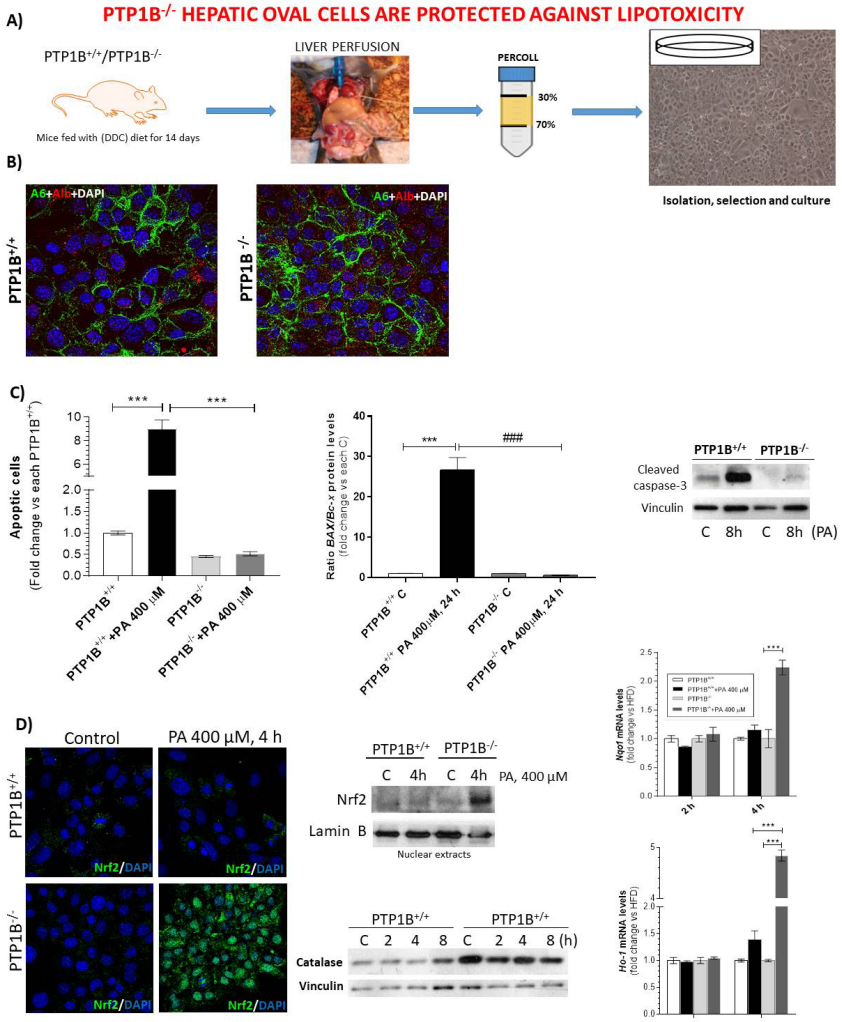


FIGURE 1. A) Schematic representation of the isolation and culture of hepatic oval cells. **B)** Representative immunofluorescence images from PTP1B$^{+/+}$ and PTP1B$^{-/-}$ oval cells with antibodies against Albumin and the oval cell marker A6. **C). (left)** Quantification of apoptotic oval cells by flow cytometry after treatment with PA for 24 (n=5). **(middle)** Quantification of Western blot analysis of Bax/BclxL ratio. **(right)** Representative Western Blot of cleaved caspase-3 in oval cells treated with PA for 8 h (n=3). **D) (left)** Representative immunofluorescence and Western blot **(middle)** showing nuclear localization of Nrf2 of and Catalase levels in PTP1B$^{-/-}$ oval cells treated with PA (n=3). **(right)** *Nqo1* and *Hmox* mRNA levels at the indicated time-periods (n=3). STATISTICAL ANALYSIS.

Conclusion: Our results revealed that liver oval cells are susceptible to lipotoxicity, an effect mediated at least in part by the induction of oxidative stress. PTP1B deficiency protects against lipotoxic cell death by mechanisms including enhancement of antioxidant defence and a major capacity to generated unsaturated lipid species stored in lipid droplets, suggesting a potential benefit in cellular regenerative therapies against NAFLD. This effects are likely mediated by autophagy-derived energy suppliers.

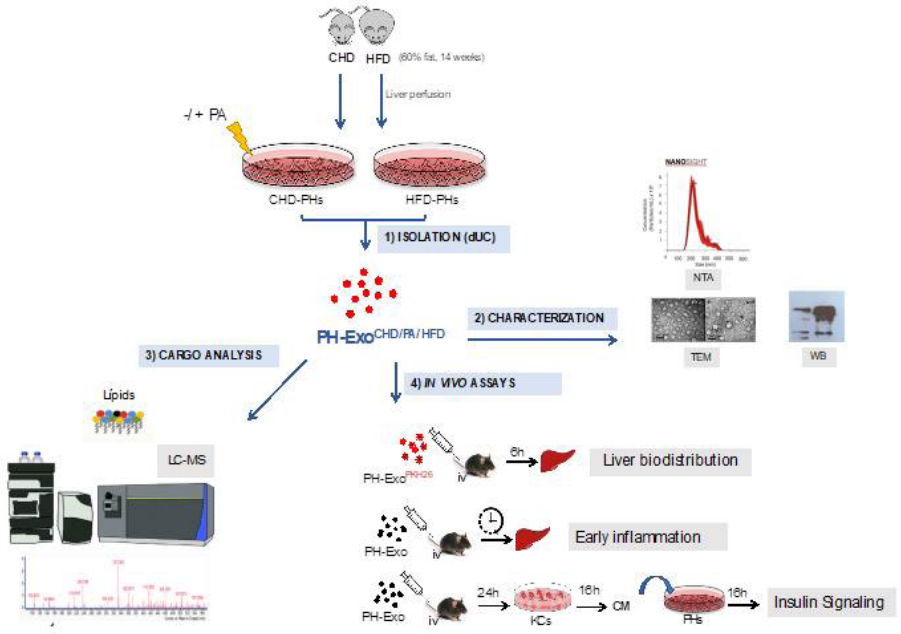
EXOSOMES: NEW MESENGERS OF THE ENDOCRINE INTREACTOME IN METABOLIC SYNDROME WITH DIAGNOSTIC POTENTIAL

Researchers involved: Irma García-Martínez, Rosa Alen, Manuel Izquierdo-Pastor, Ángela M. Valverde

Background and Aims: Cell-to-cell communication by extracellular vesicles (EVs) is an emerging issue in non-alcoholic fatty liver disease (NAFLD). In this project we aimed to characterize the exosome fraction of the EVs secreted by the hepatocytes under lipotoxic conditions of NAFLD and the impact of lipotoxic exosomes in macrophages/Kupffer cells inflammation, as well as in insulin signaling in hepatocytes.

Methods: C57BL/6J male mice were fed a chow (CHD) or high-fat diet (HFD) for 14 weeks. Exosome-enriched fraction (Exos) was isolated from: 1) hepatocytes from CHD-fed mice in the absence (Exo^{CHD}) or presence of palmitic acid (PA) (Exo^{PA}), 2) hepatocytes from HFD-fed mice (Exo^{HFD}). Exosomes were characterized as described in the previous report. Lipidomic analysis was conducted to identifying the traffic of saturated fatty acids (SFAs) between hepatocytes and macrophages mediated by exosomes. To analyze the *in vivo* effects of lipotoxic exosomes on liver inflammation

and insulin signaling, C57BL/6J male mice were injected intravenously with hepatocyte-derived exosomes **(Figure).**



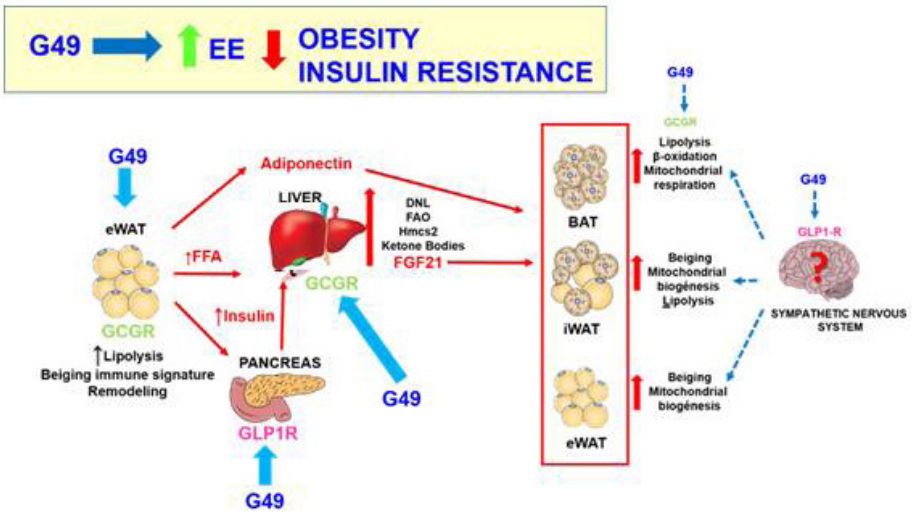
Results: The lipidomic analysis revealed an increase in C:16 (PA) and C:18 (stearic acid, SA) in exosomes released by hepatocytes under lipotoxic stress (Exo^{PA} or Exo^{HFD}) compared to Exo^{CHD}. Similar profile of intracellular PA, but not SA, was found in the secreting hepatocytes (sPH), suggesting the presence of fatty acid elongases in the exosomes cargo. Moreover, total content of MUFAs was increased in Exo^{PA} and Exo^{HDF} fractions. We also analyzed SFAs content in the recipient macrophages (rM) and a substantial elevation in PA was detected in macrophages loaded with Exo^{PA}, whereas macrophages receiving Exo^{HFD} contained less PA, but higher SA. These results point to transfer of SFAs cargo of the exosomes from secreting hepatocytes to recipient macrophages.

Livers from mice injected with Exo^{PA} and Exo^{HFD}, but not Exo^{CHD}, showed enhanced proinflammatory signaling cascades and proinflammatory gene expression together with NF-kappaB nuclear translocation at short-time periods post-injection. Immnunofluorescence analysis revealed the presence of exosomes in Kupffer cells at 2 h post-injection. We also investigated if Kupffer cells mediate insulin resistance in hepatocytes in response to *in vivo* injections of lipotoxic exosomes. To achieve this, mice were injected Exo^{PA} or Exo^{CHD} and after 24 h Kupffer cells were isolated and cultured for 16 h. Then, the conditioned medium CM ($CM\text{-}Exo^{CHD}$ or $CM\text{-}Exo^{PA}$) was collected and used to treat primary hepatocytes for 16 h after which insulin signaling was analyzed. We found that Akt phosphorylation (Ser473 and Thr308) was decreased in primary hepatocytes exposed to $CM\text{-}Exo^{PA}$, suggesting that lipotoxic exosomes induce hepatocyte insulin resistance at least in part through activation of hepatic resident macrophages.

Conclusion: We identified SFAs as essential cargo of the exosomes released by the hepatocytes under lipotoxic conditions that are transferred to macrophages/Kupffer cells, thereby triggering inflammation. In mice receiving injections of lipotoxic exosomes, liver inflammation concurred with the activation of Kupffer cells and hepatocyte insulin resistance.

Therapies based on dual agonism for GLP-1 and glucagon receptors agonists to combat obesity and co-morbidities.

Researchers involved: Pilar Valdecantos, Laura Ruiz, Angela M. Valverde.



Bariatric surgery is an effective surgery for the treatment of obesity and type 2 diabetes mellitusremission. Pharmacological approaches which exert similar metabolic adaptations are an active area of research. We investigated the effects of G49, an oxyntomodulin (OXM) analog and GCGR/GLP-1R dual agonist, in preventing diet-induced obesity (DIO) and its underlying molecular mechanisms.

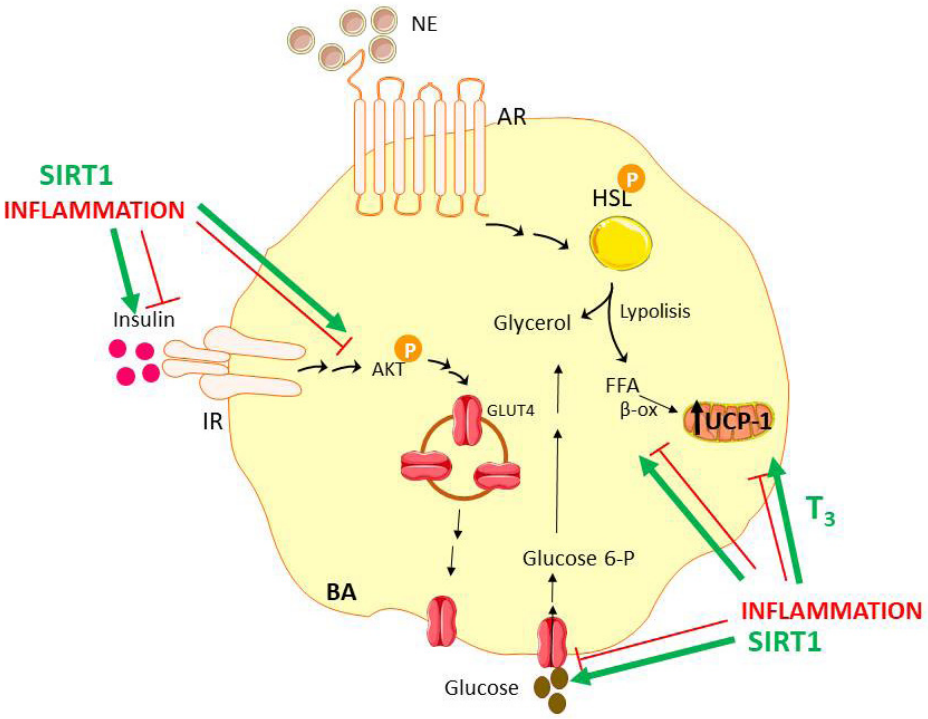
G49 reversed obesity and insulin resistance in DIO mice to a greater extent than Fc-GLP-1, a GLP-1R agonist. G49 triggers an inter-organ crosstalk between adipose tissues (WAT and BAT), pancreas, and liver initiated by a rapid release of free fatty acids (FFAs) by

epidydimal WAT and subsequent elevations in adiponectin and FGF21 resulting in beiging in WAT depots, BAT activation and increased energy expenditure. OXM elevation and similar metabolic profile were found in plasma from obese patients after malabsorptive bariatric surgery. We suggest that treatment of obesity with G49 represents a potential pharmacological alternative to bariatric surgery.

Inflammation linked to obesity in Brown Adipose Tissue: molecular mechanisms and therapeutic approaches

Researchers involved: Nuria Pescador, Patricia Vázquez, Carmen Escalona, Ángela M. Valverde

Objective: Metainflammation is a chronic low-grade inflammatory state induced by obesity and associated comorbidities, including peripheral insulin resistance. Brown adipose tissue (BAT), a therapeutic target against obesity, is an insulin target tissue sensitive to inflammation. Therefore, it is demanding to find strategies to protect BAT against inflammation and its effects in energy balance. In this research line we have explored the impact of moderate Sirtuin 1 (SIRT1) overexpression in insulin sensitivity and β-adrenergic responses in BAT and brown adipocytes under pro-inflammatory conditions.



Methods: The effect of inflammation in BAT functionality was studied in obese *db/db* mice and lean wild-type (WT) mice or mice with moderate overexpression of SIRT1 ($SIRT1^{Tg+}$) injected a low dose of bacterial lipopolysaccharide (LPS) to mimic endotoxemia. We also conducted studies in differentiated brown adipocytes (BA-WT and BA-$SIRT1^{Tg+}$) exposed to a macrophage-derived pro-inflammatory conditioned medium (CM) to evaluate the protection of SIRT1 overexpression in insulin signaling and glucose uptake, mitochondrial respiration, fatty acid oxidation (FAO), as well as norepinephrine (NE)-mediated-modulation of uncoupling protein-1 (UCP-1) expression.

Results: BAT from *db/db* mice was susceptible to metabolic inflammation manifested by activation of pro-inflammatory signaling cascades, increased pro-inflammatory gene expression, tissue-specific insulin resistance and reduced UCP1 expression. Impairment of insulin and noradrenergic responses were also found in lean WT mice upon LPS injection. By contrast, BAT from mice with moderate overexpression of SIRT1 ($SIRT1^{Tg+}$) was protected against LPS-induced activation of pro-inflammatory signaling, insulin resistance and defective thermogenic-related responses upon cold exposure. Importantly, the drop of triiodothyronine (T_3) levels both in circulation and intra-BAT after exposure of WT mice to LPS and cold was markedly attenuated in $SIRT1^{Tg+}$ mice. *In vitro* experiments in BA from the two genotypes revealed that upon differentiation with a T_3-enriched medium and subsequent exposure to a macrophage-derived pro-inflammatory CM, only BA-SIRT1Tg+ fully recovered insulin and noradrenergic responses.

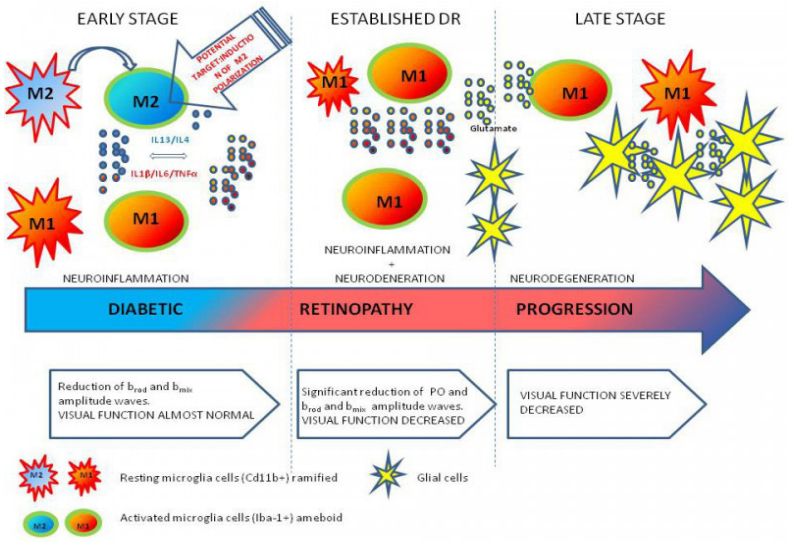
Conclusion: This study has unraveled the benefit of moderate overexpression of SIRT1 to confer protection against defective insulin and β-adrenergic responses caused by inflammation in BAT. Our results have potential therapeutic value proposing combinatorial therapies of BAT-specific thyromimetics and SIRT1 activators to combat metainflammation in this tissue.

Inflammation as an early stage of diabetic retinopathy: terapeutical approaches.

Researchers involved: Ana I Arroba, Angela M. Valverde

Somatostatin (SST) is a neuroprotective peptide but little is known regarding the potential role of its anti-inflammatory effects on retinal neuroprotection. In a previous study, we provided first evidence that topical (eye drops) administration

of SST prevents retinal neurodegeneration in streptozotocin (STZ)-induced diabetic rats. However, STZ by itself could cause neurotoxicity, thus acting as a confounding factor. The aims of the present study were: 1) To test the effect of topical administration of SST in the db/db mouse model, a spontaneous model of type 2 diabetes, thus avoiding the confounding effect of STZ on neurodegeneration. 2) To further explore the anti-inflammatory mechanisms of SST in glial cells. This task was performed by using mouse retinal explants and cell cultures. In summary, we confirm that SST topically administered was able to prevent retinal neurodysfunction and neurodegeneration in db/db mice. Furthermore, we found that SST prevented the activation of the classical M1 response of Bv.2 microglial cells upon LPS stimulation as a potent pro-inflammatory trigger. The anti-inflammatory effect of SST in Bv.2 cells was also observed in response to hypoxia. In conclusion, we provide evidence that the neuroprotective effect of SST in diabetic retina can be largely attributed to anti-inflammatory mechanisms.



Metabolic side effects of long treatment with antipsichotics: TREATMENT

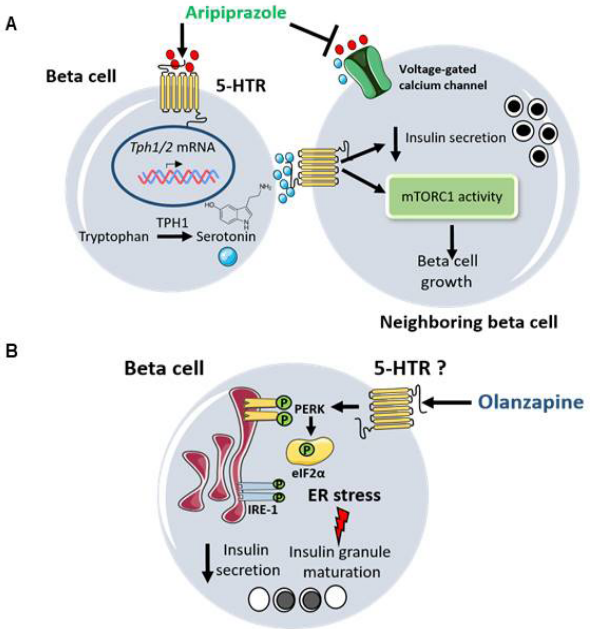
Researchers involved: Vitor da Silva, Diana Grajales, Patricia Vázquez

We are evaluating the metabolic side effects of second generation antipsychotics (SGAs) (olanzapine and aripiprazole) in whole body insulin sensitivity and glucose homeostasis in mice treated with this drugs. We are focusing in peripheral tissues (fat, liver and skeletal muscle) as well as in pancreatic beta cells.

Regarding SGAs effects on beta cells we analyzed the functional adaptations of beta cells in 12-weeks-old C57BL/6J x 129 sv female mice fed an olanzapine- or aripiprazole-supplemented diet (5.5-6 mg/kg/d) for 6 months. Glucose and insulin tolerance tests (GTT and ITT, respectively), *in vivo* glucose-stimulated insulin secretion (GSIS) and indirect calorimetry were performed at the end of the study. The effects of SGAs on beta cell plasticity and islet serotonin levels were assessed by transcriptomic analysis and immunofluorescence on pancreatic sections. Static incubations of islets and INS-1 (832/13) cells were performed for measuring Ca^{2+} currents and insulin secretion. Endoplasmic reticulum (ER) stress was analyzed by Western blot.

Results: Six-month treatment of female mice with olanzapine or aripiprazole induced weight gain ($p<0.01$ and $p<0.05$, respectively), glucose intolerance ($p<0.01$) and impaired insulin secretion ($p<0.05$). Olanzapine, but not aripiprazole, induced ER stress in both INS-1 cells and pancreatic islets. Treatment of islets with the chemical chaperone Tauroursodeoxycholic acid (TUDCA) prevented olanzapine-induced ER stress and the decline in insulin secretion ($p<0.01$). Aripiprazole induced serotonin production in beta cells by increasing tryptophan hydroxylase 1 (TPH1) expression and inhibited Ca^{2+} signaling and insulin secretion. Of note, aripiprazole increased beta cell size and mass along with activation of mTORC1/S6 ($p<0.05$), however without preventing beta cell dysfunction.

Conclusions/interpretation: Olanzapine and aripiprazole induce weight gain and beta cell dysfunction leading to glucose intolerance via distinct mechanisms. These deleterious metabolic effects should be considered while treating patients with these SGAs as they may increase the risk for metabolic syndrome and diabetes.



Proposed models for aripiprazole and olanzapine effects in beta cell function. (A) Aripiprazole, probably through a 5-HTR receptor, increases TPH1 expression, promoting serotonin production in the beta cells. The release of serotonin can, in turn, activate the mTORC1 signaling pathway inducing beta cell growth and also inhibit insulin secretion. **(B)** Olanzapine activates ER stress and may impair the maturation of the insulin-containing granules reducing insulin secretion.

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Doctoral theses and other works

Carmen Escalona Garrido

"Protective role of SIRT1 activation against insulin resistance and defective thermogenesis associated to inflammation in brown adipose tissue". Universidad Autónoma de Madrid. Facultad de Medicina. 2020. Supervisor/s: Ángela María Martínez, Patricia Vázquez. Calificación: Sobresaliente Cum Laude

Diana Grajales Abellán

"Effects of the Second Generation Antipsychotics olanzapine and aripiprazole in beta cell functionality and pancreatic islet plasticity". Universidad Autónoma de Madrid. Facultad de Medicina. 2020. Supervisor/s: Ángela María Martínez. Calificación: Sobresaliente Cum Laude

Funding

Ángela María Martínez Valverde

"Training European Network: Metabolic Dysfunctions associated with Pharmacological Treatment of Schizophrenia." Financiado por: European Union. Año 2017-2021

"Molecular Mechanisms and Intertissular communication in insulin resistance." Financiado por: Comunidad de Madrid. Año 2018-2021

"Extending the knowledge on cellular and molecular players in the progression and treatment of non-alcoholic liver disease associated to obesity (FaTLiV)." Financiado por: ministerio de ciencia e innovacion. Año 2019-2021

"New messengers in the intreactome between hepatic and extra-hepatic cells in non-alcoholic fatty liver disease with diagnostic value." Financiado por: Fundación Ramón Areces. Año 2019-2022

"Identification of metabolic biomarkers for chronic diseases and their treatments." Financiado por: Ministerio de Ciencia e Innovación. Año 2020-2022

Irma García Martínez

"Searching biomarkers of response to intravitreal injections of anti-VEGF and corticosteroid in the treatment of Diabetic Macular Edema." Financiado por: CIBERdem-Instituto de Salud Carlos III. Año 2020-2021

Patricia Rada Llano

"Role of the succinate/SUCNR1 axis in the

physiopathology of NAFLD." Financiado por: CIBERdem. Instituto de Salud Carlos III. Año 2020-2021

Awards

"Mejor comunicación presentada en la Reunión Anual de CIBERdem." Año 2019

Role of mitochondrial dysfunction in the development of metabolic diseases

Principal Investigator
Monsalve Pérez, María

Investigators under contract
García Gómez, Raquel
Prieto Arroyo, Ignacio Borja

Predoctoral
Selinger Galant, Leticia
Patel-, Gaurangkumar Arvindbhai
Kramar-, Barbara
Yildiz-, Ramazan

Undergraduate Students
Canales Alandi, Ivan
Córdova Ortiz, Isabel
Guerrero Morillo, Desirée
Gómez López, Ainhoa
Martínez Santamaría, José Carlos
Quintela García, César

Master Students
Bayo Jiménez, María Teresa

Visiting Investigation
Navarro González de Mesa, Elisa
Fierro Fernández, Marta
Blanco Ruiz, Eva María

Keywords: Mitochondria, oxidative stress, metabolism, cardiovascular diseases, non-alcoholic fatty liver disease, diabetes, cancer, toxicology, nutrition.

Research Lines

Oxidative Metabolism and Tumor Development

Researchers involved: Quintela, C., Prieto, IB., Martínez, JC., Guerrero, D., García, R., Córdova, I., Bayo, MT., Monsalve, M.

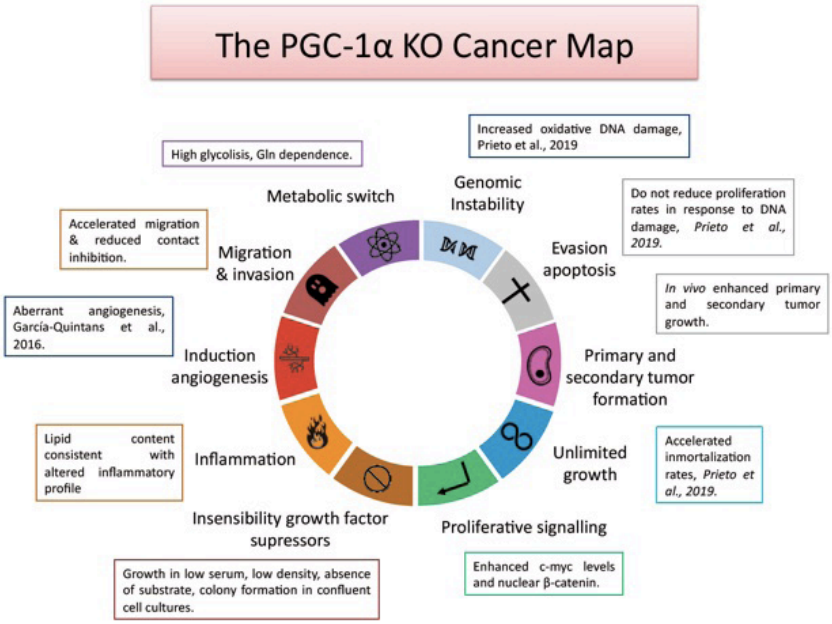
Obesity is considered the worldwide pandemia of the XXI century. It's association with cardiovascular diseases and type 2 diabetes has been clearly established, more recently it has also being recognized a s relevant risk factor for tumor development. One study, using NCI Surveillance, Epidemiology, and End Results (SEER) data, estimated that in 2007 in the United States, about 34,000 new cases of cancer in men (4 percent) and 50,500 in women (7 percent) were due to obesity. The percentage of cases attributed to obesity varied widely for different cancer types but was as high as 40 percent for some cancers. **Obesity not only increases cancer incidence, it also worsens prognosis, increasing the risk of metastasis and reducing the effectiveness of therapy.**

However, the mechanisms that link tumor development to metabolic control are still very poorly understood and the use of metabolic biomarkers or metabolism based therapeutical approaches is very limited in common medical practice.

The molecular basis of the association between metabolism and cancer could be dependent on the regulation of tumor suppressor genes by factors that control metabolic pathways. Both tumors and obese subjects are characterized by a suppression of oxidative metabolism and mitochondrial production of ATP, relying heavily on glycolysis as the major source of ATP and reducing equivalents. Suppression of mitochondrial activity is also associated with elevated production of mitochondrial reactive oxygen species (ROS) and subsequent activation of NOX activity. These elevated levels of ROS

are functionally relevant in tumor progression since they facilitate cellular proliferation and migration having been associated with growth factor independent cell proliferation, epitelium-mesenquimal transition, tumor angiogenesis and metastasis.

Control of oxidative metabolism, mitochondrial activity and cellular antioxidant capacity are under the regulation of the transcriptional coactivator PGC-1α. PGC-1α activity is induced by caloric restriction and reduced in obesity, and would be expected to be reduced in tumor cells. Importantly, PGC-1α regulates the activity of the tumor suppressors p53 and TLS. PGC-1α activity is particularly high in metabolically active tissues.



However, PGC-1α activity has been proposed to be associated with poor prognosis, because it induces the expression of genes that promote resistance to radiation and chemo-therapeutic agents, including antioxidant enzymes, xenobiotic catabolism enzymes, and solute carriers, that promote cellular extrusion of bioactive drugs and to induce pro-angiogenic factors that could promote tumor angiogenesis and facilitate tumor growth.

Therefore, **our aim was to evaluate how the control of oxidative metabolism mediated by PGC-1α impacts on tumor development, and identify metabolic biomarkers of tumor development that could be applied to provide a personalized medicine approach to cancer treatment.** To that end we used a translational perspective, starting from in vitro data to provide an much needed understanding of the mechanism of action (MOA), the implications will be validated in a mouse model of disease and finally its applicability to clinical practice will be tested through the validation of non invasive metabolic biomarkers in humans.

Metabolic Side Effects of Pharmacological Treatments

Researchers involved: Yildiz, R., Patel, GA., Kramar, B., Monsalve, M.

Long term administration of pharmacologycal drugs may result in relevant metabolic side effects.

The molecular basis of these alterations is poorly characterized but likely related to the liver catabolism of the pharmaceutical drugs and hence to liver toxicity.

Drug detoxification mechanisms vary widely with the chamistry of the drug and among individuals, and hence there is currenly a lack of diagnostic procedures that allow to detect the subjects at risk and adjust the chemistry to the patient.

We aim to characterize the early signals of stress induced by drug administration that could allow us to predict adverse cardiovascular outcomes of long term drug administration.

Health impact of Alkaline water consumption

Researchers involved: García, R., Monsalve, M., Prieto, IB.

The tap water that most of the Spanish population drinks, despite having excellent sanitary conditions, suffers from an ionic imbalance, which could have a negative long-term effects on human health.

This has led to the development of filtering systems for domestic use, such as the one that is owned intellectual of the Spanish company ALKANATUR. The poor implementation of these Systems in Spain it gives on the one hand a window of opportunity for the economic development of this area, and for other health improvement and its socio-economic impact especially in terms of diseases associated with aging.

In this context, ALKANATUR, a Spanish company, is in a position of socio-economic advantage. For the development of thispotentiality it is necessary to make direct assessments on the impact of the filtering of ALKANATUR water in health, and specifically in pathologies with high socio-economic cost, such as cancer and diabetes.

Preclinical models and new therapies

Principal Investigator
Zapata Hernández, Juan Manuel

Associate Investigators
Adrados de LLano, Magdalena
Aldea Romero, Marcos

Investigators under contract
Pérez Chacón, Gema
Carr Baena, Pablo Miguel

Undergraduate Students
Rueda Huélamo, María
Peña Gutiérrez, Irene

Master Students
Cerro Pardo, Isabel

Collaborations
Compte Grau, Marta

Visiting investigator
Balbi Santana, Oriana

Keywords: New therapies, immunotherapy, antibody-drug complex, ADC, small chemicals, poly-functional antibodies, chronic lymphocytic leukemia, CLL, lymphoma, cancer, inflammation, autoimmunity, transgenic mice, knock-out mice, TNF-R associated factors, TRAF, Toll like receptors, TLR, NLR, RLR, TRIM37.

Preclinical models

TNF-Receptor Associated Factors (TRAFs) in physiology and disease: Role of TRAF2 and TRAF3 in B cell lymphomagenesis.TRAF2 and TRAF3 are master regulators of B cell homeostasis and function. They bind and regulate various proteins involved in the control of innate and adaptive immune responses. Using transgenic mice, we have shown that dysregulation of TRAF2 and TRAF3 expression in B cells results in B cell transformation.

1. *The Traf2DNxBCL2 mouse model of chronic lymphocytic leukemia (CLL) and small lymphocytic lymphoma (SLL).* We have shown that transgenic mice overexpressing in B cells BCL2 and a TRAF2 mutant that causes endogenous TRAF2-depletion develop chronic lymphocytic leukemia (CLL) and small lymphocytic lymphoma (SLL) with high incidence. The analysis of the VHDJH rearrangements expressed by the expanded CLL/SLL clones demonstrates that this mouse model of CLL recapitulates the biased VHDJH family usage, BCR stereotypia and antigen-specific HCDR3 selection of its human counterpart.

2. *The Traf3xBCL2 mouse model of mature (post-germinal center) non-Hodgkin lymphomas.* We have generatedtransgenic mice overexpressing in B cells TRAF3 and BCL2. These *Traf3xBCL2*-double tg mice developed a variety of mature B cell neoplasms, mostly mature B cell neoplasms consistent with diffuse large B cell lymphoma and plasma cell neoplasms. In contrast, mouse littermates representing all the other genotypes *(TRAF3-/BCL2-; TRAF3+/BCL2- and TRAF3-/BCL2+)* did not develop any lymphadenopathy within the observation period of 20 months. The analysis of the VHDJH rearrangements expressed by the *Traf3xBCL2*+/+ and *Traf3xBCL2*+/- B cells demonstrated a large representation of HCDR3 sequences highly similar to those recognizing pathogen-associated molecular patterns and damage-associated molecular patterns, thus supporting a role for TRAF3 in promoting B cell differentiation in response to these antigens. Our results are consistent with a role for TRAF3, perhaps by promoting exacerbated B cell

responses to certain antigens, and BCL2, presumably by supporting survival of these clones, acting as a tandem to induce mature B cell neoplasms in transgenic mice.

New Therapies

1.*Development of a new CD13-based antibody-drug complex (ADC)*

In collaboration with Pharma Mar, the Universidad Autónoma de Madrid and the Universidad de Barcelona we have developed a new antibody drug complex (ADC) (MI130110) based on an anti-CD13 mAb and the marine compound PM050489. CD13 seems an attractive ADC target as it shows a differential pattern of expression in various human

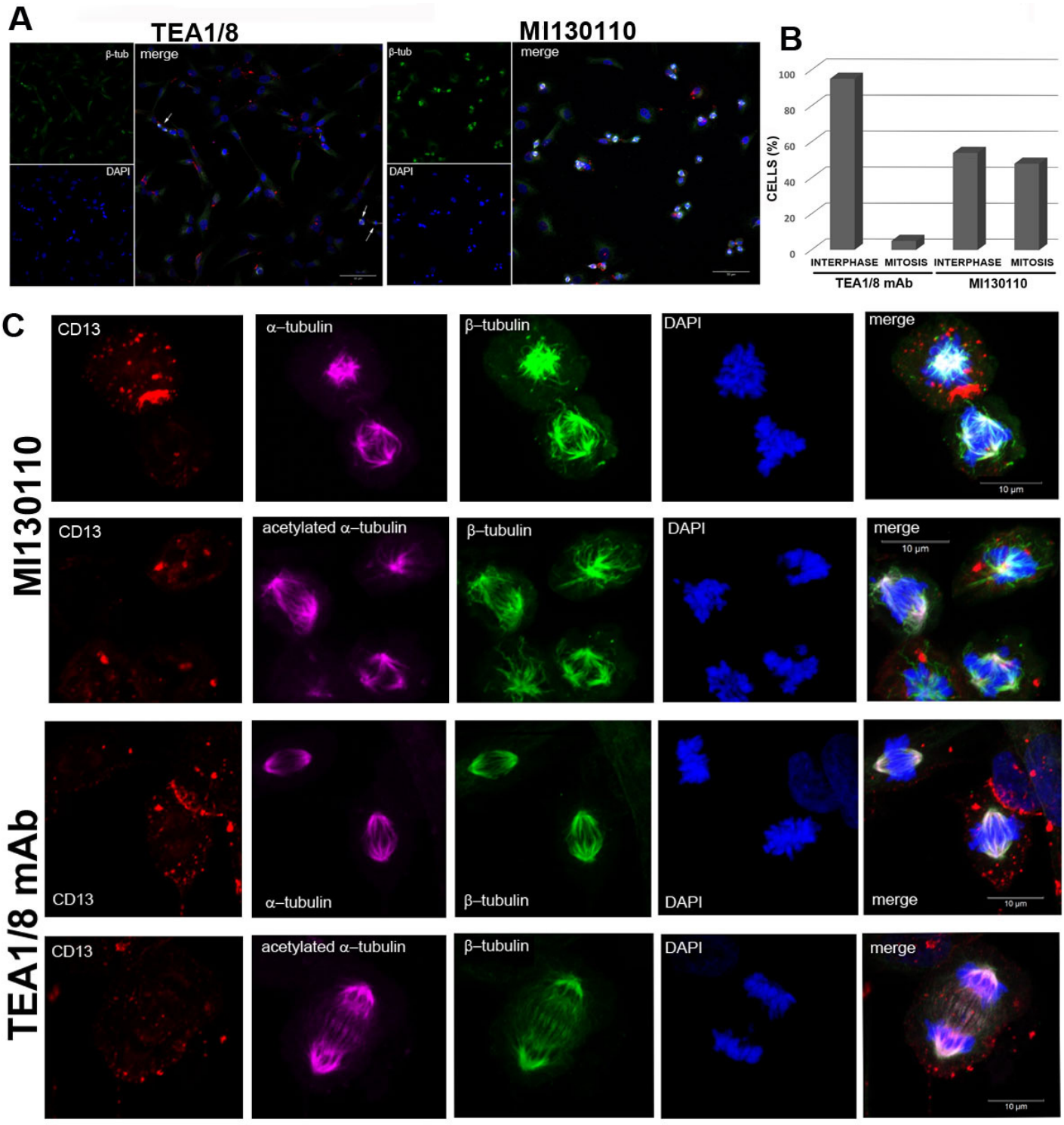


Figure 1. A: HT1080 cells incubated for 24 h at 37 ºC with MI130110 (5 µg/mL) but not with anti-CD13 mAb (5 µg/mL) results in mitotic arrest. The expression of β tubulin, DAPI-staining of DNA and a merged composition of representative fields is shown. B: Quantification of cells in interphase and mitosis after 24 h treatment with anti-CD13 mAb (5 µg/mL) or MI130110 (5 µg/mL). C: MI130110 treatment causes mitotic catastrophe. Figure shows representative cells undergoing mitosis (128 x optical magnification) treated either with MI130110 or with naked anti-CD13 mAb. Staining of CD13 (red), α-tubulin and acetylated α-tubulin (purple), β-tubulin (green) and chromosomes (blue) and a fluorescence merged image (merge) is shown. Scale bars are shown.

tumor types with bad prognosis and in tumor cell lines and because it is internalized upon engagement with a suitable monoclonal antibody. PM050489 is a marine cytotoxic compound tightly binding tubulin and impairing microtubule dynamics which is currently undergoing clinical trials for solid tumors.

The MI130110 ADC showed remarkable activity and selectivity in vitro on CD13-expressing tumor cells causing the same effects than those described for PM050489, including cell cycle arrest at G2, mitosis with disarrayed and often multipolar spindles (Figure 1), consistent with an arrest at metaphase and induction of cell death. In contrast, none of these toxic effects were observed in CD13-null cell lines incubated with MI130110. Furthermore, in vivo studies showed that MI130110 exhibited excellent antitumor activity in a CD13-positive fibrosarcoma xenograft murine model, with total remissions in a significant number of the treated animals. Mitotic catastrophes, typical of the payload mechanism of action, were also observed in the tumor cells isolated from mice treated with MI130110. In contrast, MI130110 failed to show any activity in a xenograft mouse model of myeloma cells not expressing CD13, thereby corroborating the selectivity of the ADC to its target and its stability in circulation. These results demonstrate the suitability of CD13 as a novel ADC target and the effectiveness of MI130110 as a promising antitumor therapeutic agent.

2. *Intratumoral expression using a NFkB-based promoter enhances IL12 antitumor efficacy*

In collaboration with el Dr. A. Rodriguez (Department of Molecular Biology, UAM), we have developed new NF-κB-driven IL12-based lentiviral vectors for intratumoral delivery that maintain their anti-tumoral activity with reduced systemic toxicity.

3. *From Ugi Multicomponent Reaction to Linkers for Bioconjugation*

In collaboration with Dr. F. Albericio (Universidad de Barcelona) we have demonstrated the suitability of the well-known Ugi reaction for the obtention of versatile bifunctional linkers for bioconjugation, useful for ADC generation.

Publications

Domínguez, JM., Pérez, G., Guillén, MJ., Muñoz-Alonso, MJ., Somovilla-Crespo, B., Cibrián, D., Acosta, B., Adrados, M., Muñoz-Calleja, C., Cuevas, C., Sánchez-Madrid, F., Avilés, P., Zapata, JM. (2020). *CD13 as a new tumor target for antibody-drug conjugates: validation with the conjugate MI130110.* J Hematol Oncol. 13(1): 32.

Ramos-Tomillero, I., Pérez, G., Somovilla-Crespo, B., Sánchez-Madrid, F., Cuevas, C., Zapata, JM., Domínguez, JM., Rodríguez, H., Albericio, F. (2020). *From Ugi Multicomponent Reaction to Linkers for Bioconjugation.* ACS Omega. 5(13): 7424-7431.

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Perez-Chacon G, Adrados M, Vallejo-Cremades MT, Lefebvre S, Reed JC, Zapata JM. (2019). *Dysregulated TRAF3 and BCL2 Expression Promotes Multiple Classes of Mature Non-hodgkin B Cell Lymphoma in Mice.* Front Immunol. 2019 Jan 11;9:3114. doi: 10.3389/fimmu.2018.03114. eCollection 2018.

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Funding

"Papel de TRAF1 en la regulación de la función de los miembros de la familia de los TNFR en linfocitos T: implicaciones en la maduración del timo y en patologías de linfocitos T." Financiado por: Instituto de Salud Carlos III. Año 2017-2019

"Factores Asociados a los receptores de TNF (TRAF)-3 y Motivo Tripartito(TRIM)-37: Nuevas funciones en control de la inmunidad innata y adaptativa en respuesta a patógenos." Financiado por: Ministerio de Ciencia e Innovación. Año 2020-2022

"Ayuda extraordinaria." Financiado por: Consejo Superior de Investigaciones Científicas. Año 2020-2020

4 Experimental Models of Human Diseases

2019-2020

4 Experimental Models of Human Diseases

Department of Experimental models for Human diseases

The development of experimental models of the different human diseases is one of the main reasons behind the present progress in medical sciences. These models allow for the study of the molecular bases of diseases and are of paramount importance for the discovery and testing of new drugs and treatments. The development of these models is the common goal of the research that takes place in our Department. The model systems used and the diseases studied are diverse. Among the diseases are mitochondrial-related pathologies, neurodegenerative, cardiovascular, muscular, bone diseases and syndromes, telomere-related diseases and cancer. Some of diseases studied are of very low prevalence and are considered rare diseases. Apart these basic studies, an important part of our work is translational and focused on patient care and attention. For example, genetic studies on patient samples aimed on the molecular diagnosis of several diseases are carried out. These clinically-oriented studies are carried out in collaboration with several Hospitals of the Spanish health system.

Human clinical samples and cell lines are used for these studies together with experimental models such as mice, Drosophila melanogaster, Saccharomyces cerevisiae or Dictyostelium dicoideum. The results obtained in recent years have allowed for the identification of new therapeutic targets, molecules with possible pharmaceutical application and biomarkers.

The Department is presently composed of 18 tenured scientists. Among the research topics are the following:

- **Cardiac electrophysiology** (Carmen Valenzuela, Teresa Gonzalez)
- **Neurodegenerative diseases** (Ana Perez-Castillo)
- **Mitochondrial biogenesis and physiopathology** (Miguel Fernandez Moreno, Juan José Arredondo, Rafael Garesse)
- **Bone and muscular hereditary diseases and ciliopathies** (Victor Ruiz and Francesc García Gonzalo)
- **Telomere-related diseases** (Rosario Perona, Leandro Sastre)
- **Protein trafficking and degradation** (Olivier Vincent, Ricardo Escalante)
- **Tumor microenvironment and metastasis** (Francisco Portillo, Pilar Eraso, María Jesús Mazón, together with Amparo Cano, of the Cancer Biology Department)
- **Translational oncology** (Juan Carlos Lacal)
- **DNA replication and repair** (Modesto Redrejo)
- **Chromosome instability and therapy response in gastric cancer** (Isabel Sanchez-Perez)

Role of EMT-TFs and Lysyl Oxidase-like 2 and 3 (LOXL2/LOXL3) in tumour progression and metastasis

Principal Investigator
Eraso Mazmela, Pilar

Research Lines

Within the main project "Role of Snail, E47 and lysyl Oxidase-like 2 and 3 (LOXL2/LOXL3) in tumor progression and metastasis" (see laboratory of Amparo Cano), we are specifically interested in LOXL2 functions. LOXL2 is a member of the lysyl oxidase family that catalyze the oxidative de-amination of lysine residues promoting covalent cross-linkages. Accumulating evidence indicates the participation of lysyl oxidases in diverse extra- and intra-cellular functions ranging from extracellular matrix maturation to tumorigenesis and metastasis. A link between intracellular LOXL2, the UPR pathway and EMT induction has been established. Increased intracellular LOXL2 expression is a poor prognosis factor in human squamous cell carcinomas and is associated to metastatic basal breast carcinomas (BBC). Our main research interest focus on further understanding the role of LOXL2 in tumorigenesis and metastasis; we aim to dissect the contribution of intracellular functions of LOXL2 and their interrelation with EMT-TFs in initiation and progression of breast carcinoma.

During the last two years (2019 and 2020), we have addressed the characterization of LOXL2 nuclear interactome in human tumor-derived cell lines with or without LOXL2 overexpression. Using immunoprecipitation of LOXL2 followed by mass spectrometry analysis we have identified several proteins of the LOXL2 nuclear interactome. Some of these proteins are involved in RNA metabolism and their interaction with LOXL2 has been confirmed by in vitro co-immunoprecipitation. Our present work is dedicated to unveil the functional consequences of these interactions for LOXL2 intracellular function.

Publications

Santamaría, PG, Mazón, MJ., Eraso, P., Portillo, F. (2019). *UPR: An Upstream Signal to EMT Induction in Cancer.* J Clin Med. 8(5).

Funding

"Contribution of LOXL2 and LOXL3 to tumor progression and metastasis." Financiado por: Ministerio de Economía y Competitividad. Year 2017-2020

Molecular mechanism of autophagy in Dictyostelium and human cell lines with a focus on rare diseases

Principal Investigator
Escalante Hernández, Ricardo

Predoctoral
Tornero Écija, Alba Rocío

Support Personnel
Ramos García, María Angeles

Undergraduate Student
Castro Mena, Natalia

Master Students
Antón Esteban, laura
Navarro Gómez, Cristina

Visiting investigator
Yuka -, Yajima

Keywords: Autophagy, autophagosome biogenesis, BPAN disease, Chorea-acantocytosis, Dictyostelium discoideum, Saccharomycces cerevisiae, VMP1, VPS13, WDR45, WIPI.

Research Lines

Autophagy is a degradative process of cellular components conserved in all eukaryotes. In certain circumstances, like starvation or cellular stress, parts of the cytoplasm are engulfed in double membrane vesicles called autophagosomes that fuse later to lysosomes where they are degraded. Autophagy is also required for the elimination of protein aggregates, dysfunctional organelles or pathogens and it is therefore of immense importance in diverse pathological processes as well as in aging.

We use several model systems to study the molecular mechanism of autophagosome formation and the role of autophagy in rare diseases, using biochemistry, genetics and cell biology techniques.

The specific aims are:

· Identification of new proteins of the autophagic machinery and its regulation
· Function of VMP1 in the origin of autophagosomes and regulation of PtdIns3P signalling
· Study of the function of VPS13 protein family. The possible role of autophagy in the associated diseases: Chorea-acanthocytosis and Cohen Syndrome
· WIPI proteins in autophagy and its role in the rare disease BPAN

Publications

Escalante, R., Cardenal-Muñoz, E. (2019). *The Dictyostelium discoideum model system.* Int. J. Dev. Biol. 63(8-9-10): 317-320.

Tábara, LC., Vincent, O., Escalante, R. (2019). *Evidence for an evolutionary relationship between Vmp1 and bacterial DedA proteins.* Int. J. Dev. Biol. 63(1-2): 67-71.

Muñoz, S., Tornero, AR., Vincent, O., Escalante, R. (2019). *VPS13A is closely associated with mitochondria and is required for efficient lysosomal degradation.* Dis Model Mech. 12(2).

"Función de los sitios de contacto entre membranas y el tráfico de lípidos en autofagia." Financiado por: Ministerio de Ciencia, innovación y universidades. Año 2019-2021

"Advancing our understanding of the molecular function of VPS13A and development of a human cellular model for preclinical studies." Financiado por: Advocacy for Neuroacanthocytosis Patients (ANP). UK.Year 2020-2023

Molecular Basis of Ciliopathies

Principal Investigator
García Gonzalo, Francesc

Investigators under contract
Sierra Rodero, María Belén
Martín Hurtado, Ana

Postdoctoral
Barbeito González, Pablo

Predoctoral
Martín Morales, Raquel

Undergraduate Students
Gallego Colastra, Leticia
Moreno de la Cruz, Paula
Palacios Blanco, Inés
Pereira Bouzas, Paula
Rodríguez Herrera, K. Gissel

Master Students
Cilleros Rodríguez, Darío

Keywords: Primary cilia, ciliopathies, phosphoinositides, GPCR, INPP5E, HTR6, SSTR3, NRF2.

Research Lines

Identification of ciliary targeting signals in INPP5E

Identification and functional analysis of ciliary targeting sequences (CTSs) in INPP5E, a phosphoinositide 5-phosphatase whose mutations cause ciliopathies such as Joubert and MORM syndromes.

Functional analysis of B9 ciliopathy proteins

Functional analysis of B9D1, B9D2 and MKS1, three proteins that localize at the ciliary transition zone, control ciliary membrane composition, and are mutated in ciliopathies such as Meckel and Joubert syndromes.

Role of INPP5E in ciliary ectovesicle release

Role of the ciliopathy-associated phosphoinositide 5-phosphatase INPP5E in growth factor-dependent ciliary ectovesicle release.

Control of ciliogenesis and Hedgehog signaling by NRF2

Mechanisms underlying how the NRF2 transcription factor, a master regulator of cellular antioxidant and stress responses, regulates ciliogenesis and Hedgehog pathway responsiveness.

Publications

Martin-Hurtado, A; Lastres-Becker, I; Cuadrado, A; Garcia-Gonzalo, FR. (2020). *NRF2 and Primary Cilia: An Emerging Partnership.* Antioxidants (Basel). 9(6): 475.

Barbeito, P; Tachibana, Y; Martin-Morales, R; Moreno, P; Mykytyn, K; Kobayashi, T; Garcia-Gonzalo, FR. (2020). *HTR6 and SSTR3 ciliary targeting relies on both IC3 loops and C-terminal tails.* Life Sci. Alliance. 4: e202000746.

Martin-Hurtado, A; Martin-Morales, R; Robledinos-Anton, N; Blanco, R; Palacios-Blanco, I; Lastres-Becker, I; Cuadrado, A; Garcia-Gonzalo, FR. (2019). *NRF2-dependent gene expression promotes ciliogenesis and Hedgehog signaling.* Sci. Rep. 9(1): 13896.

Funding

"Sintonizando la Antena Celular: Mecanismos Moleculares de Control de la Composición de Cilios Primarios." Financiado por: Ministerio de Ciencia e Innovación (MICINN). Año 2020-2024

Ion channels (II)

Principal Investigator
González Gallego, Teresa

Predoctoral
Vera Zambrano, Alba

Undergraduate Students
Berlinches López, Judith
Baena Nuevo, María

Master Students
Baena Nuevo, María

Research Lines

Kv1.5 channelosome

Kv1.5 channels generate the outward potassium current IKur, responsible for the atrial repolarization process and the atrial action potential duration in humans. These channels represent a pharmacological target for the development of antiarrhythmic drugs useful in the treatment of supraventricular arrhythmias, such as atrial fibrillation. Ion channels form signalling complexes or channelosomes and we are interested in the protein composition of the human cardiac Kv1.5 channelosome, as it is an essential feature for an optimal, fast and efficient transmission of signals either from the extracellular or from intracellular medium.

Ion channels pharmacology

Ion channels are ubiquitous proteins involved in many different physiological and pathophysiological processes. They are suitable drug targets for a plethora of drugs useful for the treatment of many diseases such as cardiac and neurological diseases. In addition, many drugs exert their undesirable effects by affecting ion channel function. We are interested in elucidate the mechanisms of action of new drugs acting on ion channels and in the determination of the binding sites of these drugs to the channels.

Channelopathies

One of our research lines is the electrophysiological characterization of new mutations on ion channels or in its modulatory subunits. These are new mutation found in the clinics that can induce cardiac arrhythmias as Long QT, Short QT and Brugada Syndromes.

Potassium channels and cancer

Ion channels are involved in other cellular functions apart from regulation of membrane potential and electrical signalling such as cell proliferation, apoptosis and differentiation. Therefore, there is an increasing interest in understanding the role of ion channels in the progression of cancer.

Role of EMT-TFs and Lysyl Oxidase-like 2 and 3 (LOXL2/LOXL3) in tumour progression and metastasis

Principal Investigator
Mazón Calpena, María Jesús

Research Lines

Role of LOXL2 in tumorigenesis and metastasis: contribution of intracellular functions of LOXL2

Within the main project "Role of Snail, E47 and lysyl Oxidase-like 2 and 3 (LOXL2/LOXL3) in tumor progression and metastasis" (see laboratory of Amparo Cano), we are specifically interested in LOXL2 functions. LOXL2 is a member of the lysyl oxidase family that catalyze the oxidative de-amination of lysine residues promoting covalent cross-linkages. Accumulating evidence indicates the participation of lysyl oxidases in diverse extra- and intra-cellular functions ranging from extracellular matrix maturation to tumorigenesis and metastasis. A link between intracellular LOXL2, the UPR pathway and EMT induction has been established. Increased intracellular LOXL2 expression is a poor prognosis factor in human squamous cell carcinomas and is associated to metastatic basal breast carcinomas (BBC). Our main research interest focus on further understanding the role of LOXL2 in tumorigenesis and metastasis; we aim to dissect the contribution of intracellular functions of LOXL2 and their interrelation with EMT-TFs in initiation and progression of breast carcinoma.

During the last two years (2019 and 2020), we have addressed the characterization of LOXL2 nuclear interactome in human tumor-derived cell lines with or without LOXL2 overexpression. Using immunoprecipitation of LOXL2 followed by mass spectrometry analysis we have identified several proteins of the LOXL2 nuclear interactome. Some of these proteins are involved in RNA metabolism and their interaction with LOXL2 has been confirmed by in vitro co-immunoprecipitation. Our present work is dedicated to unveil the functional consequences of these interactions for LOXL2 intracellular function.

Publications

Santamaría, PG, Mazón, MJ., Eraso, P., Portillo, F. (2019). *UPR: An Upstream Signal to EMT Induction in Cancer*. J Clin Med. 8(5).

Funding

"Contribution of LOXL2 and LOXL3 to tumor progression and metastasis." Financiado por: Ministerio de Economía y Competitividad. Year 2017-2020

Identification of new therapeutic targets for the treatment of neurodegenerative diseases. Regulation of adult brain neurogenesis

Principal Investigator
Pérez Castillo, Ana María

Co-Principal Investigator
Morales García, Jose Ángel

Associate Investigators
Gine Domínguez, Elena
Santos Montes, Angel

Investigators under contract
Sierra Magro, Ana
Van Bulck, Michiel

Support Personnel
Sanz San-Cristóbal, Marina
Alonso Gil, Sandra

Undergraduate Students
Carnicero Senabre, Daniel
Martínez Cotrina, Aitana
Muriel González, Alicia

Master Students
Lozano Muñoz, David
Alarcón Gil, Jesús
Cimpean -, Anda
Lombardo Cristina, Víctor
Moreno Rupérez, Álvaro
Orihuel Bañuls, Gracia
Carceller Lopez, Elena

Keywords: Alzheimer, APP, Ayahuasca, C/EBPβ,LRRK2, neurogenesis, neuroinflammation, neuroprotection, Parkinson.

Research Lines

Identification and analysis of new therapeutic targets forthe development of new treatments with neuroprotective, anti-inflammatory and neurogenic effect.

Our research is **focused on identification of new therapeutic targets for the treatment of neurodegenerative diseases.** To that purpose our work is based on the use of several preclinical models that mimics some of the aspects that characterize Alzheimer's and Parkinson's diseases. Using these models we identify and analyze potential cellular targets in order to develop new drugs for the treatment of these diseases. We have studied the processes that characterize these pathologies describing the involvement of several genes in different brain disorders such as C/EBPβ, whose inhibition has been shown to have a potent neuroprotector in a Parkinson's model. Some other gen of interest we have analyzed is PDE7, which codes for an enzyme involved in the degradation of cAMP. Our results suggest that this gene is expressed early in degenerative processes that affect the dopaminergic neurons of the substantia nigra, as well as promotes the appearance of pro-inflammatory phenomena.

Also, a main focus of the lab concerns research **on neurogenesis and aging**. In this regard we are expanding our previous observations that describe the neurogenic effect of certain components of the brew known as Ayahuasca. In this sense, we are currently working in the role of different new cellular targets which can expand our knowledge of the processes that lead to improved neurogenesis and that can be of use for a better understanding and new treatments of aging-related disorders. In fact, we have demonstrated that N,N-dimethyltrypatamine compond found in th hellucinogenic tea Ayahuasca, as well as the alkaloids of Banisteriopsis caapi, the other plat source of this brew, regulates adult neurogenesis in vitro and in vivo.

Concerning Parkinson disease,through metabolomic analysis we have also shown that in the development of this disease, there are some significant changes in mice and patients with familiar and idipiopathis Parkinson.

Publications

Herrera-Arozamena, C., Estrada-Valencia, M., Pérez, C., Lagartera, L., Morales, JÁ., Pérez-Castillo, A.M, Franco-Gonzalez, JF., Michalska, P., Duarte, P., León, R., López, MG., Mills, A., Gago, F., García, ÁJ., Fernández, R., Cuadrado, A., Rodríguez-Franco, MI. (2020). *Tuning melatonin receptor subtype selectivity in oxadiazolone-based analogues: Discovery of QR2 ligands and NRF2 activators with neurogenic properties.* Eur J Med Chem. 190: 112090.

Sethi, J., Van, M., Suhail, A., Safarzadeh, M., Pérez-Castillo, A.M, Pan, G. (2020). *A label-free biosensor based on graphene and reduced graphene oxide dual-layer for electrochemical determination of beta-amyloid biomarkers.* Mikrochim Acta. 187(5): 288.

Sethi, J., Van, M., Suhail, A., Safarzadeh, M., Pérez-Castillo, A.M, Pan, G. (2020). *Correction to: A label-free biosensor based on graphene and reduced graphene oxide dual-layer for electrochemical determination of beta-amyloid biomarkers.* Mikrochim Acta. 187(6): 338.

Yakhine-Diop, SMS., Morales, JÁ., Niso-Santano, M., González-Polo, RA., Uribe-Carretero, E., Martinez-Chacon, G., Durand, S., Maiuri, MC., Aiastui, A., Zulaica, M., Ruíz-Martínez, J., López de Munain, A., Pérez-Tur, J., Pérez-Castillo, A.M, Kroemer, G., Bravo-San Pedro, JM., Fuentes, JM. (2020). *Metabolic alterations in plasma from patients with familial and idiopathic Parkinson's disease.* Aging (Albany NY). 12.

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Funding

"CCAAT/Enhancer binding protein β (C/EBPβ) como modulador de la neuroinflamación. Una nueva diana terapéutica en la enfermedad de Parkinson." Financiado por: Ministerio De Economia, Industria Y Competitividad. Year 2018-2020

"Blood Biomarker-based Diagnostic Tools for Early-stage Alzheimer's Disease." Financiado por: Union Europea / Marie Skłodowska-Curie Actions. Year 2016-2021

"Bases metabólicas de la degeneración." Financiado por: Comunidad de Madrid. Year 2018-2021

Patents

"Combination product for the treatment of neurological and/or psychiatric disorders." Year 2020

Study of diseases related to telomeric shortening. Diagnosis and new therapies

PRINCIPAL INVESTIGATOR
Perona Abellón, Rosario

ASSOCIATE INVESTIGATOR
Ibáñez de Cáceres, Inmaculada

POSTDOCTORAL
Pintado Berninches, Laura
García Arias-Salgado, Elena
Benítez Buelga, Carlos

PREDOCTORAL
Fernández Varas, Beatriz

SUPPORT PERSONNEL
Manguán García, Cristina

Keywords: Telomere, dyskeratosis congenita, pulmonary fibrosis, aplastic anemia, DUSP1, DUSP6, cancer.

Research Lines

Diagnosis and new therapies in dyskeratosis congenita and idiopathic pulmonary fibrosis

The group has been focussed in the last years in the study of **telomeres and telomere-related diseases.** Telomeres are nucleo-protein complexes at the terminal end of the chromosomes and protect them from degradation. The human telomere sequence consists in tandem repetitions of the AATGGG sequence that is recognized by a protein complex named shelterin to form a stable heterochromatin structure. Placed at the end of a lineal DNA molecule, telomeres cannot be completely replicated by DNA polymerases and that results in shortening in every replication cycle. This process is prevented by the activity of the telomerase complex that elongates telomeres in embryonic, germinal and tissue stem cells. In most cells of somatic tissues telomerase activity is not expressed and their telomeres are shortened with the aging of the individual. Excessively short telomeres produce cell-cycle arrest, senescence or apoptosis that is one of the causes of aging.

There are a number of rare diseases that are being studied by our group, including dyskeratosis congenita, aplastic anemia and pulmonary fibrosis that harbour mutations in genes coding for components of the telomerase, shelterin complexes and auxiliary proteins. Shortening of telomeres is also observed in related diseases such as Ataxia telangiectasia.

Our research is focussed in the following topics:

1. **Study of the GSE4 peptide as possible therapy for telomere-biology disorders.** Our laboratory has shown that a small peptide derived from dyskerin, a component of the telomerase complex can increase telomerase activity in cells obtained from dyskeratosis congenita patients. Expression of GSE4 in patient's cells increases expression of telomerase components, telomerase activity and cell proliferation. In addition, DNA damage and oxidative stress get decreased. Similar results have been obtained in cells from Ataxia Telangiectasia patients and mor recetly with idiopathic pulmonary fibrosis animal model.
2. **Diagnosis of telomere-related diseases.** We are collaborating with several hospitals in the diagnosis of telomere-related diseases that are very difficult to diagnose based exclusively on clinical criteria. Telomere length is first determined since patients affected by these diseases have very short telomeres, below the 10% and frequently below the 1% of the control population. Once diagnosed, the molecular cause of the disease is studied by determining possible pathological variants in the genes coding for proteins involved in telomere homeostasis. Massive sequencing

of a panel of related genes and exome sequencing are used in these studies. Finally, we perform in vitro studies to determine the functional relevance of the possibly pathogenic variants.

Role of the MAPK phosphatase DUSP1 in squamous skin cell carcinoma

DUSP1 is a member of DUSPs, highly expressed in different types of human tumors, including non-small-cell lung cancer (NSCLC), breast, ovarian, bladder, osteosarcoma and in prostate cancer in early stages of disease. We have previously demonstrated that DUSP1 has an essential function in NSCLC biology, both in tumor growth and in the response to cisplatin treatment. Cutaneous Squamous cell Carcinoma (cSCC) is the second most frequent type of nonmelanoma skin cancer, with 38.16 cases per 100,000 person-years in Spain. Combined with such a high frequency, even a 1% mortality rate means it will kill over 200 people per year in Spain alone. Still, its treatment hasn't improved over the years– it's the 2nd worst treated cancer in Spain. Moreover, because of changes in lifestyle and the environment, the incidence of skin cancer is steadily increasing. The main causative agent of skin cancer is the UV component of sunlight. UV radiation (UVR) produces two major lesions in DNA, the cyclobutane pyrimidine dimer (CPD) and the (6-4) photoproduct [(6-4) PP], both of which are mutagenic and carcinogenic in animal model systems and are thought to be the primary cause of skin cancer in humans. Our preliminary findings support a role of DUSP1 as a tumor suppressor gene in non-melanoma skin cancer. We found that upon DMBA/TPA induction DUSP1-/- mice are more prone to progress from papilloma to invasive cSCC. Also, we found that deficient DUSP1 mice showed an increased proliferation of basal cells in the epidermis in response to TPA induction, supporting a role of DUSP1 as tumor suppressor. cSCCs are maintained by basal skin stem cells, also called Tumor Propagating Cells (TPCs). Interestingly, our preliminary results showed not only that DUSP1 depletion in mice is associated with higher proliferation in cSCC but also that DUSP1 mRNA levels are significantly reduced in keratinocytes obtained from patients with invasive basal carcinoma and cSCC compared to normal skin from healthy donors. Based on these preliminary findings we propose to study the mechanisms involved in the inhibition of DUSP1 expression in cSCC by using human biopsies and animal models, together with chromating remodeling, RNAseq and Chipseq studies.

Publications

Peña-Chilet M, Roldán G, Perez-Florido J, Ortuño FM, Carmona R, Aquino V, Lopez-Lopez D, Loucera C, Fernandez-Rueda JL, Gallego A, García-Garcia F, González-Neira A, Pita G, Núñez-Torres R, Santoyo-López J, Ayuso C, Minguez P, Avila-Fernandez A, Corton M, Moreno-Pelayo MÁ, Morin M, Gallego-Martinez A, Lopez-Escamez JA, Borrego S, Antiñolo G, Amigo J, Salgado-Garrido J, Pasalodos-Sanchez S, Morte B; Spanish Exome Crowdsourcing Consortium, Carracedo Á, Alonso Á, Dopazo J and Spanish Exome Crowdsourcing Consortium: Fátima Al-Shahrour, Rafael Artuch, Javier Benitez, Luis Antonio Castaño, Ignacio Del Castillo, Aitor Delmiro, Carmina Espinos, Roser González, Daniel Grinberg, Encarnación Guillén, Pablo Lapunzina, Esther Lopez, Ramón Martí, Montserrat Milá, José Mª Millán, Virginia Nunes, Francesc Palau, Belen Perez, Luis Pérez Jurado, Rosario Perona, Aurora Pujol, Feliciano Ramos, Antonia Ribes, Jordi Rosell, Eulalia Rovira, Jordi Surrallés, Isabel Tejada, Magdalena Ugarte. (2020). *CSVS, a crowdsourcing database of the Spanish population genetic variability.* Nucleic Acids Res.

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Moncho-Amor, V. Pintado-Berninches, L. Ibañez de Caceres, I. Martin-Villar, E. Quintanilla, M. Chakravarty, P. Cortes-Sempere, M. Fernández-Varas, B. Rodriguez-Antolín, C. de Castro, J. Sastre, L. Perona, R. (2019). *Role of Dusp6 Phosphatase as a Tumor Suppressor in Non-Small Cell Lung Cancer.* Int J Mol Sci. 20: 2036.

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(2019). *Understanding the evolving phenotype of vascular complications in telomere biology disorders.* Angiogenesis. 22: 95-102.

Doctoral theses and other works

Beatriz Fernández Varas

"Uso del péptido GSE4 como posible tratamiento de la fibrosis pulmonar".

Autonoma de Madrid. Medicina. 2020. Director/es: Rosario Perona. Calificación: Apto Cum laude

Funding

"DUSP1 as a key regulator in the progression of invasive cutaneous squamous cell carcinoma." Financiado por: ISCIII FIS Co-IP L Sastre. Año 2020-2022

"Neumonitis y fibrosis pulmonar inducidas por radioterapia. Posibles tratamientos con peptidos encapsulados en nanoparticulas."

Financiado por: ISCIII FIS Co_IP L. Sastre. Año 1918-1920

"Acortamiento telomérico y variantes de genes de la telomerasa asociados a neumonitis y fibrosis pulmonar secundaria a radioterapia en pacientes con cáncer de pulmón de celula no pequeña." Financiado por: SEPAR. Año 2019-2020

"Estudio de la longitud telomérica en pacientes con FP como criterio para conocer las bases genéticas y adecuado consejo genético familiar de la enfermedad." Financiado por: FEDER Federacion Española de Enfermedades raras. Año 2019-2020

Role of EMT-TFs and Lysyl Oxidase-like 2 and 3 (LOXL2/LOXL3) in tumour progression and metastasis

PRINCIPAL INVESTIGATOR
Portillo Pérez, Francisco

Research Lines

LOXL2 and LOXL3 proteins belong to the lysyl oxidase family, constituted by lysyl oxidase (LOX) and four lysyl oxidase-like paralogs (LOXL1 to LOXL4). **LOX family members are lysine-tyrosylquinone-dependent copper amine oxidases** that catalyze the oxidative de-amination of the ε-amino group in certain peptidyl lysine residues promoting covalent cross-linkages.

Accumulating evidence indicates the participation of lysyl oxidases in a plethora of biological extra- and intra-cellular functions ranging from extracellular matrix maturation to tumorigenesis and metastasis. Our previous studies described that intracellular LOXL2 and LOXL3 regulate Snail1 stability and functionality being in the case of LOXL2 independent of its catalytic activity, and further studies indicated that LOXL2 plays also Snail1-independent roles in EMT and cooperates with other EMT-TFs, like E47/TCF3. LOXL2 perinuclear localization is a poor prognosis factor in human squamous cell carcinomas and it is associated to metastatic basal breast carcinomas (BBC). Regarding LOXL3, our studies identified LOXL3 overexpression in a broad cohort of human melanoma samples and an essential role for LOXL3 in melanoma cell survival.

Our main research interest **focus on a deep understanding of the role of LOXL2 and LOXL3 in tumorigenesis and metastasis.**

In particular we aim to dissect the contribution of their intra and extracellular functions to initiation and/or progression of breast carcinoma and melanoma, respectively. To this end, we have develop genetically modified mouse models (GEMs) for conditional deletion of Loxl2 and Loxl3, as well as conditional overexpression of Loxl2 in specific mouse cancer model, as breast and melanoma, together with a broad range of mouse and human tumor-derived cell lines manipulated for Loxl2 or Loxl3 expression. We have also generated and characterized GEM with conditional deletion of E2A gene (coding for E47/E12 EMT-TF) in the context of PyMT breast cancer.

During the last two years (2017-2018) the main objectives addressed are summarized as:

1. Characterization of LOXL2 action in breast tumors. In vivo and in vitro studies have allowed identify the prominent action of Loxl2 in lung breast cancer metastasis. Mechanistically, Loxl2 induces invasion by regulating Snail1 stability and favors the generation of the lung pre-metastatic niche.
2. Characterization of LOXL3 action in melanoma. In vitro analyses in human melanoma cell lines identified LOXL3 pro-survival action mediated by regulation of DNA repair and mitotic exit contributing to maintain the high genetic instability of melanoma. Further studies on GEM of melanoma support a role for Loxl3 in melanoma initiation and lymph node metastatic dissemination
3. Characterization of E47/E12 action in breast cancer initiation and metastasis. The GEM of E2A KO gene in the PyMT breast cancer model has provided strong evidence for the participation of E47 EMT-TF in breast cancer initiation and lung metastasis. At least, some of the E47 actions are mediated by functional interaction with Snail1 as well as with Loxl2.

Human Genetics and Molecular Pathology

Principal Investigator
Ruiz Pérez, Víctor Luis

Associate Investigators
González Casín, Roberto Angel
Gómez Carmona, Ricardo

Postdoctoral
Rivera Barahona, Ana

Predoctoral
Jiménez Estrada, Juan Andrés
Palencia Campos, Adrián

Support Personnel
Fernández Núñez, Elisa

Master Students
Guerrero Espinosa, Erika Marisol

Keywords: Ellis-van Creveld syndrome, EVC, EVC2, primary cilia, hedgehog signaling, GLI1, osteogenesis imperfecta, bone disorders.

Research Lines

ELLIS VAN-CREVELD SYNDROME AND PHENOTYPICALLY OVERLAPPING CILIOPATHIES

Ellis-van Creveld syndrome (EvC) is an autosomal recessive condro-ectodermic dysplasia primarily caused by mutations in *EVC* or *EVC2*. The proteins encoded by these genes form a protein complex localized at the base of the primary cilium and act as a positive mediators of hedgehog signaling (Hh). In vertebrates, the transduction of the Hh signal is completely dependent on the primary cilium, a structure that has received enormous attention in recent years due to the large number of human disorders associated with mutations in proteins that localize to this organelle. Our goal is to continue progressing in the understanding of the molecular physiopathology of EvC and other phenotypically overlapping conditions and the function of Evc and Evc2 within the Hh pathway.

OSTEOGENESIS IMPERFECTA AND BONE FRAGILITY

Osteogenesis imperfecta (OI) is a bone-related disorder characterized by an increased risk of fractures. The large majority of OI cases have a dominant mode of inheritance and are caused by mutations in *COL1A1* or *COL1A2*, the genes encoding the procolagen type I peptidic chains. However, there is a small fraction of cases with mutations in other genes which have resulted to be highly genetically heterogeneous. Our goal in this line of research is to ivestigate the molecular pathomechanisms leading to this condition and other forms of bone fragility.

Publications

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Aglan, M., Jønch, AE., De Luca, A., Mortier, G., Cormier-Daire, V., Ziegler, A., Wallis, M., Lapunzina, P., Herberg, FW., Taylor, SS., Ruiz-Perez, V.L (2020). *Germline and Mosaic Variants in PRKACA and PRKACB Cause a Multiple Congenital Malformation Syndrome.* Am J Hum Genet.

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Ballesta-Martínez, M., Rosell, J., Aglan, MS., Temtamy, S., Otaify, GA., Cuevas-Catalina, L., Torres-Saavedra, M., Nevado, J., Tenorio, J., Lapunzina, P., Bermejo-Sánchez, E., Ruiz-Perez, V.L (2019). *Heterozygous pathogenic variants in GLI1 are a common finding in isolated postaxial polydactyly A/B.* Hum. Mutat.

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Doctoral theses and other works

Adrián Palencia Campos

"Caracterización molecular de pacientes con el síndrome de Ellis-van Creveld: Análisis de variantes e identificación de nuevos genes responsables de esta patología".

Universidad Autonóma de Madrid (UAM). Facultad de Medicina. 2019. Director/es: Víctor Luis Ruiz. Calificación: Sobresaliente Cum Laude

Funding

"Avances en el conocimiento de las bases genéticas y moleculares de enfermedades raras asociadas a defectos congénitos." Financiado por: Ministerio de Ciencia e Innovación. Año 2020-2023

"Caracterización de procesos moleculares del desarrollo óseo implicados en enfermedades esqueléticas de base genética." Financiado por: Ministerio de Economía y Competitividad. Año 2016-2020

Chromosome Instability & Therapy

PRINCIPAL INVESTIGATOR
Sánchez Pérez, María Isabel

ASSOCIATE INVESTIGATOR
Gómez Quiroga, Adoración

POSTDOCTORAL
Pajuelo Lozano, Natalia
Melones Herrero, Jorge

MASTER STUDENTS
Cama García, Marta
Menéndez Ribes, Marta

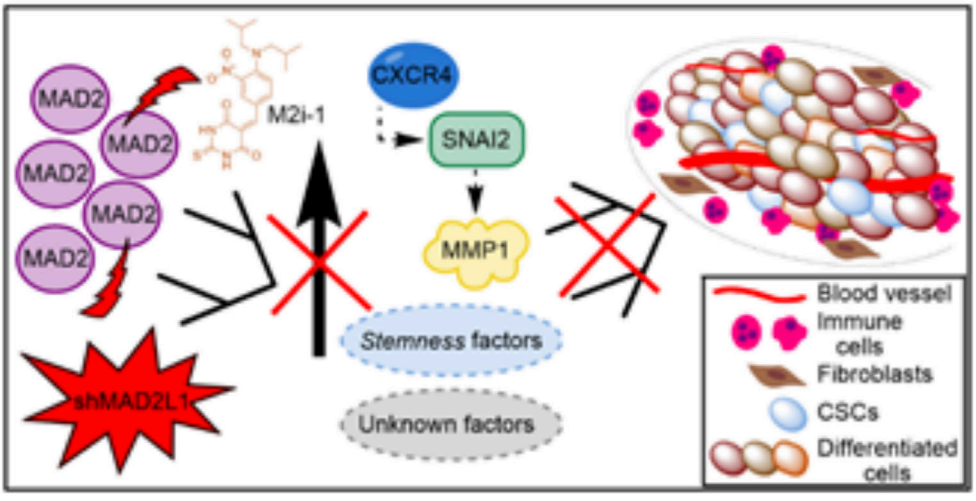
Keywords: Cell cycle, mitosis, chromosome instability, metalodrugs, cancer therapy.

Research Lines

Overview

Our group centres its research on understanding how deregulation of mitotic checkpoint drives tumorigenesis and threrapy response in gastric cáncer (GC). GC is still the fourth main cause of cancer related death all over the world. In GC, adenocarcinomas gastrectomy, and adjuvant radio/chemotherapy are the election treatment. Our focus of interest is to gain insight into the molecular pathways that condition the response to specific therapies in GC. Furthermore, GC is asolid tumor characterized by high rates of chromosomal instability (CIN) and aneuploidy. One of the causes of CIN and aneuploidy is a failure in mitosis due to weakness or overactivation of the mitosis control point known as spindle assembly checkpoint (SAC). Throughout the years, our laboratory has analyzed the expression levels of the different components of SAC, correlating the key mitotic proteins Mad2 and BubR1 with CIN. In addition, the interference of the

A



B

corresponding genes modifies the migration and invasion of cells derived from GC, so our studies are directed to the molecular mechanism that intervenes in this effect. Cancer stem cells are responsable for recidives, heterogenity and treatment resistance of tumor. Mitosis plays important roles balancing stem cells between self-renewal and differentiation to progenitor cells by regulating symmetric and asymmetric division. Mitotic checkpoint proteins play an important role maintaining the CSC population, for example Mad2 (Figure A).

Our studies have enhanced our understanding of the impact of Mad2 on GCSC and have provided novel promising cisplatin drugs with antitumoral potential against these cells (Figure B). We selected a series of aliphatic amine platinum compounds bearing chloride and/or iodide as the leaving groups. The complexes' cytotoxicity and interaction with DNA indicated differences in the reactivity. Now, we are studying the molecular mechanism of action on tumoral cells. Our data reveals differences between them. Chlorido drugs showed similar behavior to cisplatin; they both required p53 to induce apoptosis but only cis-ipa showed DNA damage requirement for apoptosis induction. On the contrary, cis and trans iodido induced cell death independent of p53 activity, and they induced cell death through Bid activation, so their toxicity could be enhanced in a combined treatment with novel Bcl-2 protein family inhibitors. These findings represent a step forward in the search for new platinum-derived agents more specific and effective in the treatment of cancer.

Publications

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Funding

"Metallic drugs with alternative structures to explore their potential in Biological Chemistry and induce cell death and specific damage in tumors." Financiado por: Ministerio de Ciencia e Innovacin (MICINN). Año 2020-2023

"AptaBreast: Desarrollo Preclínico de un Aptámero para el Tratamiento del Cáncer." Financiado por: Ministerio de Ciencia e Innovacin (MICINN). Año 2020-2023

"3. Network for Equilibria and Chemical Thermodynamics Advanced Research (NECTAR)." Financiado por: Universidad de Mesina. Año 2018-2020

Telomeres and telomere-related diseases

Principal Investigator
Sastre Garzón, Leandro

Co-Principal Investigator
Perona Abellón, Rosario

Associate Investigators
Iarriccio Silva, Laura
García Arias-Salgado, Elena
Guerrero López, Rosa

Predoctoral
Bolelli -, Aronne
Carrascoso Rubio

Undergraduate Student
Mendoza Lupiáñez, Lucía

Master Students
Olalla Chantal, Carmen

Collaborations
Manguán García, Cristina.
Fernández Varas, Beatriz
Molina Molina, Maria

Keywords: Telomere, Dictyostelium discoideum, dyskeratosis congenita, pulmonary fibrosis, aplastic anemia.

Research Lines

Telomeres and telomere-related diseases

Researchers involved: García, E., Guerrero, R., Fernández, B., Carrascoso, C., Manguán, C., Perona, R., Sastre, L.

The group has been centred during these years in the study of telomeres and telomere-related diseases. Telomeres are nucleo-protein complexes that constitute the terminal end of the chromosomes and protect them from degradation. Human telomere sequence is formed by tandem repetitions of the AATGGG sequence that are recognized by a protein complex named shelterin to form a stable heterochromatin structure. Placed at the end of a lineal DNA molecule, telomeres cannot be completely replicated by DNA polymerases and would get shorter every replication cycle. This process is prevented by the activity of the telomerase complex that elongates telomeres in embryonic, germinal and tissue stem cells. Most cells in the somatic tissues do not express telomerase activity and their telomeres are shortened with the age of the individual. Excessively short telomeres produce cell-cycle arrest, senescence or apoptosis and is one of the causes of aging. Mutations in genes coding for components of the telomerase and shelterin complexes and auxiliary proteins cause a number of rare diseases that are being studied by our group, including dyskeratosis congenita, aplastic anemia and pulmonary fibrosis and also related diseases such as Ataxia telangiectasia. In particular, our projects are focussed in the following topics:

1. **Diagnosis of telomere-related diseases.** We are collaborating with several hospitals in the diagnosis of telomere-related diseases that are very difficult to identify based exclusively on clinical criteria. Telomere length is first determined since patients affected by these diseases have very short telomeres, below the 10% and frequently below the 1% of the control population. Once diagnosed, the molecular cause of the disease is studied by determining possible

pathological variants in the genes coding for proteins involved in telomere homeostasis. Massive sequencing of a panel of related genes and exome sequencing are used in these studies. Finally, we perform in vitro studies to determine the functional relevance of the possibly pathogenic variants.

2. **Telomere structure and extension in *Dictyostelium discoideum.*** Our group has experience in the use of this organism and we are using it as model system for the study of telomere-related diseases. As a firs step, we have determined the structure of D. discoideum telomeres and studied the elongation mechanism. Later on, we have generated dyskerin mutants that genocopy mutations found in dyskeratosis congenita patient to study the pathophysiology of these gene variants.
3. **Study of the GSE4 peptide as possible therapy for telomere-biology disorders.** Our laboratory has shown that a small peptide derived from dyskerin, a component of the telomerase complex can increase telomerase activity in cells obtained from dyskeratosis congenita patients. Expression of GSE4 in patient's cells increases expression of telomerase components, telomerase activity and cell proliferation. In addition, DNA damage and oxidative stress get decreased. Similar results have been obtained in cells from Ataxia Telangiectasia patients.

Role of the MAPK phosphatase DUSP1 in squamous skin cell carcinoma

Researchers involved: Guerrero, R., Manguán, C., Perona, R., Sastre, L.

DUSP1 is a member of the dual-specificty protein phosphatases, highly expressed in different types of human tumors, including non-small-cell lung cancer (NSCLC), breast, ovarian, bladder, osteosarcoma and in prostate cancer in early stages of disease. We have previously demonstrated that DUSP1 has an essential function in NSCLC biology, both in tumor growth and in the response to cisplatin treatment. Cutaneous Squamous cell Carcinoma (cSCC) is the second most frequent type of non-melanoma skin cancer, with 38.16 cases per 100,000 person-years in Spain. Combined with such a high frequency, even a 1% mortality rate means it will kill over 200 people per year in Spain alone. Still, its treatment hasn't improved over the years – it's the 2nd worst treated cancer in Spain. Moreover, because of changes in lifestyle and the environment, the incidence of skin cancer is steadily increasing. The main causative agent of skin cancer is the UV component of sunlight. UV radiation (UVR) produces two major lesions in DNA, the cyclobutane pyrimidine dimer (CPD) and the (6-4) photoproduct [(6-4) PP], both of which are mutagenic and carcinogenic in animal model systems and are thought to be the primary cause of skin cancer in humans. Our preliminary findings support a role of DUSP1 as a tumor suppressor gene in non-melanoma skin cancer. We found that upon DMBA/TPA induction DUSP1-/- mice are more prone to progress from papilloma to invasive cSCC. Also, we found that deficient DUSP1 mice showed an increased proliferation of basal cells in the epidermis in response to TPA induction, supporting a role of DUSP1 as tumor suppressor. cSCCs are maintained by basal skin stem cells, also called Tumor Propagating Cells (TPCs). Interestingly, our preliminary results showed not only that DUSP1 depletion in mice is associated with higher proliferation in cSCC but also that DUSP1 mRNA levels are significantly reduced in keratinocytes obtained from patients with invasive basal carcinoma and cSCC compared to normal skin from healthy donors. Based on these preliminary findings we propose to study the mechanisms involved in the inhibition of DUSP1 expression in cSCC by using human biopsies and animal models, together with chromatin remodeling, RNAseq and Chipseq studies.

Publications

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"Neumonitis y Fibrosis pulmonar inducidas por radioterapia. Posibles tratamientos curativos con péptidos encapsulados en nanopartículas, papel de JNK/MKP1 y del acortamiento telomérico." Financiado por: Fondo de Investigaciones Sanitarias. Año 2018-2020

"Acortamiento telomérico y variantes de genes de la telomerasa asociados a neumonitis y fibrosis pulmonar secundaria a radioterapia en pacientes con cáncer de pulmón de célula no pequeña." Financiado por: SEPAR. Año 2019-2020

"Estudio de la longitud telomérica en pacientes con FP como criterio para conocer las bases genéticas y adecuado consejo genético familiar de la enfermedad." Financiado por: FEDER. Año 2019-2020

Ion channels laboratory (I)

PRINCIPAL INVESTIGATOR
Valenzuela Miranda, Carmen

POSTDOCTORAL
Prieto Folgado, Ángela

PREDOCTORAL
Peraza Pérez, Diego Alberto

de Benito Bueno, Ángela
García Socuéllamos, Paula

SUPPORT PERSONNEL
Arias Sánchez, Sara

UNDERGRADUATE STUDENTS
Díez de Hoz, Sara

Márquez Marín, Isabel
Sánchez Ruz, Andrea

MASTER STUDENTS
González Merinero, Yaiza

Keywords: Ion channels, IKur, cardiac arrhytmias, Kv1.5 channels, regulatory subunits.

Research Lines

Our working group studies the modulation, both physiological and pharmacological, of ion channels present in the membrane of human cardiac myocytes. In order to record the activity of these ion channels, we use the patch-clamp technique. Ion channels are membrane proteins capable of generating and maintaining the duration of the action potential, as well as maintaining the membrane potential. Thus, they are responsible, among other effects, of the muscle contraction, heart rate and synaptic transmission. In particular, our group focuses on the study of potassium channels present in the human myocardium.

In the heart, the IKur current constitutes the most important potassium output current in the atrial repolarization process. The IKur is generated after the activation of Kv1.5 channels. Since these channels are responsible for cardiac atrial repolarization, they constitute potential drug targets for drugs useful in the treatment of cardiac arrhythmias.

Publications

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Moreno, C., Oliveras, A., Bartolucci, C., Muñoz, C., Cruz, ADL., Peraza, DA., Gimeno, JR., Martín-Martínez, M., Severi, S., Felipe, A., Lambiase, PD., González, T., Valenzuela, C. (2019). *Corrigendum to Supplemental Material: 'D242N, a KV7.1 LQTS mutation uncovers a key residue for IKs voltage dependence'* [Journal of Molecular and Cellular Cardiology, Volume 110, September 2017, Pages 61-69]. J. Mol. Cell. Cardiol. 128: 25.

Khemili, D., Valenzuela, C., Laraba-Djebari, F., Hammoudi-Triki, D. (2019). *Differential effect of Androctonus australis hector venom components on macrophage KV channels: electrophysiological characterization.* Eur. Biophys. J. 48(1): 1-13.

Doctoral theses and other works

Diego Alberto Peraza Pérez

"Modulación de la polarización de macrófagos por fármacos antitumorales. Estudio electrofisiológico". Universidad Autónoma de Madrid. Facultad de Medicina. 2020. Director/es: Carmen Valenzuela. Calificación: Sobresaliente cum laude por unanimidad

Yaiza González Merinero

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Andrea Sánchez Ruz

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Sara Díez de Hoz

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Funding

"Estudio en profundidad del canalosoma cardiaco de Kv1.5." Financiado por: MICINN. Año 2016-2019

"SAFETOLL - Desarrollo de nuevas aplicaciones terapéuticas de ApTOLL en enfermedades vasculares y autoinmunes." Financiado por: MICINN. Año 2018-2020

"Estudio de las proteinas integrantes de los canalosomas kv1.5 Y kv4.3 Como dianas para la fibrilacion auricular." Financiado por: MICINN. Año 2020-2023

"Ayudas extraordinarias para la preparación de proyectos 2019." Financiado por: Consejo Superior De Investigaciones Cientificas. Año 2020-2020

Patents

"Modulating Compounds Of Kchip2 And Its Use For The Treatment Of Cardiovascular Pathologies." Año 2019

Protein trafficking and Degradation

Principal Investigator
Vincent, Olivier

Predoctoral
Bueno Arribas, Miranda

Support Personnel
Cruz Cuevas, Celia

Master Students
Zapata del Baño, Antonia

Andreo López, Juana

Visiting investigator
Navas Hernández, Mª Angeles

Keywords: Autophagy, VMP1, VPS13, WIPI, BPAN, Chorea-acanthocytosis, Saccharomyces cerevisiae.

Research Lines

Our main goal is to characterize molecular mechanisms that control protein trafficking and degradation, and are potentially involved in human diseases.

In the past years, we have studied the regulatory mechanisms of arrestin-related proteins that function as endocytic adaptors for receptors and transporters at the plasma membrane.

More recently, we started a project to characterize the function of VMP1, VPS13 and WIPI proteins in the process of autophagy.

Autophagy is an intracellular degradation mechanism involved in several pathologies such as neurodegenerative diseases ChAc (Corea-acanthocytosis) and BPAN (Beta-propeller Protein-Associated Neurodegeneration). Mutations in the VPS13A and WIPI4/WDR45 genes are responsible for these diseases.

We aim to determine the function of these proteins in autophagy. VPS13 and WIPI proteins are evolutionarily conserved and we use the model organism S. cerevisiae to analyze their function and to identify their interactome by using the two-hybrid system.

The specific goals are:

· Identification of new VMP1 and VPS13 interactors and analysis of their possible role in lipid trafficking.
· Characterization of the molecular mechanisms involved in WIPI-mediated recruitment of the autophagic machinery to the autophagosomal membrane.
· Functional analysis of a new Atg2 interactor potentially involved in autophagy.
· Generation by CRISPR/Cas9 genome editing and characterization of WIPI KOs in HeLa cells.

Publications

Vincent, O., Gutierrez-Nogués, A., Trejo-Herrero, A., Navas, M. (2020). *A novel reverse two-hybrid method for the identification of missense mutations that disrupt protein-protein binding.* Sci Rep. 10(1): 21043.

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Funding

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