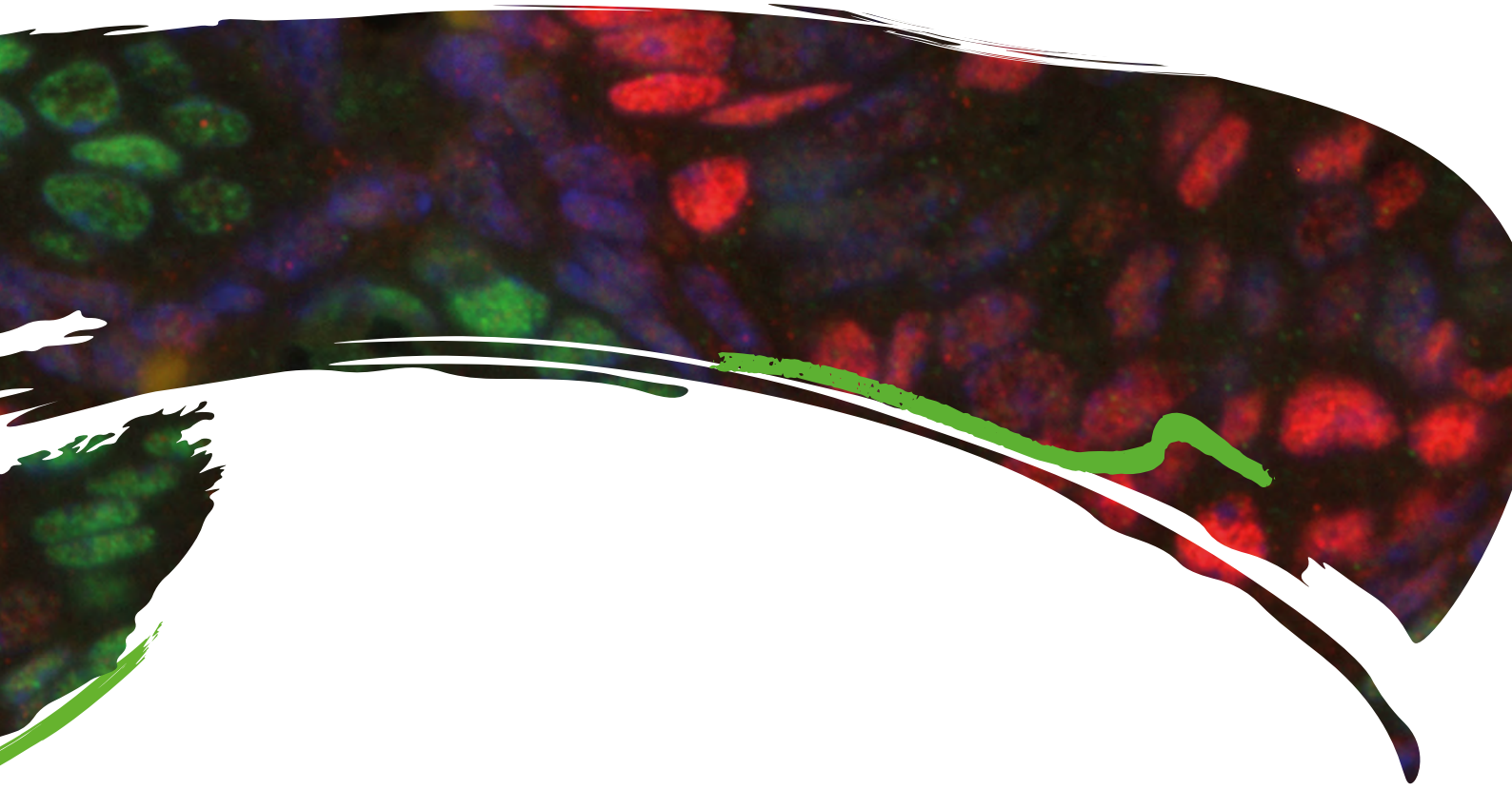
cabimer

Scientific **Report**

2023/2024



Junta de Andalucía

CSIC
Consejo Superior de Investigaciones Científicas

Universidad de Sevilla

Universidad Pablo de Olavide Sevilla

cabimer

Scientific **Report**

2023/2024

Content

Scientific Report 2023-2024

Director (ended Dec 2024)
Prof. Andrés Aguilera

Welcome

It is my pleasure to present the scientific report of CABIMER (Centro Andaluz de Biología Molecular y Medicina Regenerativa / Andalusian Centre of Molecular Biology and Regenerative Medicine) for 2023 and 2024. As a groundbreaking multidisciplinary biomedical research center in Andalusia, CABIMER draws together basic and applied research with the aim of transforming the results of the scientific work into direct improvements for citizens' health and quality of life. CABIMER provides a rich intellectual environment to support individual researchers and to foster collaborations among faculty members, postdoctoral fellows, graduate students, technicians, visiting scientists and trainees. A large number of scientists working at the Centre contribute to a stimulating and international atmosphere with international seminars taking place in the Center on a regular frequency all year round, improving the recognition and visibility of its research and researchers.

During this 2-year period there has been a real improvement of CABIMER activities and infrastructural facilities to support the science undertaken by the 29 actual Principal Investigators (PI's), which include 5 emerging PIs after the incorporation of two new PIs, Daniel Rico and Patricia Altea-Manzano, coming from UK and Belgium. In 2024, CABIMER as a Centre and all its PIs and groups were evaluated in situ during a 3-days visit by its international Scientific Advisory Board formed by prestigious European Scientists. They stressed the sustained quality of the scientific research accomplished in the last 5 years and provided highly valuable recommendations to gain more international status to better succeed with international funding.

Some highlights of the past 2 years are the success of CABIMER researchers in obtaining funding from competitive calls from national and international agencies, including an ERC Starting grant and an AXA Research grant. The center has significantly improved the quality of the publications and grant incomes, as well as the number and quality of PhD students and postdocs. During these two years, the center produced 13 PhD theses and 97 publications, a relevant number of which in top journals of the Nature group, Cell Press or the AAAS, and the research groups have obtained more than 12.5 million euros in competitive grants, multiplying by 4 the funds obtained in the 2-years periods before 2022.

In the 2023-24 period CABIMER has updated the 9 fully-functional core services including the Biological Research Unit with a special unit for the generation of genetically modified mice, the Genomic platform for the use of external and internal services, the advanced Imaging unit as well as Histology and Model organism services to support the different research activities of the Center using the most modern and high-tech molecular and cellular technologies. Importantly, in 2023 we created the new Unit of Bioinformatics to give support to all groups and are ready to start a new Unit of Proteomics, In this context, CABIMER has been highly successful obtaining competitive national funds above 3.2 million euros, which are being used to create a new Proteomics Unit and to update the Image system with a Super-resolution microscope, among other equipment. To stimulate the research activity and commitment of young scientists CABIMER has created together with the Foundation BioMol the Prizes CABIMER-Biomol for young scientists that has celebrated the first two editions in 2023 and 2024. Three young PhD students/postdocs have been honored every year.

CABIMER was awarded the Prize of Research in Cancer awarded by the AECC (Asociación Española Contra el Cancer/Spanish Association Against Cancer) and its scientists have been awarded national and regional grants for young scientists, among it is worth highlighting the National Prize "Gabriella Monreale" for young researchers from the Spanish Government awarded to Patricia Altea-Manzano. Continuing its series of biennial international meetings, we held the Third CABIMER International Workshop in March 2024 on "New Frontiers in Metabolism: from Cell to System Biology" with highly recognized invited speakers and attendants from all over the world that had a high international repercussion.

CABIMER is successfully increasing its reputation as an International Research Center of Excellence and a major center of biomedical research in Spain. To accomplish these goals and improve its capabilities in the next future CABIMER will continue expanding the number of research groups and research lines, with special emphasis on young researchers and the incorporation of well-established and successful groups. We are proud of the effort and dedication of all our PI's and researchers, as well as the support staff who have all contributed to the success of CABIMER as a referent in Molecular Biology and Biomedical research in Spain, with an increasing international visibility. We still have a long way to go and many objectives to accomplish, now under the guidance of the new director Jose Carlos Reyes; many new exciting discoveries lie ahead of us. I hope the information summarized in our Scientific Report conveys this ambition.

Director
Dr. Jose C. Reyes

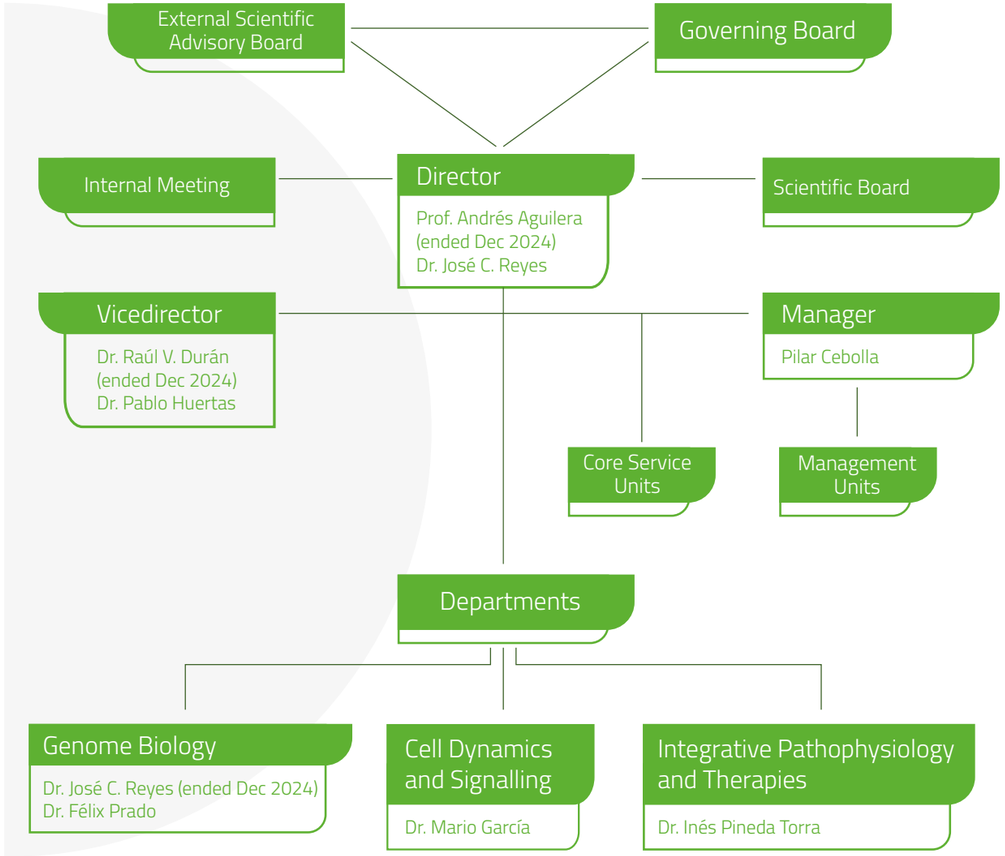
Welcome

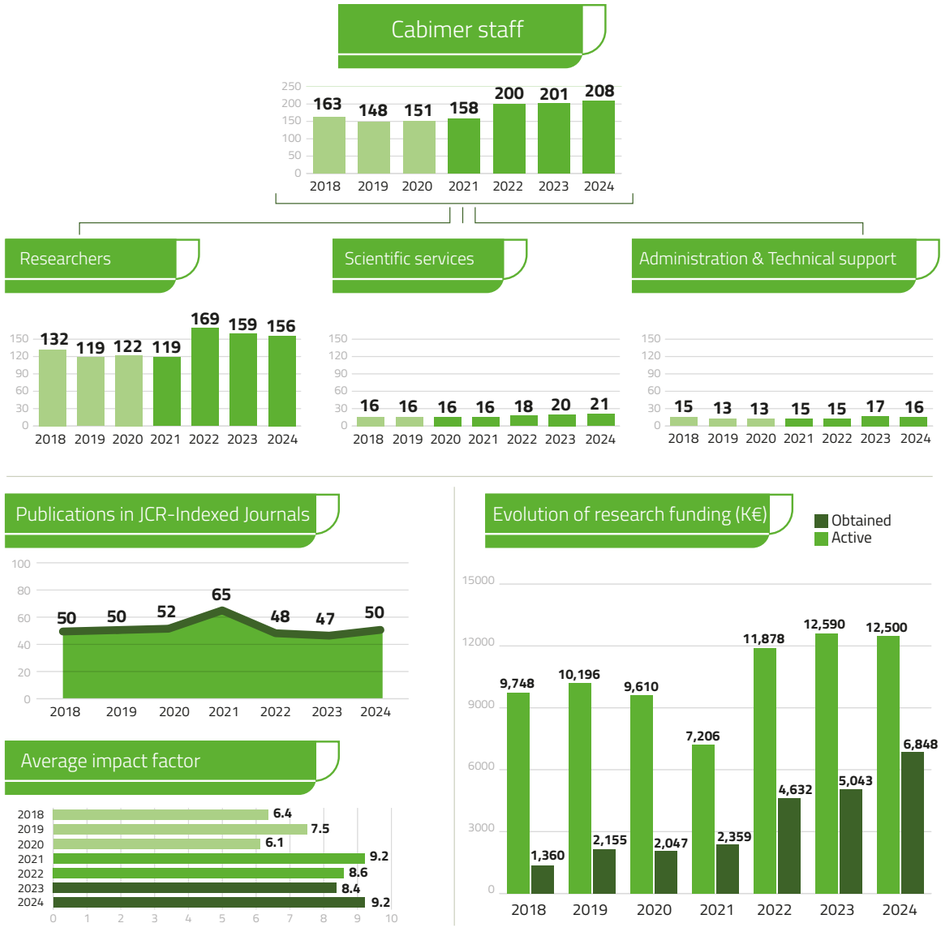
It is an honor and a huge challenge for me to take over the torch from Professor Andrés Aguilera as Director of CABIMER. The first thing I would like to do is to thank him for his enormous work and dedication to CABIMER in the eight years in which he has been our Director. As Prof. Aguilera has described in his words, CABIMER is probably in one of the best moments of its history, with consolidated groups with notable scientific production and international recognition and several extremely competitive emerging groups with an excellent future ahead of them. I therefore see the future with optimism. But CABIMER also faces some important challenges. Several of the CABIMER groups are small in size and an increase in their critical mass is necessary for them to be able to have the appropriate international impact. This requires an increase of the Center area dedicated to laboratories, which we will try to undertake in the coming years, with the help of the institutions that make up the Governing Council of CABIMER. Other future challenges such as greater internationalisation, the ever-increasing need to present our progresses to society, the increased presence of female researchers as group leaders in order to close the gender gap, or how to use the huge capabilities of Artificial Intelligence for our research are tasks that will occupy us intensely in the coming years.

The main scientific objective of CABIMER since its creation has been to coordinate and merge in a single Centre basic research of excellence together with useful top-level translational research oriented towards the transfer of knowledge to the biosanitary sector. We want this to continue to be the main philosophy of CABIMER's research. Therefore, the scientific strategy in the coming years will concentrate, on the one hand, on the study of the molecular and cellular bases of diseases, especially cancer, metabolic diseases, rare diseases of genetic origin, neurodegenerative diseases, and ageing; and on the other, on the development of therapies aimed at the treatment of these diseases. To do this, we will pursue a greater connection with the clinicians of the hospitals in our area.

In 2026, it will be the 20th anniversary of the creation of CABIMER. Twenty years of high-level research, training of a large number of students, technicians and researchers distributed throughout the national and international geography and discoveries in multiple branches of biomedicine. CABIMER is an example of success in which five different institutions at local, regional and national levels cooperate to generate knowledge, economic activity and advances in health. I am convinced that if our institutions continue supporting us as they have done up to now, the next 20 years will be even better, if possible, than the last. Our enthusiasm, dedication and love of knowledge are guaranteed to achieve this.

Organization and Outreach





Genome Biology

The Department of Genome Biology focuses on elucidating the molecular mechanisms that regulate chromosome expression, maintenance, and progression throughout the cell cycle, and how dysregulation of these processes contributes to cancer and genetic disorders. Research at this Department encompasses key aspects of genome dynamics, including genome instability, DNA replication, recombination, repair, and the cellular response to DNA damage. Additional areas of focus include chromatin integrity, epigenetic modifications, and gene expression, with an emphasis on integrating genomics and epigenomics approaches through advanced computational biology tools. Genomic instability, a hallmark of several congenital syndromes, rare genetic diseases, and somatic conditions—particularly cancer and aging—is a central theme of the department's research. Similarly, understanding the regulatory networks underlying transcriptional and epigenetic control during processes such as cell differentiation, tissue plasticity, and cell signalling is vital for uncovering the mechanisms that preserve cellular homeostasis and fitness. Dysregulation in these pathways is a significant driver of disease pathology. In addition, the department employs three-dimensional genome analysis to investigate the spatial organization of chromosomes and chromatin interactions, exploring how these structural features influence gene regulation and genome function. These research topics are pursued by a diverse set of multidisciplinary teams, each bringing unique conceptual and technological expertise. This diversity fosters strong collaborative efforts, promoting synergistic advancements across the Department's thematic areas within the broader context of CABIMER. This integrative approach maximizes the collective strengths of the groups, enhancing both innovation and discovery.

HEAD OF DEPARTMENT

Dr. Félix Prado

RESEARCH GROUPS

1. **Genome Instability & Cancer**
   Prof. Andrés Aguilera
2. **Epigenetics and Gene Expression**
   Dr. José C. Reyes
3. **Chromatin Integrity and Function**
   Dr. Félix Prado
4. **Mitochondrial Plasticity and Replication**
   Dr. Ralf E. Wellinger
5. **DNA Double Strand Break Repair**
   Dr. Pablo Huertas
6. **Molecular Oncology and Targeted Therapies**
   Dr. Andrés López-Contreras
7. **DNA Damage Response During Meiosis**
   Dr. Tatiana García-Muse
8. **Transcription and mRNA Processing**
   Dr. Silvia Jimeno-González
9. **Replication and Nuclear Dynamics**
   Dr. Cristina González-Aguilera
10. **Replication and Endogenous DNA Damage**
    Dr. Iván V. Rosado
11. **Chromatin Modifications**
    Dr. Gonzalo Millán-Zambrano
12. **Computational Epigenomics and Cell Identity**
    Dr. Daniel Rico

Principal Investigator

Dr. José C. Reyes

Epigenetics and Gene Expression

Group Leader

Current position

- Scientific Researcher CSIC.
- Director of CABIMER.

Group Members

Postdocts

- Katiuska González Arzola (Assoc. Prof., US).
- Álvaro Gallego-Martínez.

PhD Students

- Patricia Navarro Cansino.
- Lorena García Bernardo.

Former members (2023-2024)

- **Postdocs:** Maria Ceballos-Chávez.
- **PhD Students:** Laura Basurto, Elena Gómez-Marín, Elena Sánchez Escabias.
- **Master students:** Francisco Montesinos, Lucía González López.
- **Technician:** María Escaño Maestre.

Research Activity

Overview

The regulation of gene expression is an essential process for cellular function and differentiation and, at a systemic level, for development, tissue homeostasis and response to external factors. Consistently, alterations in gene expression patterns are both cause and consequence of a multitude of diseases. Probably the most important and regulated step of the gene expression process is transcription. Chromatin, which is the nucleoprotein complex formed by DNA and histone proteins, plays a fundamental role in regulating transcription. The main goal of our group is to understand how changes in the chromatin of regulatory elements and gene bodies occur during transcription, how they are regulated and inherited, and which protein factors are responsible for them. We especially investigate how alterations of these mechanisms are involved in human diseases.

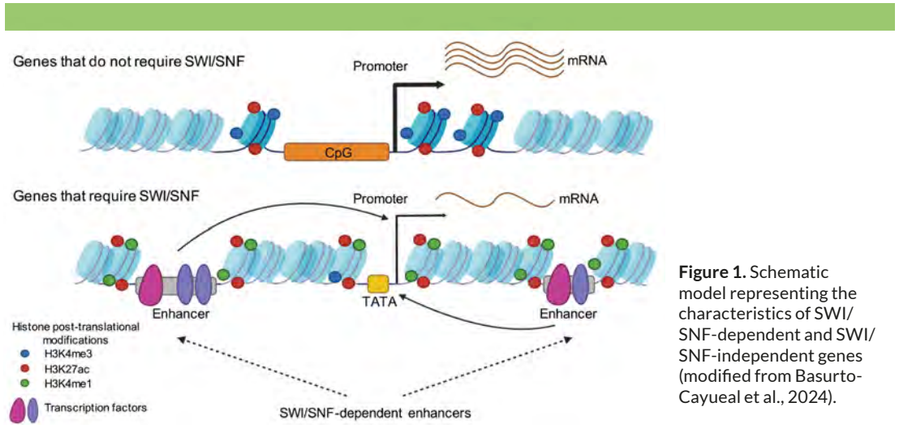


Figure 1. Schematic model representing the characteristics of SWI/SNF-dependent and SWI/SNF-independent genes (modified from Basurto-Cayueal et al., 2024).

Research Highlights

1.- What determines that some genes require the SWI/SNF complex for their expression and others do not?

The building block of the chromatin is the nucleosome, in which an octamer of histones is surrounded by about 150 bp of DNA. Nucleosomes can play positive or negative roles in transcription and therefore, in order to control transcription, several multiprotein machineries carry out changes in the structure and positioning of nucleosomes at regulatory elements (mostly promoters and enhancers). The SWI/SNF complex was the first chromatin remodelling machinery to be discovered and has been extensively investigated since. However, numerous aspects of its regulation and activity are poorly understood. In mammals, there is not a single SWI/SNF complex (also known as the BAF complex), but rather a polymorphic family of complexes. Each of these complexes is formed by 10 to 13 subunits. The enzymatic motor of the complexes is one of the two mutually exclusive ATPases of the SNF2 family called BRM/SMARCA2 and BRG1/SMARCA4. BRG1 plays important and context-specific roles in cancer, appearing either mutated or over-expressed in different types of tumors. Many important questions are debated about the SWI/SNF complex such as: Which regulatory regions are regulated by SWI/SNF activity, and which are not? Is SWI/SNF activity required to sustain a promoter's chromatin accessibility in mammals? What factors determine SWI/SNF activity dependence? Why SWI/SNF inhibition only mildly altered the gene

expression, with about one-third of the genes being upregulated?

To address these questions, we have used BRM014, an inhibitor of the ATPase activity of the complexes to characterize the features of the SWI/SNF-dependent and -independent genes. We found that SWI/SNF-independent genes are frequently highly expressed genes with very accessible promoters enriched in active histone posttranslational modifications and with well-phased nucleosomes. These genes tend to be surrounded by a small number of enhancers. Almost all housekeeping genes match this category and are SWI/SNF-independent. In contrast, SWI/SNF-dependent genes are typically low expressed genes, that have promoters with low accessibility, low levels of active histone marks, low nucleosomal phasing and enriched in TATA-box motifs. These genes encode generally signal transduction, developmental and cell identity genes and were surrounded by enhancers whose accessibility was very sensitive to SWI/SNF inhibition (Figure 1). Interestingly, chromatin accessibility of enhancers was dependent on SWI/SNF-activity while accessibility of about 90% of promoters was SWI/SNF-independent. Finally, we have used machine learning models trained with datasets of the chromatin characteristics of promoters and close regulatory regions to efficiently predict SWI/SNF-dependent or independent genes in two different cell lines, indicating that the chromatin landscape is essential to establish SWI/SNF dependency (Basurto-Cayueal et al., 2024).

2.- Role of the *Serpine1* gene transcript in controlling EMT and tumor infiltration.

Epithelial-to-mesenchymal transition (EMT) is a biological process by which epithelial cells acquire mesenchymal properties. This process occurs during embryonic development, tissue remodeling, wound healing, and it is the origin of cancer metastasis. EMT is consequence of an intense transcriptomic and epigenomic reorganization that we have been studying during the last years. In collaboration with Dr. José A. Pintor-Toro´s group, from CABIMER, we have recently shown that the Serpine1 mRNA per se, independent of its protein-coding function, confers mesenchymal properties to the cell, promoting migration, invasiveness, and increasing glycolytic activity by sequestering miRNAs. We computationally analyzed iCLIP-seq experiments to identify AGO2-bound mRNAs and discovered that Serpine1 mRNA acts as a natural miRNAs sponge, dampening the EMT inhibitory activity of miRNAs. Finally, Dr. Pintor-Toro also showed an inverse association between human SERPINE1 mRNA expression and the presence of CD8+ T cells in colon adenocarcinomas (Polo-Generelo et al., 2024).

Grants (starting or ending 2023-2024)

- 2021-2024:PID2020-118516GB-I00. Ministerio de Ciencia e Innovación.
- 2023-2025: BIOT22_00018_2. Junta de Andalucía.
- 2013-present: VEC – 001/2014 FVEC-FPS. Fundación Vencer el Cáncer.

Publication Highlights

Lara-Ureña N, Gómez-Marín E, Reyes JC, García-Domínguez M. **2024**. SARS-CoV-2 E protein interacts with BET SEED domain and alters transcription in a different way than BET inhibition. **Cellular and Molecular Life Sciences**. 81(1):313.

Polo-Generelo S, Rodríguez-Mateo C, Torres B, Pintor-Tortolero J, Guerrero-Martínez JA, König J, Vázquez J, Padillo-Ruiz J, de la Portilla F, Reyes JC, Pintor-Toro JA. **2024**. Serpine1 mRNA confers mesenchymal characteristics to the cell and promotes CD8+ T cells exclusion from colon adenocarcinomas. **Cell Death Discovery**. 10(1):116.

Basurto-Cayuela L, Guerrero-Martínez JA, Gómez-Marín E, Sanchez-Escabias E, Escaño-Maestre M, Ceballos-Chávez M, Reyes JC. **2024**. SWI/SNF-dependent genes are defined by their chromatin landscape. **Cell Reports**. 43(3):113855.

Sola-García A, Cáliz-Molina MA, Espadas I, Petr M, Panadero-Morón C, González-Morán D, Martín-Vázquez ME, Narbona-Pérez AJ, López-Noriega L, Martínez-Corrales G, López-Fernández-Sobrino R, Carmona-Marin LM, Martínez-Force E, Yanes O, Vinaixa M, López-López D, Reyes JC, Dopazo J, Martín F, Gauthier BR, Scheibye-Knudsen M, Capilla-González V, Martín-Montalvo A. **2023**. Metabolic reprogramming by Acly inhibition using SB-204990 alters glucoregulation and modulates molecular mechanisms associated with aging. **Communications Biology**. 6(1):250.

Principal Investigator

Prof. Andrés Aguilera

Genome Instability and Cancer
Group Leader

Current position

- Full Professor of Genetics, University of Seville (US).
- Director (ended in December 2024).

Group Members

Senior Researchers

- Rosa Luna (Assoc. Prof., US).
- Ana G. Rondón (Assoc. Prof., US).
- Belén Gómez-González (Assist. Prof., US).

Postdocs

- María García-Rubio (Assoc. Prof., US).
- Emilia Herrera-Moyano (Assist. Prof., US).
- Sonia Barroso.
- M. Angeles Ortiz-Bazán.
- Sara Priego-Moreno.
- Nibal Badra-Fajardo.
- Victoria Sánchez-Martín.

PhD Students

- Iván Núñez-Martín.
- Mar Bustamante-Sequeiros.
- Cristina Acedo-Rubio.
- Sandra Trujillo Sierra.

Technician

- Pablo Cano Jiménez.

Former Members (2023-2024)

- **Postdoc:** Cristina Guillén-Mendoza.
- **PhD students:** M. Eugenia Soler-Oliva, Javier Marqueta-Gracia, Pablo Maraver-Cárdenas.
- **Master students:** Salomé Spaag (*Erasmus+*), Sheila Luna-González, Xavier Madrid-González.
- **Visiting Scientists:** Prof. J. Lucas Argueso (*Colorado State Univ, Forth Collins, CO, USA*), Nibal Badra-Fajardo (*PhD student, Patras Univ, GR*).

Genome Biology

Research Activity

Overview

The key role of genome instability in tumorigenesis and a number of rare cancer-prone genetic diseases has made it a major subject in basic biological research, cancer biology and biomedicine. Our research is focused on the factors and mechanisms responsible for genome instability associated with replication stress and replication-born DNA breaks, including those caused by transcription-replication conflicts and R-loops, as key players in cancer. Our specific goals have been to identify and decipher the

1. mechanisms by which cells prevent genotoxic R-loop accumulation
2. main factors protecting and repairing replication fork stalls and breakage
3. links between transcription-replication conflicts and R-loops with cancer;

Research Highlights

1. Prevention of genotoxic R-loop accumulation. During this period we have contributed to understand three different modes of preventing genotoxic R-loops: i) by protecting the RNA by assembly; ii) by the action of chromatin remodelers and modifiers, and iii) by removing unscheduled R-loops formed co-transcriptionally. We have uncovered a new resolvase of R-loops, DICER, which resolves R-loops specifically via its RNase activity. Despite being able to bind R-loops and simple DNA-RNA hybrids, it only resolves R-loops, being the only enzyme so far reported to have such specificity (Camino et al, Mol Cell 2023). In addition, we completed a screening

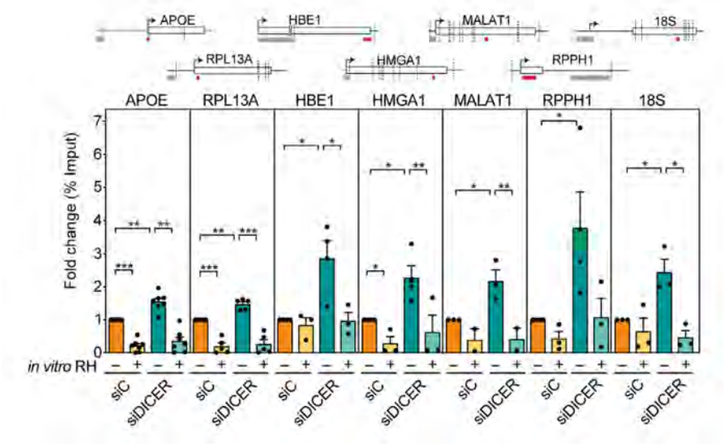


Figure 1. DNA-RNA immunoprecipitation followed of qPCR (DRIP-qPCR) showing the increase in R-loop accumulation in DICER-depleted cells (*from Camino et al, Cell Rep 2023*).

of nuclear factors as potentials R-loop suppressors that led to the identification of new RNA metabolism and chromatin related factors, the top candidate being DDX47. We have shown that this enzyme resolves R-loops and DNA-RNA hybrid by a newly uncovered DNA-RNA unwinding activity in vitro that suppress genotoxic R-loops in vivo (Marchena-Cruz et al, Cell Rep 2023). In the same line we have found that the ALYREF nuclear chaperon, an RNA-binding factor involved in RNA metabolism together with the THO complex, stimulates the DNA-RNA unwinding activity of the UAP56/DDX39B RNA helicase (Badhari et al, J Biol Chem 2024). Moreover, we have shown that the yeast transcription terminator factor Rat1 is required for RNAPII to terminate transcription when stacked at an R-loop. Finally, we have contributed to show and map specific regions and mechanism by which defective TopoI increases R-loops and genome instability differentially at early times of treatment versus late times (Duardo et al, Sci. Adv. 2023)

2. Replication fork stalling and breakage, and DNA repair. Using drugs to induce replication fork breakage, we have found that histone deacetylation by SIN3A is required for replication fork protection. We have shown that at fork blocks, compaction of chromatin by histone deacetylation is required to prevent the unscheduled action of nucleases,

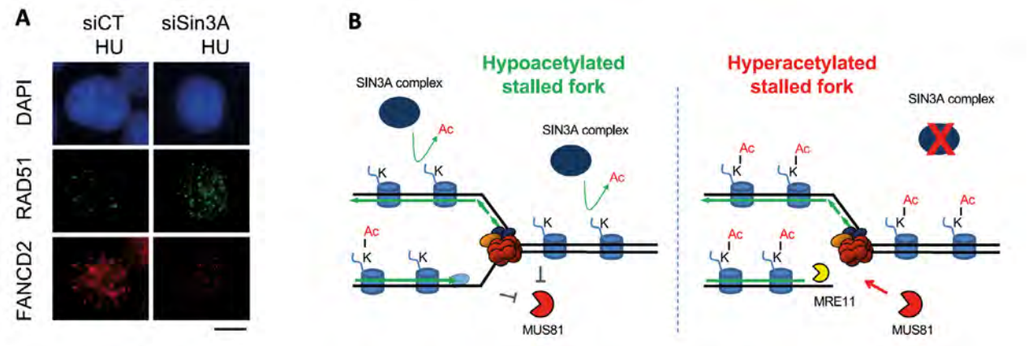


Figure 2. Results showing the role of histone deacetylation by SIN3A in protecting stalled replication forks under replications stress. **A.** IF showing that in the absence of SIN3, blocked forks, as visualized by FANCD2 foci, are reduced as they break leading to an increase of RAD51 foci. **B.** Model to explain how SIN3-mediated histone deacetylation protect forks by contributing to a transiently compacted chromatin that prevents the action of nuclease MUS81, thus preserving genome integrity (*from Muñoz et al, Cell Rep 2024*).

in particular MUS81, to preserve genome integrity (Muñoz et al, Cell Rep 2024). We also found that human RecQL5 interacts with yeast RNAPII impairing transcription and cause transcription-dependent genome instability, supporting its pivotal role at conflicts (Lafuente et al, Mol Genet Genom 2024). Finally, we have contributed to collaborative studies that have uncovered new roles for histone variant H2A.Z on transcription-coupled nucleotide excision repair (Gaillard et al, PLos Genet 2024), a biased deamination of C to U to the non-transcribed strand during transcription (Williams et al, DNA Repair 2023), as well as a role for actin nucleator protecting replication forks by limiting nascent DNA strand degradation (Nieminuzscy et al, Nucl Acids Res 2023).

3. Transcription-replication conflicts and cancer. We have reported a key role for chromatin remodelers in preventing transcription-associated genome instability and the accumulation of genotoxic R-loops. By comparative analysis with cancer databases of our genome-wide data we have shown that R-loop-prone and transcription-replication conflict regions are mutation hotspots, that are significantly increased in cancer samples from patients, supporting a role for R-loops as an intermediate in the origin and progression of cancer (Bayona-Feliu et al, Nat Commun 2023).

In addition to those highlights and others, we have contributed with 2 invited reviews on R-loop homeostasis factors (Luna et al, Genes

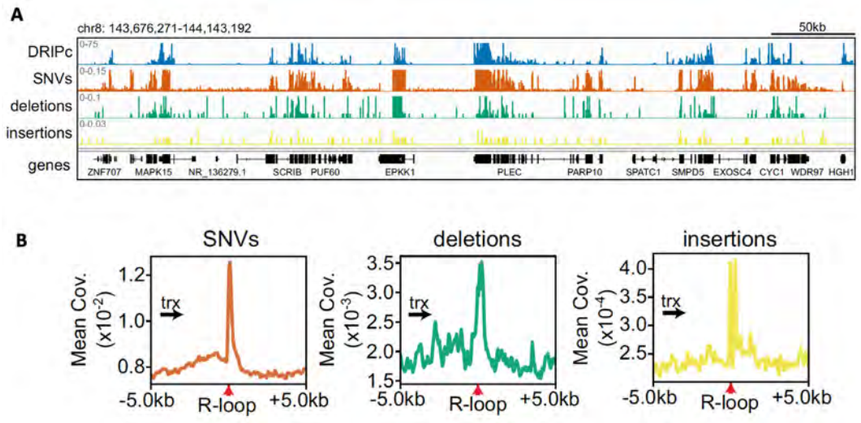


Figure 3. Comparative genome-wide analysis showing that R-loop-prone regions detected in SWI/SNF-depleted cells are hotspots of mutations found in cancer cells. A. R-loop and mutation profiles of selected genomic regions, showing coincidence of peaks. B. Metagenomic analysis showing the increase of mutation frequency around R-loop-prone regions in cancer cells (*from Bayona-Feliu et al, Nat Commun 2023*).

Dev 2024) and break-induced RNA-DNA hybrids (Gómez-González et al, EMBO Rep, 2023).

Grants

- Since 2013-2026: VEC001/2014 FVEC-FPS. "Vencer el Cáncer" Foundation.
- 2021-2023: US-1380058 Junta de Andalucía-US.
- 2021-2023: P18-FR-655 (PAIDI) Junta de Andalucía.
- 2020-2023: PID2019-104270G-100 Ministry of Science and Innovation.
- 2021-2023: BIO102. Junta de Andalucía-Univ. Seville.
- 2022-2025: FIUS22/01788, Foundation Investigation Univ. Seville.
- 2022-2025: HR22-00073. Caixa Research Foundation.
- 2022-2025: PID2022-138251NB-I00 Ministry of Science, Innovation and Universities.
- 2024-2027: PPIT2024-31756 (for support to the whole center). Univ Seville.
- 2023-2025: RED2022-134164-T (coordinator of 15 group network). AEI, Ministry of Science.

Publication Highlights

(Original articles; corresponding author(s) indicated by *)

Muñoz S, Barroso S, Badra-Fajardo N, Marqueta-Gracia JJ, García-Rubio ML, Ubieto-Capella P, Méndez J, Aguilera A*. **2024**. SIN3A histone deacetylase action counteracts MUS81 to promote stalled fork stability. **Cell Rep**. 43(2):113778.

Mérida-Cerro JA, Maraver-Cárdenas P, Rondón AG*, Aguilera A*. **2024**. Rat1 promotes premature transcription termination at R-loops. **Nucleic Acids Res**. 52(7):3623-3635.

Bayona-Feliu A*, Herrera-Moyano E, Badra-Fajardo N, Galván-Femenía I, Soler-Oliva ME, Aguilera A*. **2023**. The chromatin network helps prevent cancer-associated mutagenesis at transcription-replication conflicts. **Nat Commun**. 14(1):6890.

Camino LP, Dutta A, Barroso S, Pérez-Calero C, Katz JN, García-Rubio M, Sung P*, Gómez-González B*, Aguilera A*. **2023**. DICER ribonuclease removes harmful R-loops. **Mol Cell**. 83(20):3707-3719.e5.

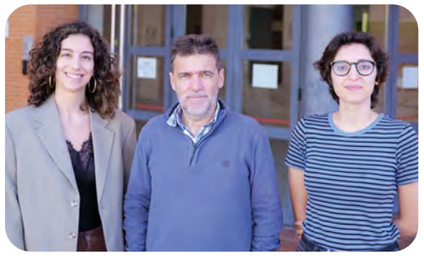
Marchena-Cruz E, Camino LP, Bhandari J, Silva S, Marqueta-Gracia JJ, Amdeen SA, Guillén-Mendoza C, García-Rubio ML, Calderón-Montaño JM, Xue X, Luna R*, Aguilera A*. **2023**. DDX47, MeCP2, and other functionally heterogeneous factors protect cells from harmful R loops. **Cell Rep**. 42(3):112148.

Principal Investigator

Dr. Félix Prado

Chromatin Integrity and Function

Group Leader



Current position

- Scientific researcher CSIC.
- Head of the Genome Biology Department.

Group Members

PhD Students

- Ana Amiama Roig.
- Macarena Alcalá Domínguez.

Former Members (2023-2024)

- **Postdocs:** Marta Barrientos Moreno, Antonia María Romero Cuadrado.
- **PhD student:** Cristina González Garrido.
- **Master student:** Gabriel Ríos Orelogio.
- **Technician:** Juan Antonio Cuevas.

Research Activity

Overview

Cells must duplicate their genomes with high fidelity and efficiency to ensure the accurate transmission of genetic information to daughter cells. This involves both the replication of DNA and its proper assembly into chromatin, processes that are vulnerable to a variety of stress conditions. These stresses can compromise DNA integrity and disrupt the pattern of nucleosome-associated epigenetic modifications, both of which are associated with genetic disorders and oncogenesis. Given the multitude of physical, chemical, and genetic factors that can interfere with the progression of replication forks, genome duplication is a complex and highly regulated process. Replication stress can lead to fork stalling or breakage, which in turn activates distinct and overlapping cellular responses aimed at preserving genomic stability. Our primary objective is to elucidate the molecular mechanisms that respond to stressed replication forks, with a particular focus on the role of chromatin assembly in safeguarding genome integrity during these challenges.

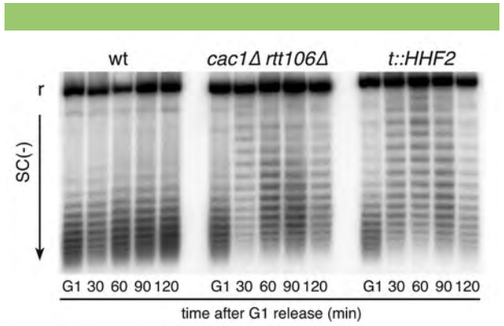


Figure 1. Defective replication-coupled histone deposition causes transient changes in DNA topology and chromatin structure of the 2µ plasmid. Plasmid topoisomer distribution of the 2µ plasmid in wild type, *cac1Δ rtt106Δ* and *t::HHF2* cells synchronized in G1 and released into fresh medium for different times.

Research Highlights

1. Restoration of Nucleosome Assembly Defects: Genome duplication is driven by the coordinated actions of DNA replication and nucleosome assembly at replication forks. Our previous work demonstrated that defective nucleosome assembly causes DNA lesions by fork breakage that need to be repaired. In addition, it causes a loss of chromatin integrity. These chromatin alterations can be restored, even though the mechanisms are unknown. We have shown that the process of chromatin restoration can deal with highly severe chromatin defects induced by the absence of the histone chaperones CAF1 and Rtt106 or a strong reduction in the pool of available histones, and that this process can be followed by analysing the topoisomer distribution of the 2µ plasmid (Figure 1). Using this assay, we have demonstrated that chromatin restoration is slow and independent of checkpoint activation, whereas it requires the action of transcription and the chromatin remodelling FACT complex. Therefore, cells

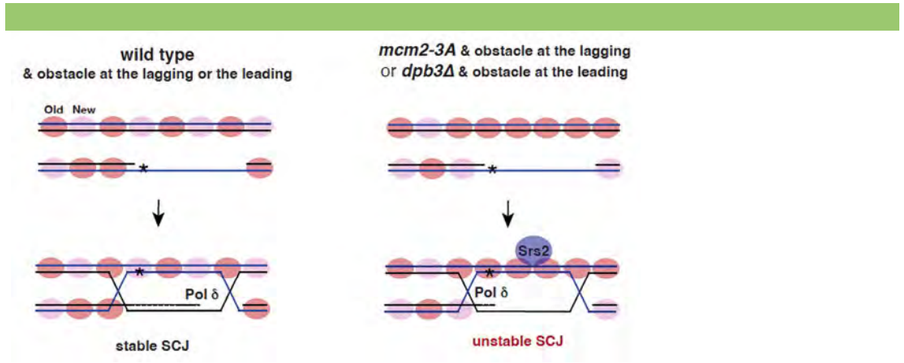


Figure 1. Parental histone distribution and location of the replication obstacle at the nascent strands regulate HR. The recombinational filling of ssDNA gaps is initiated by a strand exchange reaction that reanneals the ssDNA gap with its complementary strand at the invaded molecule, leading to the formation of a sister chromatid junction (SCJ). Defective transfer of parental histones at a nascent strand (mcm2-3a and dpb3 mutants) causes a deficit of parental histones in that strand and a concomitant accumulation of parental histones in the sister strand. Parental histone excess at the invaded strand allows strand invasion but destabilizes the SCJ, which seems to be mediated by an unscheduled recruitment/activity of the antirecombinogenic helicase Srs2.

are able to "repair" not only DNA lesions but also chromatin alterations associated with defective nucleosome assembly.

2. Impact of Parental Histone Recycling on DNA Repair Mechanisms: Consistent with the co-regulation of DNA synthesis and nucleosome assembly, we have demonstrated that mutations impairing parental histone recycling during DNA replication result in defects in the recombinational repair of single-stranded DNA gaps. These gaps arise from DNA adducts that impede replication and are subsequently filled by translesion synthesis. These recombination defects are in part due to an excess of parental nucleosomes at the invaded strand that destabilizes the sister chromatid junction formed after strand invasion through a Srs2-dependent mechanism. In addition, we have shown that a dCas9*/R-loop is more recombinogenic when the dCas9*/DNA-RNA hybrid interferes with the lagging than with the leading strand, and this recombination is particularly sensitive to problems in the deposition of parental histones at the strand that contains the hindrance. Therefore, parental histone distribution and location of the replication obstacle at the lagging or leading strand regulate homologous recombination (Figure 2).

3. Non-Canonical Roles of the MCM Helicase and the RNR Ribonucleotide Reductase in Replication Stress Response: We have previously reported physical interactions between the recombination proteins Rad51 and Rad52 with the MCM helicase in nuclease-insoluble scaffolds enriched with replication and repair factors. These MCM/Rad51/Rad52 complexes form in G1 to assist stressed replication forks via non-recombinogenic functions. To further investigate the role of MCM in the DNA damage response, we explored its interaction partners under conditions of replicative stress. Our results reveal that MCM physically interacts with the ribonucleotide reductase (RNR) complex and its regulators, including the Dun1 kinase. These interactions, observed in small MCM and RNR subpopulations, are independent of their primary subcellular locations, increase in response to DNA damage, and, in the case of the Rnr2 and Rnr4 subunits of RNR, depend on Dun1. Notably, partial disruption of MCM/RNR interactions leads to defective release of Rad52 from DNA repair centers—without affecting RPA release—despite successful repair of the lesions. This phenotype is associated with hypermutagenesis, though independent of dNTP levels. Our findings suggest that a specifically regulated pool of MCM and RNR complexes plays a non-canonical role in maintaining genomic stability by preventing persistent Rad52 foci and reducing hypermutagenesis.

Grants

- 2022-2025: PID2021-127486NB-I00. Ministerio de Investigación, Ciencia e Innovación.
- 2021-2023: PY20_00750. Consejería de Conocimiento, investigación y Universidad.

Publication Highlights

Yáñez-Vílchez A, Romero AM, Barrientos-Moreno M, Cruz E, González-Prieto R, Sharma S, Vertegaal A, Prado F.* **2024**. Physical interactions between specifically regulated subpopulations of the MCM and RNR complexes prevent genetic instability. **PLoS Genetics.** 20(5): e1011148.

Barrientos-Moreno M, Maya-Miles D, Murillo-Pineda M, Fontalva S, Pérez-Alegre M, Andújar E, Prado F*. **2023**. Transcription and FACT facilitate the restoration of replication-coupled chromatin assembly defects. **Sci. Rep.** 13: 11397.

González-Garrido C, Prado F.* **2023**. Parental histone distribution and location of the replication obstacle at nascent strands control homologous recombination. **Cell Rep.** 42: 112174.

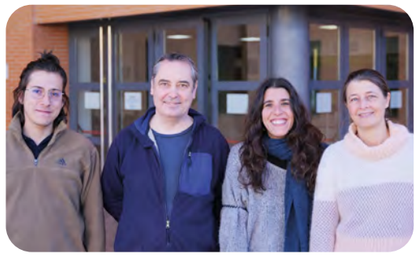
González-Garrido C, Prado F.* **2023**. Novel insights into the roles of Cdc7 in response to replication stress. **FEBS J**. 290: 3076-30-88.

Principal Investigator

Dr. Ralf E. Wellinger

Mitochondrial Plasticity and Replication

Group Leader



Current position

- Full Professor of Genetics, University of Seville (US) .

Group Members

Senior Researcher

- Helene Gaillard (Assoc.Prof., US).

Postdoc

- Inés García de Oya.

Former Members (2023-2024)

- **Visiting:** Karan Lohmaneeratana (RW).
- **Master student:** Rocío de los Ángeles Domínguez Sierra (HG).

Research Activity

Overview

Nutrigenomics initially referred to the study of the effects of nutrients on the expression of an individual's genetic makeup. More recently, this definition has been broadened to encompass nutritional factors that protect the genome from damage, or that expose the genome to damage. Main objectives of our research include the impact of micronutrients, such as manganese, iron or selenium, on human health and lifespan. We are also interested in the potential of micronutrients as a therapeutic target for cancer or some genetically inherited disorders. We aim at understanding the role of micronutrients as co-factors for the activation and correct function of enzymatic activities, as well as the impact of uncontrolled micronutrient homeostasis in DNA damage occurrence, signalling and processing. Finally, we are generally interested in how chromatin and DNA-templated processes such as transcription or replication modulate the repair of endogenous and exogenous DNA lesions.

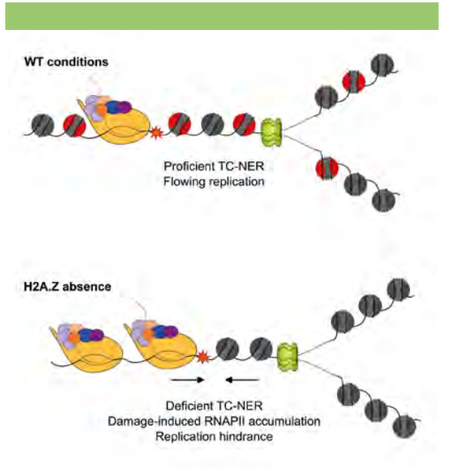


Figure 1. *Model of H2A.Z function in TC-NER and at replication forks.* Schematic drawing illustrating the function of H2A.Z-enriched chromatin in the vicinity of RNAPII stalled at a bulky DNA lesion and at replication forks in wild-type (WT) and htz1Δ cells. The RNAPII is drawn in yellow, the replisome schematized as green helicase and H2A.Z-containing nucleosomes highlighted in red.

Research Highlights

DNA Repair-Optimized Chromatin and Genome Stability.

The genome of living organisms is constantly challenged by intrinsic and extrinsic DNA damaging agents. The resulting DNA lesions must be readily repaired to maintain genome integrity. This is particularly important for bulky DNA lesions, such as those produced by UV light, as they will block the progress of elongating RNA polymerases on transcribed genes. These DNA lesions are repaired by a specific pathway called transcription-coupled nucleotide excision repair (TC-NER), the dysfunction of which is associated with severe

human diseases. By a genetic screening we discovered that the HTZ1 gene, encoding the histone variant H2A.Z, is required for efficient DNA repair by TC-NER. Our molecular and genetic analyses showed that in the absence of H2A.Z, RNA polymerases persist on damaged DNA, causing interference with DNA replication and genome instability (see Figure 1). Our findings further highlight the importance of chromatin plasticity for the maintenance of genome integrity.

Improvement Shotgun Sequencing of Genomic DNA from Pathogens

Shot gun sequencing of genomic DNA can be used for the detection or mutational analysis of pathogens. Our group collaborated with the research group Prof. Arinthip Thamchaipenet from the Kasetsart University in Bangkok to detect the pathogenic bacterium Phytoplasma sacchari in infected sugarcane plants. Sugarcane is an economically important plant in Thailand as well as Spain and since phytoplasma DNA is often much less abundant compared with the host plant's DNA, it can be difficult to isolate and analyse. By improved enrichment of non-methylated DNA from small genomes, we were able to provide a high coverage of the phytoplasma genome by shot gun sequencing. Consequently, these methodological improvements will be applied for the mutational analysis of INDELs in budding yeast genomes.

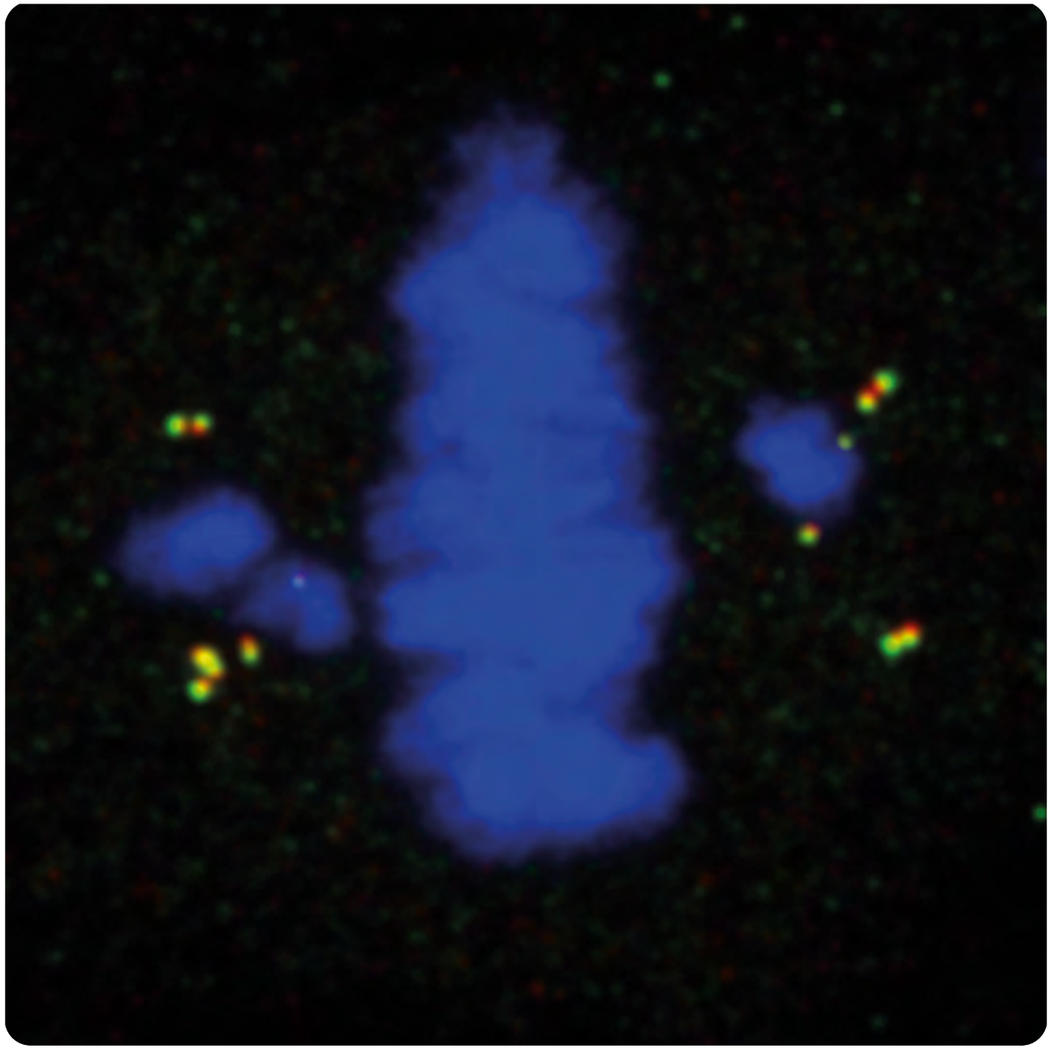
Grants (starting or ending 2021-2022)

- 2022-2025: PID2022-140466NB-I00. Ministerio de Ciencia e Innovación.

Publication Highlights

Gaillard H*, Ciudad T, Aguilera A, Wellinger RE. **2024**. Histone variant H2A.Z is needed for efficient transcription-coupled NER and genome integrity in UV challenged yeast cells. PLoS **Genetics.** 20(9), e1011300.

Lohmaneeratana K, Gutiérrez G, Thamchaipenet A*, Wellinger RE*. **2024**. Phytoplasma DNA Enrichment from Sugarcane White Leaves for Shotgun Sequencing Improvement. **Plants.** 13(21), 3006.



Principal Investigator

Dr. Pablo Huertas

DNA double strand breaks repair and human disease

Group Leader

Current position

- Associate Professor, Associate Professor University of Seville (US).
- Vicedirector CABIMER.

Group Members

Senior Researcher

- Sonia Jimeno (Assoc.Prof.,US).
- Fernando Romero Balestra (Assist.Prof.,US).
- Néstor García Rodríguez.

Postdocs

- Maikel C. Pozo.
- María Jesús Fernández Ávila.
- Rosario Prados Carvajal.

PhD Students

- Nieves Iria Domínguez García.
- Maria del Carmen Domínguez Pérez.
- Lucía González López.
- María Isabel Jimenez López.
- Amador Romero Franco.

Former Members (2023-2024)

- **PhD Students:** Rosa Camarillo Daza, Andrés Domínguez Calvo, Andrea Moo Bajo, Guillermo Rodríguez Real.

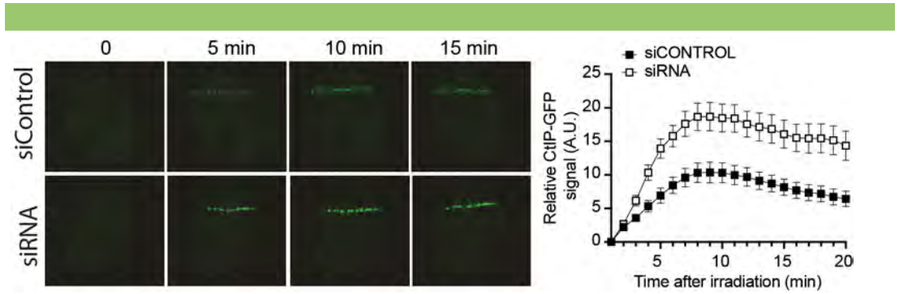


Figure 1. Accumulation of the DNA repair protein CtIP at sites of DNA double strand break using laser micro irradiation. The accumulation on a control cell line or upon depletion of a critical factor is shown.

Research Activity

Overview

Repairing double strand breaks (DSBs) is crucial for cell and organismal survival. Failure to repair DSBs results in embryonic death or cell loss, while mutations impairing repair contribute to cancer and genetic disorders. DSBs are fixed by two main mechanisms: non-homologous end joining (NHEJ), where the broken ends are simply rejoined, and homologous recombination (HR), a more complex pathway that involves processing of the ends. The balance between these pathways is tightly regulated, and any disruption can lead to chromosomal abnormalities and, as a consequence, to disease. Despite its significance, the mechanisms that control the choice between NHEJ and HR are not well understood. In our lab, we focus on understanding how this decision is made, its role in survival and disease, and how it might serve as a therapeutic target for cancer and genetic disorders.

Research Highlights

The 2023-2024 period has been very fruitful in international collaborations, but also in paving the research of the next years. We have very actively participated in the study of the role of BRCA1 as a DNA repair protein in disease, focusing in cancer. In addition to our own publication on the role of this protein in repairing replication stress, we have contributed to 3 additional papers on the subject. We have also reported a novel connection between DNA repair and centriole Biology. Articles related to new findings regarding the impact of the circadian clock, cell-to-cell communication or RNA

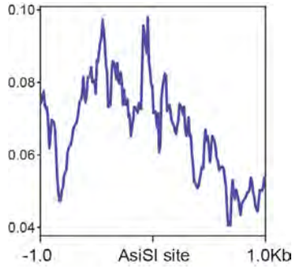


Figure 2. Accumulation of the DNA repair protein CtIP at sites of DNA double strand break using seq. DSB were caused by expression of the restriction enzyme AsiSI.

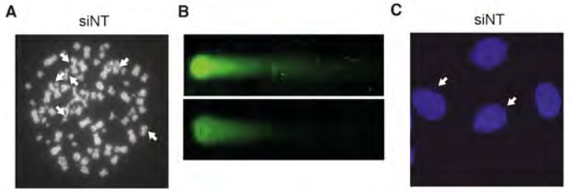


Figure 3. Different ways to measure genomic instability upon exposure to DNA damage. A, mitotic spread showing chromosomal defects is shown. White arrow mark chromosomal abnormalities. B, Comet assay showing the presence of unprepared DSBs. C, White arrow show the appearance of micronuclei.

metabolism are currently in preparation. Regarding the connection with disease, we have contributed in the understanding of cancer development and continue working in rare diseases such as the genetically inherited Aicardi-Goutieres Syndrome that we propose can be explored for the search of therapeutics interventions.

Grants (starting or ending 2023-2024)

- 2020-2023: PID2019-104195G9. Ministerio de Ciencia e Innovación.
- 2020-2023: P18-RT-1204. Junta de Andalucía.
- 2023-2026: PID2022-136791NB-I00. Ministerio de Ciencia e Innovación.
- 2024-2027: PRYGN246626HUER. Asociación Española Contra el Cáncer (AECC).

Publication Highlights

García-Rodríguez N, Domínguez-García I, Domínguez-Pérez MDC, Huertas P. **2024.** EXO1 and DNA2-mediated ssDNA gap expansion is essential for ATR activation and to maintain viability in BRCA1-deficient cells. **Nucleic Acids Res.** 52(11):6376-6391.

Rodríguez-Real G, Domínguez-Calvo A, Prados-Carvajal R, Bayona-Feliú A, Gomes-Pereira S, Balestra FR, Huertas P. **2023**. Centriolar subdistal appendages promote double-strand break repair through homologous recombination. **EMBO Rep**. 24(10):e56724.

Ceppi I, Dello Stritto MR, Mütze M, Braunshier S, Mengoli V, Reginato G, Võ HMP, Jimeno S, Acharya A, Roy M, Sanchez A, Halder S, Howard SM, Guérois R, Huertas P, Noordermeer SM, Seidel R, Cejka P. **2024**. Mechanism of BRCA1-BARD1 function in DNA end resection and DNA protection. **Nature.** 634(8033):492-500.

Salas-Lloret D, García-Rodríguez N, Soto-Hidalgo E, González-Vinceiro L, Espejo-Serrano C, Giebel L, Mateos-Martín ML, de Ru AH, van Veelen PA, Huertas P, Vertegaal ACO, González-Prieto R. **2024**. BRCA1/BARD1 ubiquitinates PCNA in unperturbed conditions to promote continuous DNA synthesis. **Nat Commun**. 15(1):4292.

Ceppi I, Cannavo E, Bret H, Camarillo R, Vivalda F, Thakur RS, Romero-Franco A, Sartori AA, Huertas P, Guérois R, Cejka P. **2023**. PLK1 regulates CtIP and DNA2 interplay in long-range DNA end resection. **Genes Dev.** 1;37(3-4):119-135.

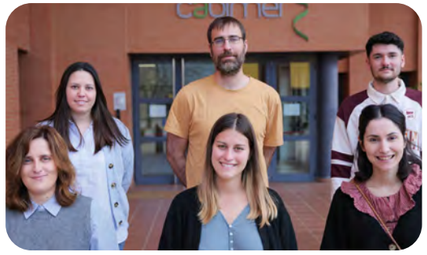
García-Vílchez R, Añazco-Guenkova AM, López J, Dietmann S, Tomé M, Jimeno S, Azkargorta M, Elortza F, Bárcena L, Gonzalez-Lopez M, Aransay AM, Sánchez-Martín MA, Huertas P, Durán RV, Blanco S. **2023**. N7-methylguanosine methylation of tRNAs regulates survival to stress in cancer. **Oncogene**. 42(43):3169-3181.

Principal Investigator

Dr. Andrés J. López-Contreras

Molecular Oncology and Targeted Therapies

Group Leader



Current position

- Scientific researcher CSIC.

Group Members

Postdocs

- Lucía Simón Carrasco.
- Paula Aguilera Aguilera.

PhD students

- Alba Guillén Benítez.
- Manuel Luque Pérez.
- Elena Pietrini.
- Alba Soledad Rodríguez Raya.

PhD candidates

- Manuel Luque Pérez.
- Alba Guillén Benítez.
- Elena Pietrini.

Former Members (2023-2024)

- **Postdoc:** María Castejón-Griñán.
- **Technician:** Loida Pérez García.
- **JAE-intro Master student:** Alba Soledad-Raya.

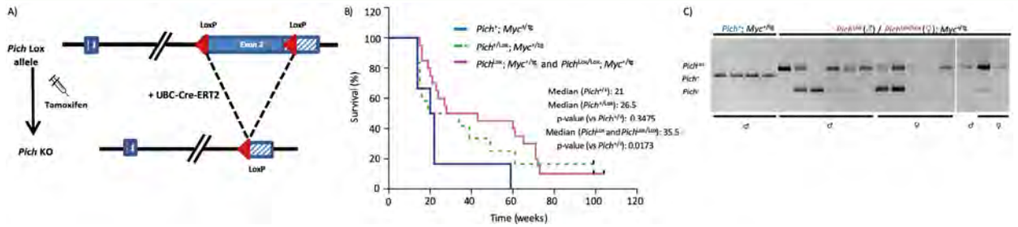


Figure 1. Pich deletion increases the survival of Eμ-Myc mice. A) Diagram of the *Pich* conditional KO allele and the expected Cre-mediated recombination. B) Survival of *Eμ-Myc* mice, without *Pich* deletion (blue line) and with *Pich* deletion in heterozygosity (green line) and homozygosity (pink line). *Pich* deletion was induced with tamoxifen injections from week 8 of age. C) Genotyping PCRs of the tumors at the humane endpoint. Of note, most tumors escaped from a complete *Pich* recombination, suggesting the relevance of PICH to sustain lymphoma progression.

Research Activity

Overview

Genome instability is a critical driver of cancer initiation, progression, and has important implications for cancer treatment. In our group, we investigate molecular factors that regulate genome stability, aiming to increase our understanding of cancer biology and identify novel therapeutic targets and strategies. Our recent research has focused on proteins involved in the regulation of replication stress and the stability of Common Fragile Sites, such as the chromatin remodelers ATRX and PICH. We employ quantitative high-content microscopy to characterize diverse cellular phenotypes, such as DNA damage, cell cycle or viability, and conduct compound screening. Our approach integrates mechanistic studies using cancer cell lines with validations in mouse models, providing insights under more physiological conditions and bridging the gap between in vitro findings and in vivo relevance.

Research Highlights

In the past few years, we have investigated the role of PICH, a chromatin remodeler and DNA translocase essential for resolving ultrafine anaphase DNA bridges, in the progression of MYC-driven B-cell lymphomas. In our study published in Blood Cancer Journal (2024), we demonstrated that PICH deficiency limits the progression of MYC-induced lymphomas. Using a conditional PICH knockout mouse model, we demonstrated that targeting PICH could serve as a promising therapeutic strategy for MYC-driven lymphomas. These findings were further validated in several human Burkitt lymphoma cell lines. Given the potential interest of PICH as a therapeutic target in cancer, we are now focused on

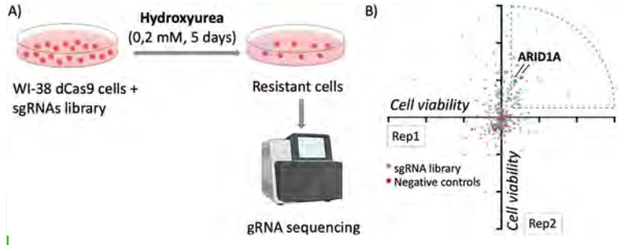


Figure 2. dCas9/CRISPR activation screening with hydroxyurea. A) Pipeline of the screening. WI-38 cells were infected with the dCas9/SAM system and a library of gRNAs targeting 68 tumor suppressor genes, with 3-5 gRNAS per gene. Cells were treated with low doses of hydroxyurea for 5 days and the resistant cells were analyzed by DNA sequencing to identify the representation of gRNAs. B) Diagram of gRNA enrichment of two replicates, each dot represents one gRNA. Two gRNAs targeting ARID1A were enriched in the two replicates performed.

Genome Biology

conducting compound screens to identify PICH inhibitors, which are currently unavailable.

We have recently published a comprehensive review about the known roles of the chromatin remodeler ATRX in regulating chromatin stability (Trends in Genetics, 2023). Given the central role of ATRX and the high frequency of ATRX mutations in human cancers, particularly brain tumors, we aim to deepen our understanding of the molecular mechanisms by which ATRX regulates chromatin stability, with the goal of identifying novel therapeutic opportunities. In this context, we have performed several drug screens to identify compounds that are synthetic lethal with ATRX deficiency, which we are currently validating in preclinical glioblastoma models.

During the past years, we have also investigated the role of FHIT in cancer, another gene frequently altered in cancer. Unlike other tumor suppressor genes, FHIT is not typically mutated; instead its entire locus is often lost due to its location within the highly unstable Common Fragile Site FRA3B. We have recently published a systematic analysis of FHIT gene alterations in cancer mining several cancer databases (Cell cycle, 2024). Our study suggests that FHIT loss is more likely a passenger event in cancer, in contrast to how its frequently described in the literature. Moreover, we are using a novel FHIT-deficient mouse model to elucidate the impact of FHIT loss in cancer and the molecular mechanism through which FHIT regulates genome stability and other pathways. As part of this research line, we have also conducted a compound screen to identify synthetic lethal interaction with FHIT loss. Although no compounds have yet been identified with synthetic lethality to FHIT, we discovered a novel cytidine analogue with potent anti-cancer activity in colon cancer cell lines. We are currently further characterizing and validating this compound in preclinical models.

Finally, we have performed CRISPR activation screens to identify genes whose overexpression

may influence the response to therapies that induce replication stress. As an example, Figure 2 illustrates the use of hydroxyurea in these screens. We have identified genes of the DNA mismatch repair pathway and others such as ARID1A, which we are currently investigating..

Grants

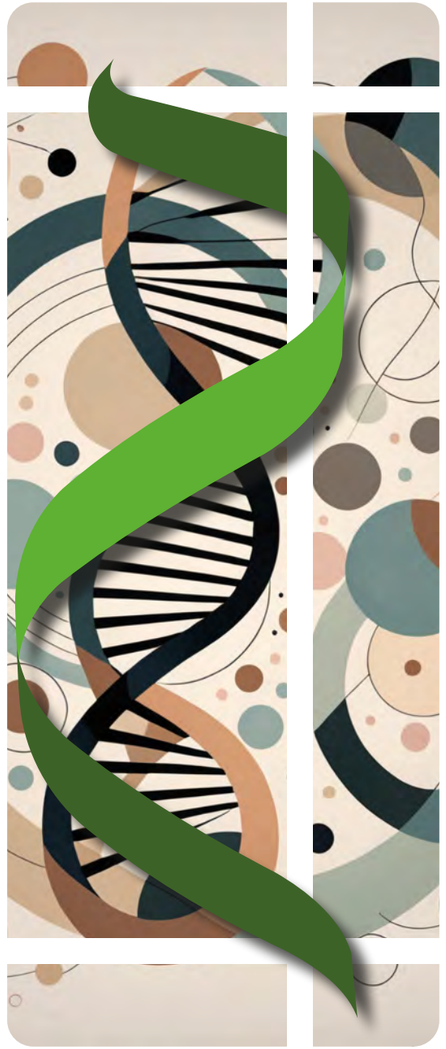
- 2021-2023: PY20-00755. Junta de Andalucía.
- 2021-2024: PID2020-119329RB-I00. Ministerio de Ciencia e Innovación.
- 022-2026: Horizon-MSCA-2021-101072903.MSCA ITN network.

Publication Highlights

Castejón-Griñán M, Albers E, Simón-Carrasco L, Aguilera P, Sbroggio M, Pladevall-Morera D, Ingham A, Lim E, Guillen-Benitez A, Pietrini E, Lisby M, Hickson ID, Lopez-Contreras AJ. **2024**. PICH deficiency limits the progression of MYC-induced B-cell lymphoma. **Blood Cancer Journal.** 14(1):16.

Simón-Carrasco L, Pietrini E, López-Contreras AJ. **2024**. Integrated analysis of *FHIT* gene alterations in cancer. **Cell Cycle.** 23(1):92-113.

Aguilera P, López-Contreras AJ. **2023**. ATRX, a guardian of chromatin. **Trends in Genetics.** 39(6):505-519.



Principal Investigator

Dr. Tatiana García-Muse

DNA Damage Response During Meiosis

Group Leader

Current position

- Associated Professor, University of Seville (US).

Group Members

Postdoc
- Mariola Chacón Rodríguez.

Student
- Nuria Fernández-Fernández.

Technician
- Carmen María Vargas Fernández.

Former Members (2023-2024)

Technicians
- Candela Caballero Fernández.
- Laura Cantalejo Carrascal.

Research Activity

Overview

Genomic DNA is exposed to both endogenous and exogenous DNA damaging agents. Without proper repair the resulting DNA damages would lead genomic instability thus affecting the faithful transmission of genetic information. In addition, defects during meiosis lead to aneuploidy, an extreme kind of genetic instability associated with fertility problems and syndromes. Since cells undergoing meiosis during oogenesis stay arrested in meiosis I for long periods of time and therefore vulnerable to DNA lesions we speculated if the increase in genome instability inferred from the increase in aneuploidy that correlates with mother age might be related to defects in DDR during meiosis. DNA damage checkpoints kinases ATR and ATM are key regulator of DDR. Our aim is to address how ATR/ATM DNA damage phosphorylations contribute to the regulation of meiosis and different DNA repair pathways to ensure genome stability.

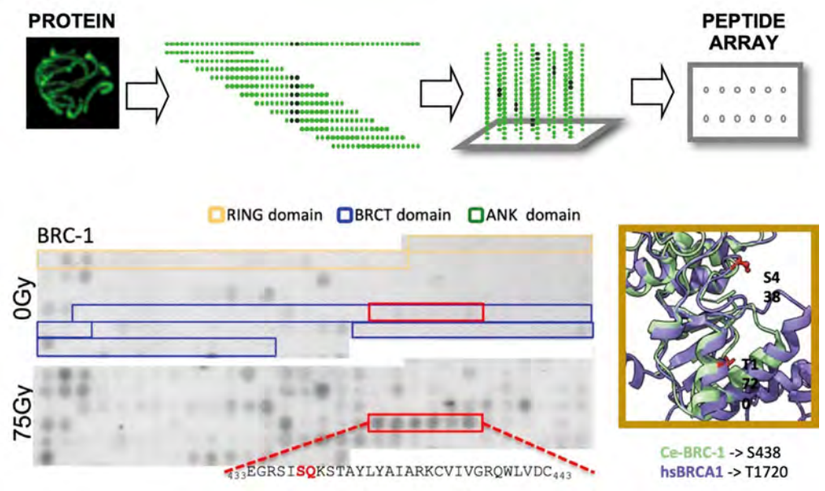


Figure 1: (Top) Scheme of the peptide array. The protein of interest can be scanned across its length by making 18 mer peptides that shifting by three residues. Each peptide is then spotted in a cellulose membrane. (Bottom left) *In vitro* phosphorylation of the BRC-1 peptide array by N2(WT) extracts with/out IR exposition. Positive serial spots (detected by autoradiography) corresponding to DNA damage-phosphorylation are boxed. The peptide sequences with specific DNA damage phosphorylation are shown with the possible phosphorylation residues highlighted in red. (Bottom right) Localization of the target residues in a structural representation of *C. elegans* and human BRAC-1.

Research Highlights

To deal with DNA damage and to prevent genomic instability cells have evolved a set of responses called the DNA damage resposponse (DDR). Phosphorylation is an essential regulator during DDR, and key kinases of DNA damage checkpoints are ATR and ATM. In order to identify residues phsophorylated in response to IR during meiosis, we performed a peptide array screening. We probed with *C. elegans* extracts, prepared before or after DNA damage,and radio labelled ATP on peptide arrays we identified all in vitro putative phosphorylation sites (Figure 1).

Among the diffent candidates we have recently focus on the *C. elegans* orthologs of tumour suppressor proteins BRCA1 (Breast Cancer 1) and BARD1 (BRCA1-associated RING domain protein. We have uncovered the

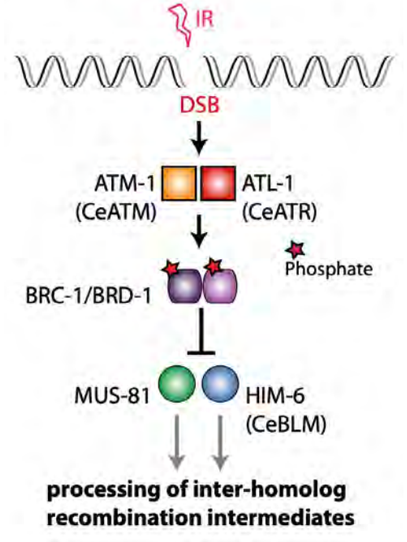
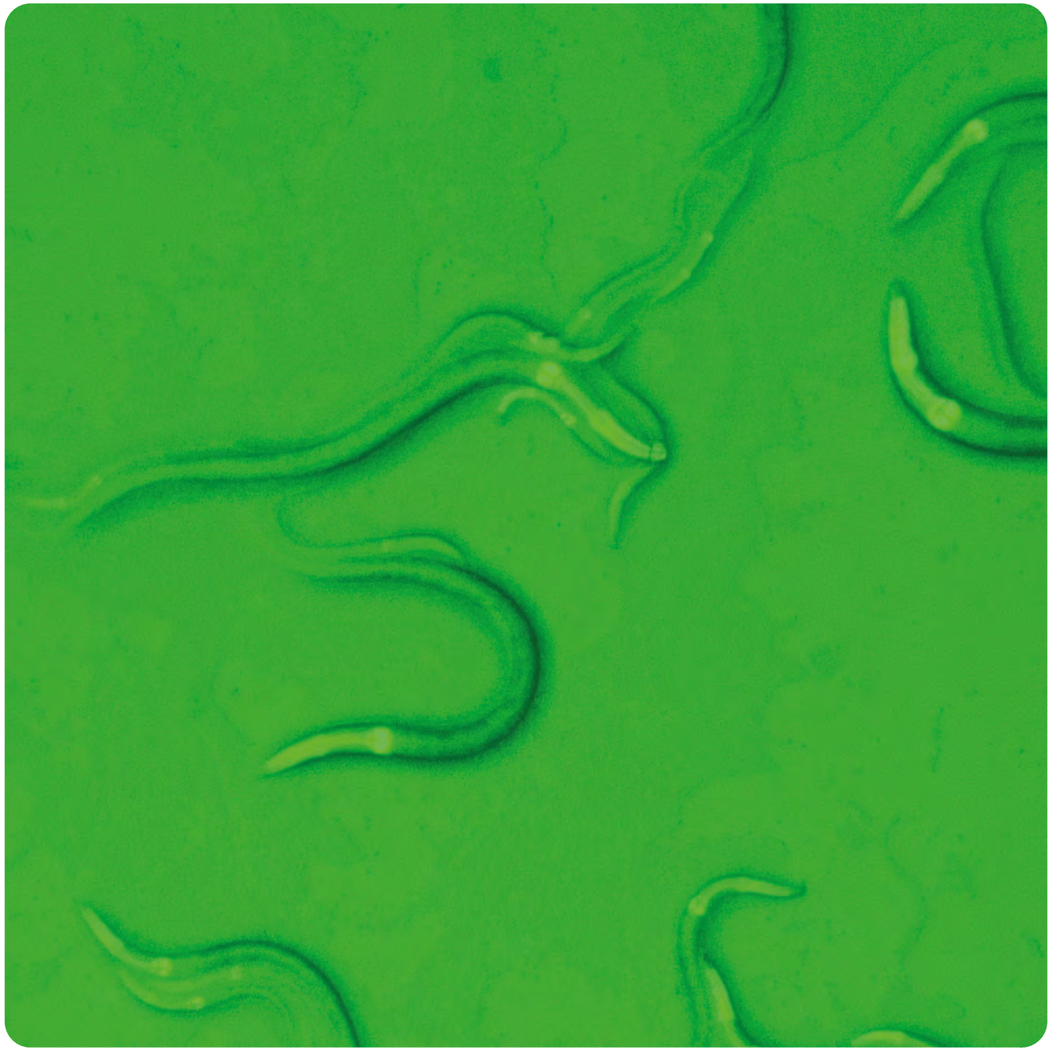


Figure 2: Proposed model. BRC-1/BRD-1 phosphorylation dependent of ATM-1/ATL-1 regulate HIM-6/MUS-81 function, correctly balancing the resolution/dissolution of recombination intermediates arising between homolog chromosomes. Through this mechanism, the cell regulates the processing and repair of excess inter-homolog crossovers, which could hinder correct chromosome segregation at the first meiotic division.

in vivo relevance of one of this DNA damage-dependent phosphorylation identified by the peptide array, specifically the posttranslational modification of both the C. elegans BRC-1 (Figure 1) and BRD-1 (not shown). We have proven that the phosphorylation of the BRC-1/BRD-1 heterodimer is essential for germline integrity in C. elegans. Failure to phosphorylate BRC-1/BRD-1 in response to DNA damage results in meiotic DSBs accumulation, chromosome breakage, catastrophic diakinesis and loss of fecundity. We further have shown that these defects are driven by the activity of C. elegans Bloom and Mus81, which catalyse Holliday junction dissolution and resolution, respectively. Hence, we propose that phosphorylation of BRC-1/BRD-1 in response to excess meiotic DSBs constitutes a key regulatory step that ensures the proper resolution of recombination intermediates required to preserve germline integrity (Figure 2).

Grants

- 2022-2025: PID2021-123850N. Ministerio de Ciencia e Innovación.
- 2022-2024: 2022/00000525. VII Plan Propio SuplemA, US.

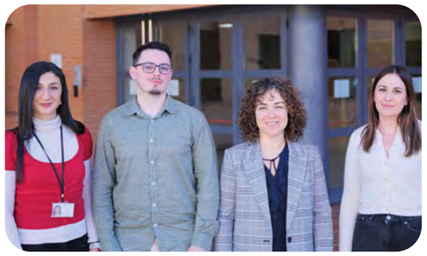


Principal Investigator

Dr. Silvia Jimeno-González

Transcription and mRNA processing

Emerging Group Leader



Current position

- Associate Professor, University of Seville (US).

Group Members

Postdoc

- Sabrina Rivero Canalejo (Assoc. Prof., US).

PhD Students

- Clara Megías Fernández,
- Alberto León Halcón.

Technician

- Irene Delgado-Sainz.

Former members (2023-2024)

- **Erasmus+ student:** Anna Giakoumidaki.

Research Activity

Overview

Topoisomerase II (TOP2) is an enzyme that regulates DNA topology, primarily by removing DNA supercoiling, which is crucial during transcription, as the movement of RNA polymerase II (RNAPII) generates torsional stress. In the last years, the role of TOP2 as a mere facilitator of transcription has been challenged, since the lack of TOP2 activity has been shown to upregulate specific genes. We aim to understand how DNA topology and topoisomerases can regulate gene expression, focusing on functions on both transcription and mRNA processing of the nascent RNA. With the use of genomic and proteomic approaches we have been able to identify novel TOP2-associated pathways involved in the coordination between transcription and mRNA processing that regulate the overall gene expression output. Our group is currently exploring the scientific and biomedical potential of these avenues of research.

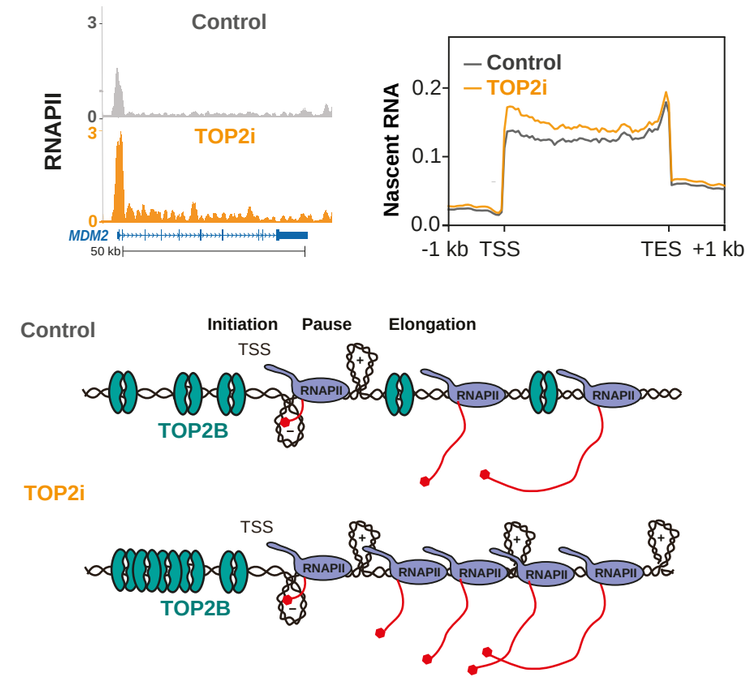


Figure 1. Transcriptional changes under TOP2 inhibition.

Research Highlights

The accumulation of topological stress in the form of DNA supercoiling is inherent to the advance of RNA polymerase II complexes and needs to be resolved to sustain productive transcriptional elongation. DNA topoisomerases are the enzymes that relax topological stress by transiently gating DNA passage, in a controlled cut-and-reseal mechanism that affects either one (type I DNA topoisomerases; mainly TOP1 in eukaryotes), or simultaneously both (type II topoisomerases; TOP2) DNA strands. Topoisomerases have been therefore traditionally considered general positive facilitators of transcription. However, the

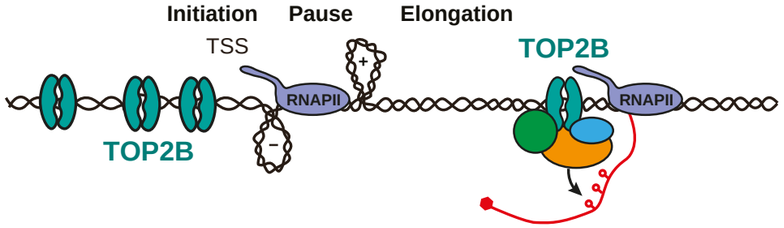


Figure 2. Dual function of TOP2 in regulation of gene expression..

function of TOP2 is not yet clearly established, since the treatment with both TOP2 catalytic inhibitors and poisons, which are molecules stabilizing cleavage complexes, increase expression levels of highly regulated genes such as stimuli-responsive genes.

To investigate the function of TOP2 in the regulation of gene expression, we have studied the consequences of TOP2 poisoning on both transcription and mRNA turnover measuring effects on nascent transcription (Figure 1) and on the decay rate of each RNA. With this approach we have identified novel functions of TOP2 in the regulation of transcription elongation and in co-transcriptional recruitment of RNA processing factors determinant for RNA fate, which is particularly relevant for genes repressed at the level of promoter-proximal pausing. We propose that TOP2 has a dual function in the regulation of paused genes, supporting basal transcription elongation levels while simultaneously coordinating modifications in pre-mRNAs (Figure 2).

Because of our previous work in which we established a connection between promoter-proximal pausing and TOP2 activity at promoters, we have also studied whether factors implicated in the regulation of transcription have a function in the repair of DSB generated by TOP2. Stabilization of the cleavage complexes produced within TOP2 catalytic cycle with TOP2 poisons produce such breaks. After DNA damage, the histone variant H2AX is phosphorylated (γ-H2AX) at the site of the break by ATM and ATR and this modification spreads over megabases, which can be observed with immunofluorescence experiments as foci in the nucleus. With ChIP-seq experiments and proteomics, we have discovered that elongation factors are specifically important for the signaling of TOP2 breaks and decisive for the recruitment of TOP2-specific repair factors.

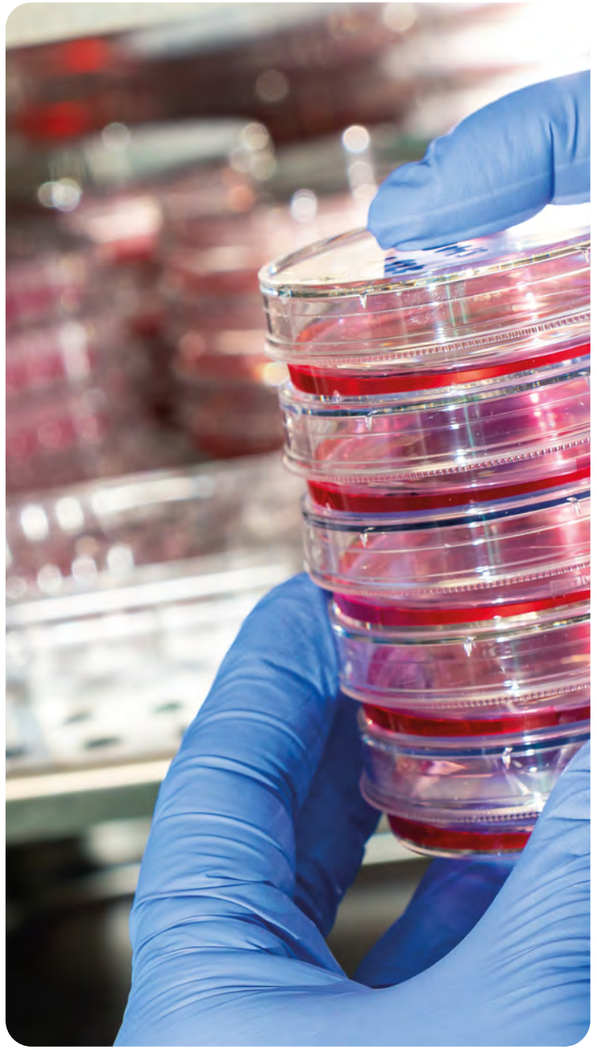
Our findings suggest that the role of topoisomerases extends beyond simply alleviating DNA supercoiling. They also play a crucial role in the intricate network involved in the complex regulation of gene expression, particularly in genes that respond to stimuli. Our future goal is to unravel the different pathways in which TOP2 is involved that are key for regulating transcription and mRNA stability.

Grants (starting or ending 2023-2024)

- 2020-2023: PID2019-104484G, Ministerio de Ciencia e Innovación.
- 2022-2025: ProyExcel_00835, Junta de Andalucía.
- 2023: VII Plan Propio Suplementaria A, Universidad de Sevilla.
- 2023-2026: PID2022-139253N, Ministerio de Ciencia, Innovación y Universidades.
- 2024: VII Plan Propio Suplementaria A, Universidad de Sevilla.

Publication Highlights

Iglesias-Ortega L, Megías-Fernández C, Domínguez-Giménez P, Jimeno-González S and Rivero S. **2023**. Cell consequences of loss of function of the epigenetic factor EHMT1. **Cellular Signalling** (Elsevier). 108:110734.

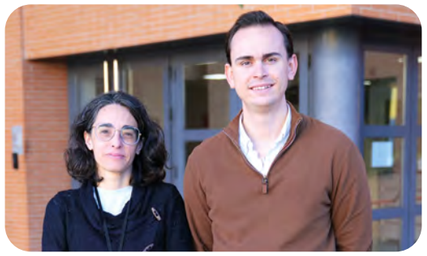


Principal Investigator

Dr. Cristina González-Aguilera

Replication and Nuclear Dynamics

Group Leader



Current position

- Tenured Scientist CSIC.

Group Members

PhD student

- Cristóbal Coronel Guisado.

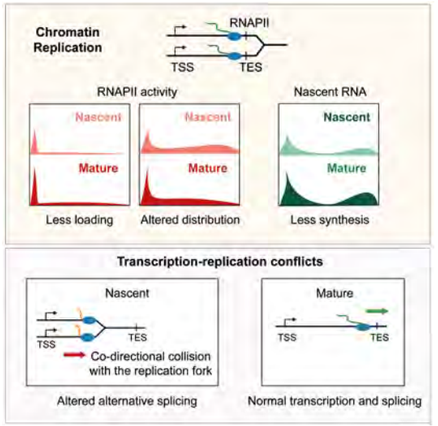
Former Members (2023-2024)

- **PhD student:** Federica Bruno.
- **Msc student:** María Marcos Simancas.

Research Activity

Overview

Chromatin replication is a necessary biological activity required to copy the genetic material that will be transferred to the daughter cells. However, it is also a very disruptive process producing a genome-wide chromatin disorganization. This includes the dilution of the epigenetic information and the reduction of DNA accessibility. Although the cell counts with specialized mechanisms to restore chromatin organization, complete restoration takes hours. This creates a window of time where transcriptional programs and gene expression may be altered. Our goal is to identify how these transient chromatin



alterations may impact gene expression regulation and nuclear dynamics and how they influence cell identity changes taking place in important physiological and pathological processes.

Research Highlights

To replicate the DNA, the replisome unwinds the two DNA strands and promotes the eviction of the parental nucleosomes ahead of the replication fork. Parental histones are recycled and distributed evenly between the two daughter strands, and together with the arrival of newly synthetized histones, allow the restoration of nucleosomes density in newly replicated chromatin. However, this movement of histones create several challenges. First, parental histones are not re-deposited in their original locations, covering, in some cases, key regulatory regions including enhancers and promoters. This results in a general reduction in chromatin accessibility. Second, newly synthetized histones lack the post-translational modifications present in parental histones, leading to a dilution of the epigenetic information by half.

To understand these processes, our laboratory has developed a cutting-edge technology, ChOR-seq (from Chromatin occupancy after replication), that enabled us to study, for the first time, the abundance and distribution of proteins bound specifically to newly replicated DNA. By labeling nascent chromatin in vivo with a nucleotide analogue, this technology provides the temporal resolution needed to capture the dynamic events occurring immediately after chromatin replication. Using ChOR-seq, our previous work showed that while cells restore chromatin organization within one cell cycle, this process is time consuming and histone mark and locus specific, creating fluctuations of the epigenetic information across the cell cycle. Thus, chromatin replication produces transient changes in chromatin organization that could significantly influence how gene expression and cellular functions are regulated.

Now, we have further demonstrated that chromatin replication transiently disrupts transcriptional activity and RNA synthesis. We discovered that transcription elongation resume within minutes after replication fork passes, meaning that transcription factors

(TFs) and RNA polymerase II (RNAPII) will interact with a chromatin that is not yet fully restored. Consequently, we observed that both the abundance and the distribution of the RNAPII were altered in nascent chromatin existing a general decrease in the level of RNAPII bound to chromatin that was associated to a general reduction in the RNA synthesis.

Moreover, we identified a new type of co-directional transcription-replication conflict (TRC) in which the progression of the RNAPII, transcribing newly replicated chromatin, is blocked by the presence of the replisome travelling ahead. This is the first demonstration that TRC can also alter transcriptional activity. These conflicts occur at the 3'end of large genes where transcription and replication move in the same orientation and we demonstrated it could have important implications in gene expression regulation since they promote changes in alternative splicing. Therefore, our findings reveal that chromatin replication not only reduces RNA synthesis but also modifies the type of isoform expressed by the cell. (Figure 1). Notably, genes affected by this novel TRC mechanism are of biological relevance being involved in key developmental and signaling pathways. This suggest that chromatin replication may serve as a regulatory mechanism for cell identity changes playing a critical role in physiological processes such as cell differentiation as well as in pathological processes like cancer.

Our current research focuses on understanding the molecular mechanisms that the cells employ to mitigate these transient disruptions in RNA synthesis to maintain cell identity and investigating how these post-replicative chromatin alterations impact cellular function. Unveiling these mechanisms, we aim to shed light on how chromatin replication influences cell identity and its implications for health and disease.

Grants (starting or ending 2023-2024)

- 2024-2026: EQC2024-008082-P. Junta de Andalucía.
- 2024-2025: 2024ICT161.CSIC. Ayuda incorporación Científicos titulares.
- 2023-2026: PID2022-140393NB-I00. Ministerio de Ciencia e Innovación.
- 2020-2023: PID2019-105742GA-100. Ministerio de Ciencia e Innovación.
- 2020-2024: RYC2018-025485-I. Ayuda Ramón y Cajal.

Publication Highlights

Bruno F, Coronel-Guisado C, González-Aguilera C. **2024**. Collisions of RNA polymerases behind the replication fork promote alternative RNA splicing in newly replicated chromatin. **Mol Cell.** 84(2):221-233.e6

Principal Investigator

Dr. Iván V. Rosado

Replication and endogenous DNA damage

Group Leader

Current position

- Associate Professor, University of Seville (US).

Group Members

Postdoct

- Marta del Rio Oliva.

PhD students

- Yaiza Rodríguez Martín.
- María José Peña-Gómez.

Former Members (2023-2024)

- **PhD students:** Paula Moreno Gordillo, María Teresa Medrano Domínguez.
- **Technicians:** Jesús Cea García, Gonzalo Pinaglia Tobaruela.

Research Activity

Overview

Our genome is constantly exposed to DNA damaging agents arising from exogenous sources or derived from our cellular metabolic reactions. Therefore, the maintenance and faithful inheritance of genetic information is essential to avoid disease. Cells have evolved several DNA repair mechanisms to preserve genetic information during DNA replication and chromosomes segregation while faithfully passing on the genome to daughter cells. Failures in these repair mechanisms can lead to genomic instability, which is a hallmark of many diseases, including cancer. Understanding these pathways is crucial for developing therapeutic strategies to mitigate the consequences of DNA damage. Of particular interest is the ultrarare genetic syndrome Fanconi Anemia (FA). FA is mainly characterized by congenital abnormalities, progressive bone marrow failure due to HSC depletion, and extreme cancer susceptibility. The FA syndrome gives name to a specific set of genes (BRCA1, BRCA2, RAD51, FANCD2…) involved in maintaining replication fork (RF) stability and in repairing complex DNA lesions such as interstrand crosslinks (ICLs). In addition to exogenous DNA damaging agents, endogenous metabolic chemicals and natural DNA transactions are emerging as novel sources of genomic instability. Our group is interested on deciphering the molecular mechanisms that avoid catastrophic consequences of damaged chromosomes, especially those operating during DNA replication.

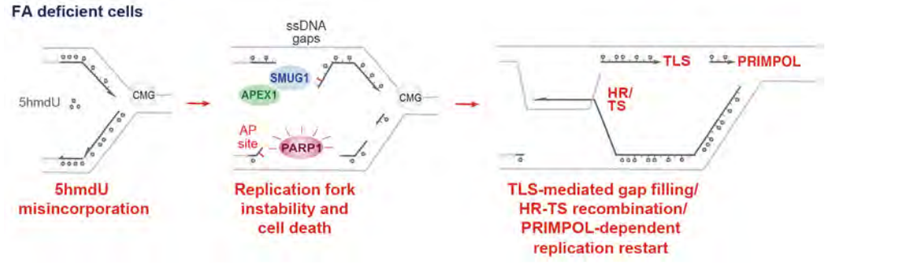


Figure 1. Proposed model of replication fork instability by 5hmdU misincorporation observed in replication fork maintenance defective FA/BRCA cells. Upon 5hmdU misincorporation (dark lollipop), the canonical SMUG1 DNA glycosylase (blue balloon) incised the ribose of the DNA backbone leaving an AP site (red stump). This AP site will be subjected to APEX1/APEX2 (green balloon) endonucleolytic cleavage introducing a ssDNA nick, which is further extended by the concerted activities of DNA2, EXO1 or APEX2 nucleases. These ssDNA gaps are signaled by PARP1 (pink balloon) to promote efficient DAN end ligation and dsDNA restoration. Malfunctioning of replication fork-associated BER induces replication fork instability in the absence of a functional FA pathway. 5hmdU-mediated stalled replication forks are resumed by alternate processes such as PRIMPOL-dependent DNA repriming or template switching recombination (TS). The overall result of these responses is the accumulation of highly cytotoxic ssDNA gaps on nascent DNA, which are further fixed by Homologous recombination (HR)- or traslesión synthesis (TLS)- dependent gap filling.

Research Highlights

Misincorporation of damaged nucleotides and depurination of adducted bases are amongst the commonest spontaneous base

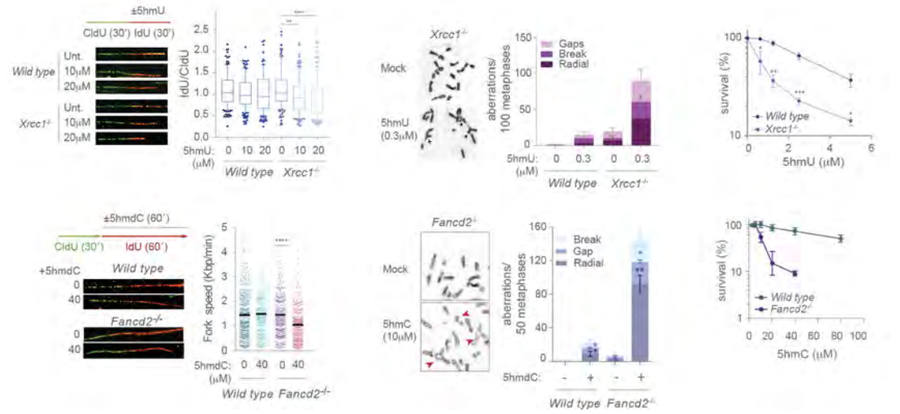


Figure 2. Cellular phenotypes of XRCC1 (top panels) or FANCD2 (*bottom panels*) deficient cells by misincorporation of 5hmdU during DNA synthesis. *Left*, replication fork instability was determined by pulse labeling replication with CldU followed by IdU. CldU/IdU or replication fork speed was calculated. *Middle*, chromosome aberration tests were assayed by exposing cells to varying dose of 5hmdU or 5hmdC for 48 h, and chromosome aberrations (breaks, gaps or radial chromosomes) were scored on metaphase spreads. *Right*, cells were exposed to a range of dose of 5hmdU or 5hmdC for 72h and viability was determined by the MTT assay.

lesions in DNA, triggering the canonical base excision repair (BER) mechanism. During removal of the damaged base by BER, AP sites generated by the SMUG1 DNA glycosylase are converted to single strand breaks (SSBs) by the AP endonuclease APEX1. Upon DNA end processing, DNA polymerases POLB or POLL conducts gap filling leaving nicked DNA ready for ligation by the XRCC1/LIG1-3 complexes. The molecular mechanisms by which deoxyuridine (dU) or modified dU, 5CldU or 5hmdU induce RF-associated genotoxicity have recently been under strong investigation (Figure 1). During dU, 5CldU or 5hmdU misincorporation, the N-glycosylase activity of UNG1/2 or SMUG1 mediates efficient excision of dU, 5CldU or 5hmdU leaving AP sites. When misrepaired, UNG- or SMUG1-mediated AP sites may persist. These BER intermediate structures can be potentially collided by the replisome resulting in BER-replication conflicts (BRCs). To fulfil DNA synthesis, cells trigger a PRIMPOL-dependent RF resumption at the expense of leaving cytotoxic ssDNA gaps. These unresolved BRCs and the subsequent accumulation of ssDNA gaps are proposed to account for the observed lethality in the absence of proficient RF maintenance

or homologous recombination (HR) pathways. The reported PARPi sensitivity of HR deficient (or deficient in RF maintenance) cells accurately correlates with the amount of ssDNA gaps. So, identifying the metabolic sources that hinder RF progression and the molecular mechanisms accounting for ssDNA gap accumulation is a fundamental but still unanswered question in the genome instability field.

Our group has recently revealed that misincorporation of the modified uracil 5´-hydroxymethyl-2´-deoxyuridine (5hmdU) is an important source of genomic instability in RF defective cells. Upon misincorporation, 5hmdU impaired RF progression in the absence of the BER and the FA factors XRCC1 and FANCD2, respectively. In addition to its role during canonical BER, XRCC1 has been found to be required for RF restart and mutagenic DNA repair in BRCA2 deficient cells. FANCD2 is redundant to BRCA2 in RF maintenance. Loss of function of either XRCC1 or FANCD2 result in heightened chromosome aberrations and loss of cell viability. These RF impairment and lethality phenotypes were exacerbated by PARP inhibitors, suggesting that impaired BER during 5hmdU removal induces BRCs and triggers the accumulation of ssDNA gaps (Figure 2). Our current research is focused on the identifying genetic factors that play a major role on the biogenesis/suppression of ssDNA gap. Moreover, understanding the molecular mechanisms triggered by BRCs that help to prevent genetic instability is paramount to a better therapeutic design. We are also addressing the physiological consequences of BRCS in tumour-prone FA mouse models..

Grants (starting or ending 2023-2024)

- 2022-2024: PID2021-128988OB-100. Ministerio de Ciencia e Innovación.
- 2023-2024: CNS2022-136055. Ministerio de Ciencia e Innovación.
- 2019-2022: P18-RT-1271. Junta de Andalucía.

Publication Highlights

Medrano M, Contreras M, Caballero-Velázquez T, Martínez L, Bejarano-García JA, Calderón-Ruiz R, García-Calderón CB, Rosado IV, Pérez-Simón JA. **2024**. Cannabinoids induce cell death in leukaemic cells through Parthanatos and PARP-related metabolic disruptions. **British Journal of Cancer.** 130 (9):1529 – 1541.

Pérez D, Moyá ML, Bautista M, León R, Molina-Márquez A, Vila M, Romero-Azogil L, Benito E, de Gracia García-Martín M, Moreno-Gordillo P, Rosado IV, Balestra FR, Huertas P, López-López M, López-Cornejo P. **2023**. A novel biocompatible polymer derived from D-mannitol used as a vector in the field of genetic engineering of eukaryotic cells. **Colloids and Surfaces B: Biointerfaces.** 224:113219

Principal Investigator

Dr. Gonzalo Millán-Zambrano

Chromatin modifications
Emerging Group Leader

Current position

- Tenured Scientist CSIC.

Group Members

Postdoc
- Sara Cea Sánchez.

PhD students
- Laura López Hernández.
- Ricardo Daniel de Arellano Casquete de Prado.

Master students
- Jesús Palmero García.
- Agustín Vera Enguídanos.

Former Members (2023-2024)

- **Postdoc:** Patrick Toolan-Kerr.
- **Master students:** Alexandra Sprenger, Laura Quintero Pantoja.

Research Activity

Overview

Chromatin structure is highly repressive to processes occurring on DNA. However, we know since pioneering studies by Vincent Allfrey that histones are subjected to a wide variety of covalent post-translational modifications (PTMs) that can modulate DNA accessibility, thereby playing key roles in many biological processes. During the past three decades, we have witnessed major advances in our understanding of the functional role of histone PTMs in key cellular processes. Although most breakthrough discoveries were driven by scientific curiosity, many of them have far-reaching implications for the treatment of human disease. This is based on the notion that, unlike genetic alterations, most of the known histone PTMs are likely reversible, which offers considerable promise for therapeutic intervention. Importantly,

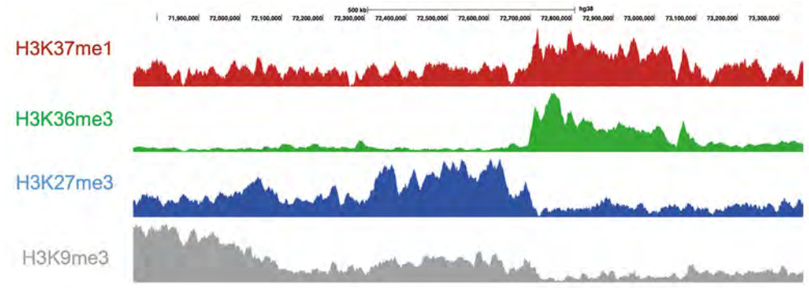


Figure 1. Genome browser view of H3K37me1, H3K36me3, H3K27me3 and H3K9me3 ChIPseq signal tracks at a representative genomic location.

there are increasing number of reports linking alterations of the histone PTM landscape to different cancer states. Our main interest is the characterization of the pathways leading to histone PTMs and their involvement in cancer.

Research Highlights

Recently, several novel histone PTMs have been identified by mass spectrometry studies. Although the evolutionary conservation of these modifications underscores their physiological relevance, the function of most of them remains to be elucidated. We previously characterized the role of a novel histone PTM, mono-methylation of histone H3 at lysine 37 (H3K37me1), in Saccharomyces cerevisiae (Santos-Rosa et al, Mol Cell 2021). We demonstrated that H3K37me1 is catalysed by Set1p and Set2p, and that it regulates DNA replication initiation. H3K37me1 prevents MCM replicative helicase interaction with chromatin, maintaining low levels of MCM loading outside of conventional replication origins. Consistently, depletion of H3K37me1 results in aberrant DNA replication initiation at cryptic genomic sites. Thus, our results indicated that H3K37me1 safeguards the correct execution of the yeast DNA replication program by protecting the genome from inappropriate origin licensing.

DNA replication stress is a major cause of genome instability, which is considered a hallmark of cancer. Importantly, DNA replication stress is not a common feature of normal cells, thereby representing a promising target for cancer-specific therapies. Cancer progression involves mutations in genes regulating cell proliferation, namely oncogenes and tumour suppressors. In this regard, it is well-established that oncogene activation can cause alterations of the DNA replication program, giving rise to replication stress. This has led to the proposal of a model for cancer development in which oncogene-

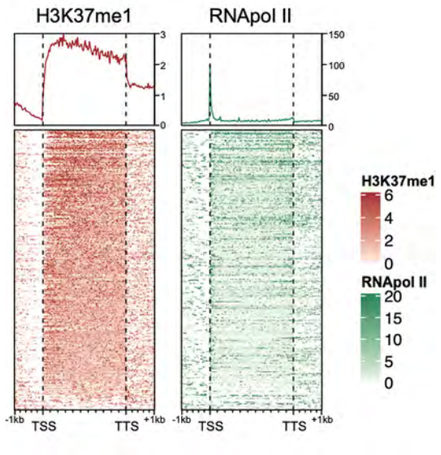


Figure 2. Metagene analysis of H3K37me1 and RNApol II ChIPseq signal distribution along transcribed genes.

induced DNA replication stress, an early driver of genomic instability in pre-cancerous cells, will in turn generate the genetic diversity necessary for cancer cells to escape apoptosis. Therefore, understanding the role of H3K37me1 in the suppression of aberrant DNA replication initiation sites has potential for clinical relevance.

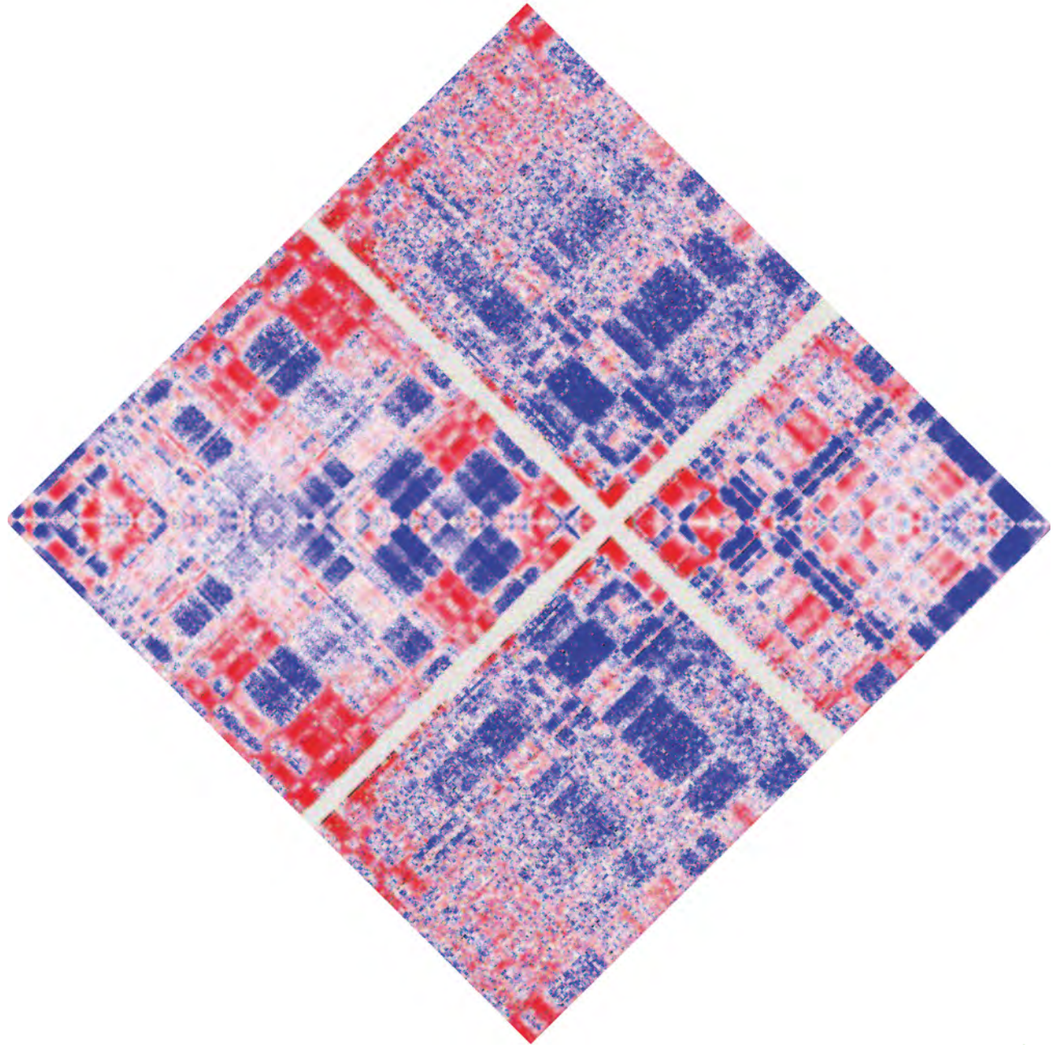
During 2023-2024 period, we have characterized the role of H3K37me1 in human cells. We found that H3K37me1 is strongly associated with transcribed genes, and that its levels are strongly correlated with those of RNA polymerase II (RNApol II). This suggest that H3K37me1 may be deposited co-transcriptionally. Interestingly, several studies have shown that, in unperturbed human cells, early DNA replication initiation sites are frequently located in non-transcribed regions adjacent to the transcription start site (TSS) of actively transcribed genes. In contrast, replication initiation within active gene bodies seems to be largely suppressed, thereby favouring co-directional orientation of replication and transcription. Our results indicates that transcription-dependent H3K37me1 supresses intragenic origin firing by destabilizing MCM chromatin association along transcribed genes.

Grants (starting or ending 2023-2024)

- 2024-2027: PID2023-151942NB-I00. Ministerio de Ciencia e Innovación.
- 2023-2025: CNS2022-135600. Ministerio de Ciencia e Innovación.
- 2022-2024: PID2021–127432NA-100. Ministerio de Ciencia e Innovación.

Publication Highlights

Terrón-Bautista J, Martinez-Sánchez M, López Hernández L, Vadusevan AA, García-Dominguez M, Williams RS, Aguilera A, Millán-Zambrano G*, Cortés-Ledesma F. **2023**. Topological regulation of the estrogen transcriptional response by ZATT-mediated inhibition of TOP2B activity. **BioRxiv**. (*Co-corresponding author).



Principal Investigator

Dr. Daniel Rico

Computational Epigenomics and Cell Identity

Group Leader

Current position

- Scientific researcher CSIC.

Group Members

PhD students

- Francisco Javier Mendoza.
- Ahmed Alsayadi (Newcastle).

Master student

- Alicia Santamaría (CSIC JAE-INTRO awardee).

Former Members (2023-2024)

- **PhD students:** Juliana Arcila (Newcastle), Jennifer Mitchell (Newcastle), Dafni Michalettou (Newcastle), Daniel Kent (Newcastle).
- **Master students:** Jack Turnbull (Newcastle), Athira Suresh (Newcastle), Juhua Su (Newcastle), Maxine Van Hagen (CABIMER).
- **Technician:** Ana López (CABIMER).

Research Activity

Overview

Daniel Rico moved his computational laboratory from Newcastle University (UK) to CABIMER in 2023. He considers himself a creative researcher and his scientific method is similar to the approach of an artist: his group continuously explores new ways to recycle, combine and analyse different types of biological data in order to understand how cells and organisms work. Their research focuses on developing computational approaches to understand the epigenomic mechanisms that generate diverse cell identities and how these processes are altered in cancer and immune-mediated diseases. They thrive working as part of bigger multidisciplinary teams, aiming for synergistic interactions with experimental biologists, physicists or clinicians that lead to ideas and discoveries that are not possible to achieve in isolation. Daniel has a strong commitment to training the next generation of computational biologists and promoting equality, diversity, and inclusion in academia.

Research Highlights

By integrating experimental studies, computational methods, and evolutionary perspectives, our group has contributed to a deeper understanding of chromatin dynamics and gene regulation. These studies offer insights into fundamental biological processes while providing tools and perspectives relevant to health and disease. Below, we summarize our key contributions from 2023 and 2024.

Computational Advances to Identify Promoter-Enhancer Regulatory Networks

To address challenges in identifying active regulatory elements, we developed Esearch3D, a computational tool that integrates chromatin architecture and transcriptional data to predict enhancer activity (Heer et al., 2023). By propagating transcriptional signals across three-dimensional genome networks, Esearch3D identifies regions with high enhancer likelihood (Figure 1A). Predictions were validated against established enhancer markers, including histone modifications and STARR-seq, demonstrating the power of combining network theory and genomic data. This method not only enables enhancer discovery but also provides a framework for exploring the dynamics of regulatory networks in different cellular contexts.

Evolution and Functional Insights into Genome Architecture and Regulation

When we look at cells with a microscope, the chromosomes look like a messy "wool ball" inside the nucleus, where it is hard to imagine how genes are organised and regulated. Only in the last 10-15 years have we been able to zoom in inside this genomic wool ball at an unprecedented resolution thanks to a technique called chromosome conformation capture (3C). 3C is based on DNA digestion, proximity ligation, deep DNA sequencing and bioinformatics analysis to identify the exact sequences that interact with each other inside the nucleus. In 3C data, we can see that the big wool ball of chromosomes is composed of smaller wool balls, sets of neighbouring genes in a linear chromosome that tend to preferentially interact with each other in three-dimensional space. These 3D genomic communities are called *topologically associated domains* (TADs). Accumulating evidence suggest that TADs could be the building blocks of the genome, enabling the coordinated activity of genes. We examined the role of TADs in organizing gene function and evolution (James et al., 2024). Our study revealed that genes within TADs are organized by evolutionary age, with older TADs enriched in conserved regulatory

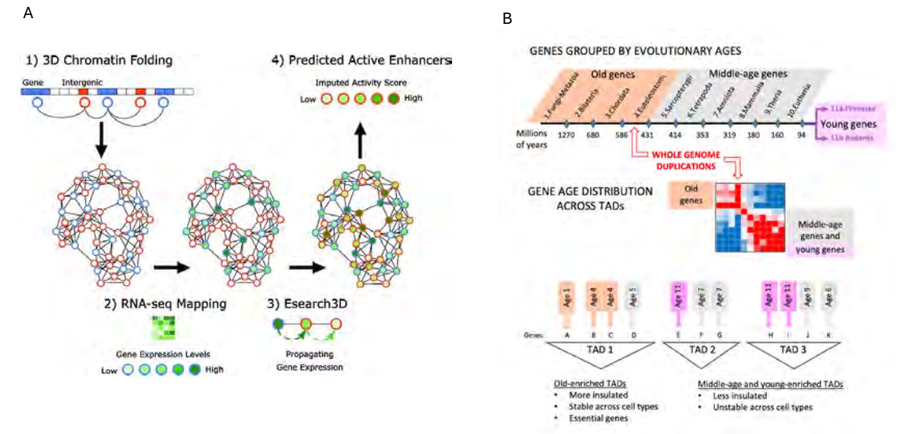


Figure 1. A) Graphical summary of the Esearch3D method (Heer et al, 2023), available at: https://doi.org/10.5281/zenodo.7737123. Gene expression is propagated in 3D genome networks as information is propagated in social networks, allowing to identify the "influencer" non-genic nodes. **B)** Schematic approach of the approach followed in James et al (2024) where we stratified genes by their evolutionary age across the human and mouse genomes.

networks (Figure 1B). Notably, younger genes interacting with ancient gene networks showed increased functional relevance, shedding light on how TADs integrate ancestral functions with genomic innovation.

Our work on the MYEOV gene provided a fascinating example of how ancient regulatory elements evolve novel functions (Davidson et al., 2024). Initially identified as a primate-specific proto-oncogene, MYEOV likely originated from a deeply conserved enhancer regulating a better known proto-oncogene: Cyclin D1 (CCND1). We demonstrated that this enhancer state is preserved across species, including mice, where MYEOV lacks an open reading frame but retains its regulatory function. These findings highlight the evolutionary trajectory of enhancer elements and their ability to acquire protein-coding species-specific roles while maintaining conserved regulatory interactions.

Chromatin Modifications, Cell Division, Cell Identity and Cancer

Histone modifications are key to regulating transcription and chromatin interactions, yet their combinatorial effects in cells remain underexplored. Building on the histone code hypothesis, we investigated the interplay between mitotic histone H3T3 phosphorylation (H3T3ph) mark and the active-promoter mark H3K4me3 (Harris et al., 2024). While H3T3ph antagonizes H3K4me3 readers *in vitro*, we demonstrated that, in cells, the transcriptional repression during mitosis occurs independently of this mechanism. This finding challenges assumptions about histone modification interactions in mitosis and suggests alternative regulatory pathways.

Finally, we are very interested in understanding the interplay between enhancer clusters, such as super-enhancers, and broad H3K4me3 domains (H3K4me3-BDs), regulatory features associated with cell identity in both normal and cancer-specific gene expression. We have reviewed our previous work this area in the context of the recent literature (Kent et al., 2023) proposing how super-enhancers and H3K4me3-BDs co-regulate tumor suppressor and oncogene expression. Our conceptual framework offers mechanistic insights and potential therapeutic strategies for cancers driven by these dysregulated regulatory elements.

Grants (2023-2024)

- 2024-2027: PID2023-148272OB-I00. Ministry of Science and Innovation.
- 2024-2025: 202420E057. Plan Intramural Especial, CSIC.
- 2020-2024: Barbour Foundation, UK.
- 2022-2023: Children's Cancer and Leukaemia Group.
- 2022-2023: The Newcastle upon Tyne Hospitals NHS Foundation Trust.
- 2019-2023: Discovery Medicine North, Medical Research Council.

Publication Highlights

Davidson B, Arcila-Galvis JE, Trevisan-Herraz M, Mikulasova A, Brackley CA, Russell LJ, Rico D. **2024.** Conserved enhancer-associated features within the MYEOV locus suggest a regulatory role for this non-coding DNA region in cancer. **Frontiers in Cell and Developmental Biology**. 12.

James C, Trevisan-Herraz M, Juan D, and Rico D. **2024**. Evolutionary analysis of gene ages across TADs associates chromatin topology with whole genome duplications. **Cell Reports.** 43, 113895.

Harris RJ, Heer M, Levasseur MD, Cartwright TN, Weston B, Mitchell JL, Coxhead JM, Gaughan L, Prendergast L, Rico D, Higgins JMG. **2023**. Release of Histone H3K4-reading transcription factors from chromosomes in mitosis is independent of adjacent H3 phosphorylation. **Nature Communications.** 14(1):72.

Heer M, Giudice L, Mengoni C, Giugno R, and Rico D. **2023**. Esearch3D: propagating gene expression in chromatin networks to illuminate active enhancers. **Nucleic Acids Research.** 51(10):e55.

Kent D, Marchetti L, Mikulasova A, Russell LJ, Rico D. **2023**. Broad H3K4me3 domains: Maintaining cellular identity and their implication in super-enhancer hijacking. **BioEssays.** 45:2200239.

Cell Dynamics

Cell Dynamics and Signaling

The main objective of the Department of Cell Dynamics and Signaling is to shed light on the mechanisms ensuring the normal function of cells and the safeguarding of tissue homeostasis. Processes like cell differentiation, migration, division, death and tissue and organ morphogenesis are essential for the integrity of the organisms. Alterations in these processes are frequently associated with the emergence of different pathologies, and cancer and degenerative diseases stand out among them. The alteration of cell division, but also of cell death and differentiation, is tightly linked to the development of cancer, which together with the enhanced migratory and invasive properties acquired by tumor cells, are classical hallmarks of cancer. Accurate coordination and timing of these processes depends on precise regulation of signaling mechanisms, able to integrate both internal and external cues to elaborate the appropriate responses to safeguard the correct cellular physiology. The main objective of the Department is to provide new knowledge on these signaling and response mechanisms, either under normal conditions as in altered or pathological situations. Thus, we aim to precisely define targets and pathways for effective therapeutic intervention to fight the aforementioned pathologies. In 2024, a new research group joined our Department, that of Dr. Patricia Altea Manzano, who obtained a position as Emerging Principal Investigator at CABIMER, while the group of Professor José Antonio Pintor retired at the end of 2023.

HEAD OF DEPARTMENT

Dr. Mario García-Domínguez

RESEARCH GROUPS

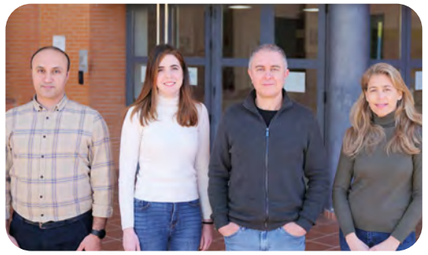
1. **Cell Differentation**
   Dr. Mario García-Domínguez
2. **Cell Death Signalling**
   Prof. Abelardo López-Rivas
3. **Cell Cycle and Oncogenesis**
   Prof. José Antonio Pintor
4. **Microtubule Dynamics in Health and Disease**
   Dr. Rosa M. Ríos
5. **Cell Division Control**
   Dr. Fernando Monje-Casas
6. **Metabolism and Cell Signaling**
   Dr. Raúl V. Durán
7. **Pancreas and Liver Development and Disease**
   Dr. Anabel Rojas
8. **Ubiquitin (-like) signaling and Proteomics**
   Dr. Román González-Prieto
9. **Metabolic Regulation and Signaling in Cancer**
   Dr. Patricia Altea-Manzano

Principal Investigator

Dr. Mario García-Domínguez

Cell Differentiation Laboratory

Group Leader



Current position

- Scientific researcher CSIC.
- Head of the Cell Dynamics and Signaling Department.

Group Members

PhD student
- Vahid Jafari.

Postdoc
- Nieves Lara Ureña.

Technicians
- Belén Torres Agrela.

Erasmus+ student
- Michele Fedele.

Research Activity

Overview

Our main research objective is deciphering the molecular mechanisms controlling cell viability and differentiation, especially concerning the nervous system. In particular, we study the post-translational modification of proteins by covalent attachment of the SUMO polypeptide (sumoylation). We mainly focus on transcriptional control and chromatin-associated factors. SUMO is essential in eukaryotes and participates in the regulation of almost all cellular processes. Knowing in detail the components of the SUMO pathway involved in the regulation of proliferation, differentiation, and cell viability, together with the associated regulatory mechanisms, is of great therapeutic interest for nervous system disorders and cancer. We have also studied the BET family of proteins, which is highly linked to proliferation and dysregulated in most cancers.

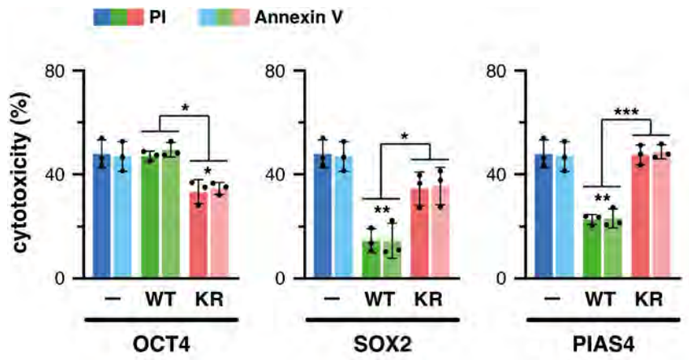
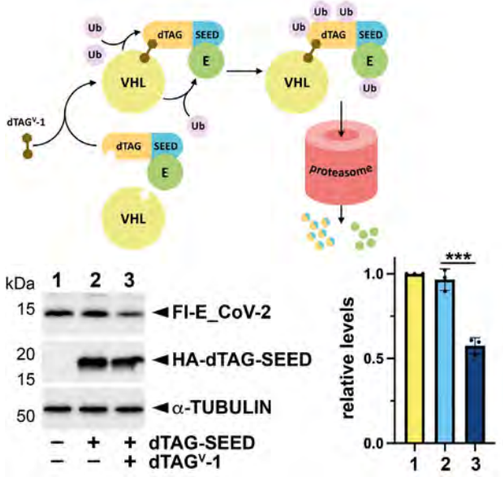


Figure 1. The sumoylation state of specific transcription factors affects cell viability. P19 cells were subjected to harmful OGD conditions (20 h) 24 h after transfection of expression vectors of the indicated factors, either the wild-type version (WT), or a version with a point mutation of the target Lys residue to Arg (KR), which renders the protein resistant to SUMO modification. Empty expression vector (–) was used as a control. The percentage of cytotoxicity was double-measured by analysis of propidium iodide (PI) incorporation (late apoptosis) and Annexin V labeling (early apoptosis). The statistical significance of differences was analyzed by ANOVA (*p<0.05, **p<0.01, ***p<0.001). Differences with control are indicated above the bars, other differences are indicated with lines.

In particular, we have recently characterized the consequences of the interaction of BET members with the envelope protein (E) of the SARS-CoV-2 virus, opening new therapeutic approaches to fight the virus.

Research Highlights

At least three functional molecules of SUMO have been described in vertebrates: SUMO1-3. SUMO2-3 are almost identical and rapidly conjugated to proteins in response to different stress conditions. A key role of SUMO modification, especially highlighted in the last decade, is preserving cell viability from harmful conditions through massive modification of proteins. In a recent proteomic study to identify proteins sumoylated in response to oxygen and glucose deprivation conditions (OGD), quite frequent inside solid tumors, we identified more than one hundred proteins undergoing modification in response to these conditions, which rapidly demodified after restoring oxygen and glucose (ROG). We found that SUMO targets responding to OGD-ROG were similar in proliferating and neuronal differentiated cells. We validated several targets and analyzed the consequences of overexpressing wild-type and sumoylation



Fusion of the SEED domain to the dTAG peptide mediates PROTAC-dependent degradation of E protein from SARS-CoV-2. Upper panel: The dTAG peptide has the ability to recruit the Ubiquitin ligase VHL in the presence of the PROTAC dTAGV-1 for ubiquitination of the fusion protein dTAG-SEED and side ubiquitination of its interactors, leading to degradation of ubiquitinated proteins in the proteasome. Lower left panel: the dTAG-SEED fusion protein was expressed in the presence or absence of the PROTAC dTAGV-1 in HEK293T cells as indicated, and cell extracts were analyzed by western blot to visualize the expression levels of E protein (tagged with a Flag epitope, Fl) and the fusion protein dTAG-SEED (tagged with an HA epitope). a-TUBULIN was registered as a protein loading control. Lower right panel: protein quantification of E protein (relative levels) from the precedent western blot. Results indicate a reduction of E protein close to 50% in the presence of the fusion protein and the PROTAC.

mutant versions of selected proteins regarding cell viability after deleterious OGD conditions. Notably, we found that overexpressing wild-type versions of SOX2 and PIAS4 factors, or a sumoylation-defective mutant of OCT4, resulted in reduced toxicity under these conditions (Fig. 1).

BET proteins are chromatin adaptors involved in transcriptional activation through the recognition of acetyl-group modifications in histones. This function is performed by two tandem N-terminal bromodomains. Besides, BET proteins are characterized by the presence of an exclusive ET domain mediating protein-protein interaction, a coiled-coil dimerization domain that we formerly characterized, and a C-terminal SEED region rich in Ser, Asp, and Glu residues. Four members compose the vertebrate family: BRD2, BRD3, BRD4, and BRDT. Recently, it was reported that the envelope protein (E) of the SARS-CoV-2 virus interacted with BRD2 and BRD4, and recent publications proposed that this is a viral hijacking mechanism since E protein interacts with bromodomains, which should block BET function, mimicking BET inhibitors. On the contrary, we have found that E protein does not interact with bromodomains but with the SEED domain of BRD2 and BRD2. Even more, other proteins reported to interact with E protein also contain SEED-like domains, which mediate the interaction. Far from acting as a BET inhibitor, which we have observed to result in the downregulation of natural immunity- and interferon response-related genes, E overexpression results in upregulation of these genes. By taking advantage of a PROTAC system and the ability of the SEED domain to selectively interact with E protein, we have been able to promote E protein degradation, which can be therapeutically exploited to fight COVID-19 disease (Fig. 2).

Grants

- 2022-2025: PID2021-125791NB-I00. Ministerio de Ciencia e Innovación.

Publication Highlights

Lara-Ureña N, Gómez-Marín E, Pozuelo-Sánchez I, Reyes JC, García-Domínguez M. **2024**. SARS-CoV-2 E protein interacts with BRD2 and BRD4 SEED domains and alters transcription in a different way than BET inhibition. **Cell Mol Life Sci**. 81:313.

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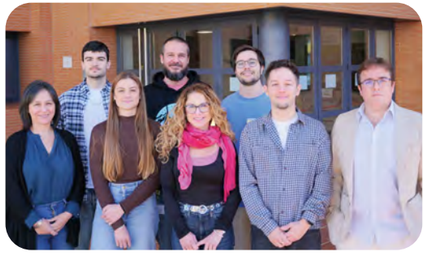
Pérez-Cabello JA, Silvera-Carrasco L, Franco JM, Capilla-González V, Armaos A, Gómez-Lima M, García-García R, Yap XW, Leal-Lasarte M, Lall D, Baloh RH, Martínez S, Miyata Y, Tartaglia GG, Sawarkar R, García-Domínguez M, Pozo D, Roodveldt C. **2023**. MAPK/MAK/MRK overlapping kinase (MOK) controls microglial inflammatory/type-I IFN responses via Brd4 and is involved in ALS. **Proc Natl Acad Sci USA**. 120:e2302143120.

Principal Investigator

Dr. Raúl V. Durán

Metabolism and cell signaling
Group Leader



Current position

- Scientific researcher CSIC.

Group Members

Senior Researcher
- Jonathan Martínez Fábregas.

Postdoctorals
- Macarena Morillo Huesca.
- Mercedes Tomé Montesinos.

PhD student
- Ryan Conesa Bakkali.
- Ignacio González López-Cepero.
- Ana Reina Bando.
- Laura Zarzuela Moncada.

Master Students / Erasmus +
- Jorge Martín-Montalvo Ruiz.

Former Members (2023-2024)

- **Senior Researcher:** Socorro Murdoch.
- **Postdoctoral:** María Jesús Fernández Ávila.
- **Master Student:** Andrea Ramos Luna.

Research Activity

Overview

The Group of Metabolism and Cell Signaling studies the crosstalk between cellular metabolic and bioenergetic flows with signaling processes, and how this interaction contributes to coordinate the growth and homeostasis of cells and tissues. In particular, we study how these interaction mechanisms are deregulated in cancer at the molecular and cellular level. During last years, the group has established the processes of interaction between the metabolism of the amino acid glutamine, the most abundant in human blood and the most important from an energetic point of view for cells, with cell signaling via the mTOR pathway,

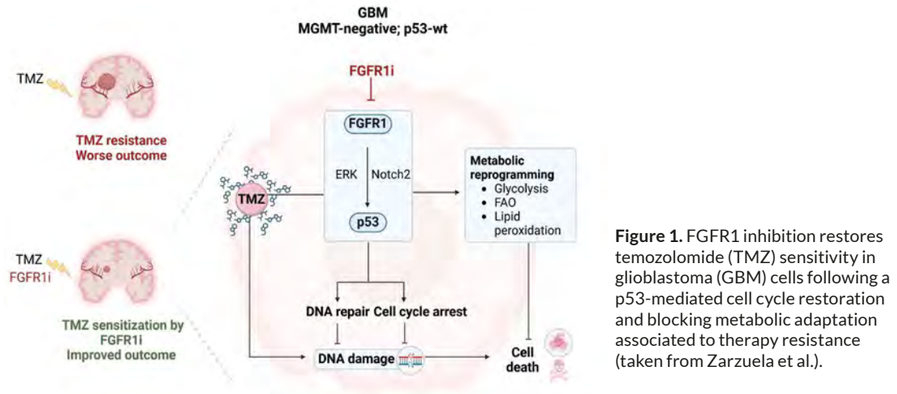


Figure 1. FGFR1 inhibition restores temozolomide (TMZ) sensitivity in glioblastoma (GBM) cells following a p53-mediated cell cycle restoration and blocking metabolic adaptation associated to therapy resistance (taken from Zarzuela et al.).

a protein complex essential in the regulation of cell growth and metabolism, and autophagy. In addition, we are also interested in the identification of the extralysosomal targets of the lysosomal proteases shown to be involved in the regulation of a plethora of physiological processes, such as mitosis, gene expression, or differentiation.

Research Highlights

Glutamine, mTOR and autophagy: a multiconnection relationship glutamoptosis

Cancer cells metabolize glutamine mostly through glutaminolysis, a metabolic pathway that activates mTORC1. The AMPK-mTORC1 signaling axis is a key regulator of cell growth and proliferation. Our recent investigation identified that the connection between glutamine and AMPK is not restricted to glutaminolysis. Rather, we demonstrated the crucial role of ASNS (asparagine synthetase) and the GABA shunt for the metabolic control of the AMPK-mTORC1 axis during glutamine sufficiency. Our results elucidated a metabolic network by which glutamine metabolism regulates the mTORC1-macroautophagy/autophagy pathway through two independent branches involving glutaminolysis and ASNS-GABA shunt.

FGFR1 inhibition improves therapy efficacy and prevents metabolic adaptation associated with temozolomide resistance in glioblastoma

Recurrent therapy resistance is a major limitation in clinical efficacy and for the outcome of glioblastoma (GBM) patients, positioning GBM among the tumor types

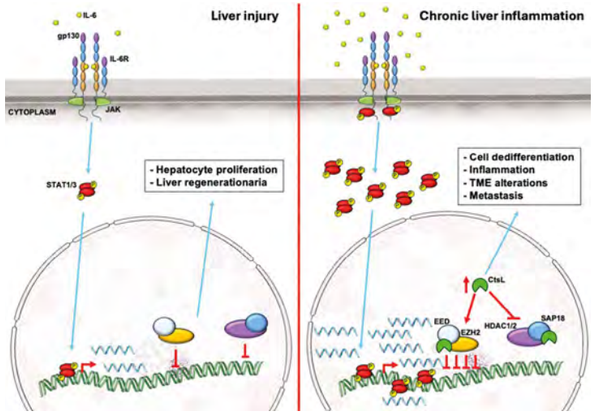


Figure 2. Transcriptional role of the IL6/CtsL axis in chronic liver disease and hepatocellular carcinoma.

with the poorest survival outcomes. In this work, we dissected resistance mechanisms in GBM, which resulted in the identification of FGFR1 pathway as a major regulator of the signaling and metabolic rewiring associated with temozolomide (TMZ) resistance in GBM. Hence, we described a mechanism of resistance that operates at two major levels. First, a p53-mediated regulation of cell cycle inducing cell cycle arrest to allow DNA repair in response to TMZ. And second, a complete metabolic rewiring promoting lipid catabolism and preventing lipid peroxidation. Both the p53-mediated response and the metabolic adaptation are controlled by FGFR1, as inhibition of the FGFR1 pathway completely abolishes this signaling and metabolic reprograming, restoring sensitivity to TMZ. Our results also indicated a correlation of FGFR1 levels with poor prognosis in GBM patients, and validated the treatment of TMZ in combination with FGFR1 inhibitors as an efficient strategy to induce tumor cell death in pre-clinical animal models. This data position the receptor FGFR1 as a very promising candidate for evaluation in future clinical approaches to limit the development of therapy resistance to TMZ in GBM patients.

Asparagine endopeptidase contributes to genotoxic stress resistance through ATR regulation in invasive ductal breast carcinoma

This project is led independently by Jonathan Martínez-Fábregas. Lysosomal proteases have frequently been implicated in the initiation and progression of cancer, but the underlying mechanisms remain poorly understood limiting the capacity to design new strategies for cancer treatment. We demonstrated that the lysosomal protease asparagine endopeptidase (AEP) accumulates in the nuclei of breast cancer cells, thereby contributing to their resistance to genotoxic stress. We demonstrated that AEP deficiency in cancer cells leads to increased sensitivity to genotoxic insults, resulting in genomic instability and cell death. Our findings also revealed that AEP specific inhibition sensitizes breast cancer cells to chemotherapy drugs cisplatin and etoposide. Interestingly, a negative correlation between AEP and ATR protein levels in breast cancer patients using data available from the TCGA database, have been found. Thus, those patients expressing high levels of AEP show low levels of ATR and poorest outcome and response to radiation therapy. Finally, all these data have been further corroborated by immunofluorescence using an independent cohort of human invasive ductal breast carcinoma samples, confirming that non-responder patients exhibited high levels of nuclear AEP leading to reduced ATR levels, in comparison to responder patients. Our data provide novel strategies for treating resistant tumors by combining AEP inhibitors with current chemo- and radiotherapy approaches, to enhance sensitivity to genotoxic insults.

Grants

- 2023-2025: RED2022-134927-T.Ministry of Science and Innovation.
- 2022-2025: PID2021-124251OB. Ministry of Science and Innovation.
- 2021-2023: US-1381282. The University of Seville.
- 2021-2023: PY20_00757. The Regional Government of Andalusia, Regional Ministry of Economy, Industry, Knowledge and Universities.
- 2023-2027: EMC21-00124. The Regional Government of Andalusia.
- 2023: RYC2021-032389. Ministry of Science and Innovation.

Publication Highlights (2023-2024)

Durán-Díaz I, Sarmiento A, Fondón I, Bodineau C, Tomé M and Durán RV. **2024**. Robust method for unsupervised scoring of immunohistochemical staining. **Entropy.** 26: 165.

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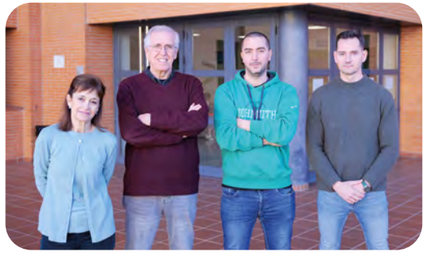
Principal Investigator

Prof. Abelardo López Rivas

Cell Death Signalling

Group Leader



Current position

- Since 2023, Ad Honorem Professor, CSIC.

Group Members

Senior Researcher

- Carmen Palacios Casanova.

PhD Student

- Younes El Yousfi El Mourabit.

Technician

- Francisco Javier Fernández Farrán.

Former Members (2023-2024)

- **Postdocs:** Rosario Yerbes Cadenas, Rocío Mora Molina.
- **Technician:** Belén Torres Agrela.

Research Activity

Overview

Physiological control of extracellular matrix (ECM) stiffness is an essential process to maintain tissue architecture and cellular homeostasis. However, alterations in the composition and mechanical properties of the ECM occur during tumor development leading to changes in its stiffness. These changes in ECM stiffness may lead to important changes in cytoskeletal dynamics, which in turn activate mechanotransduction signaling pathways that are fundamental in tumor progression. In addition to cell-intrinsic alterations, the uncontrolled proliferation of tumor cells in solid tumors results in the generation of different stress factors in the tumor microenvironment such as hypoxia, nutrient scarcity and acidosis that can affect the correct folding of proteins in the endoplasmic reticulum (ER) of the tumor cells, leading to persistent ER stress. Taking into account that a possible outcome of the

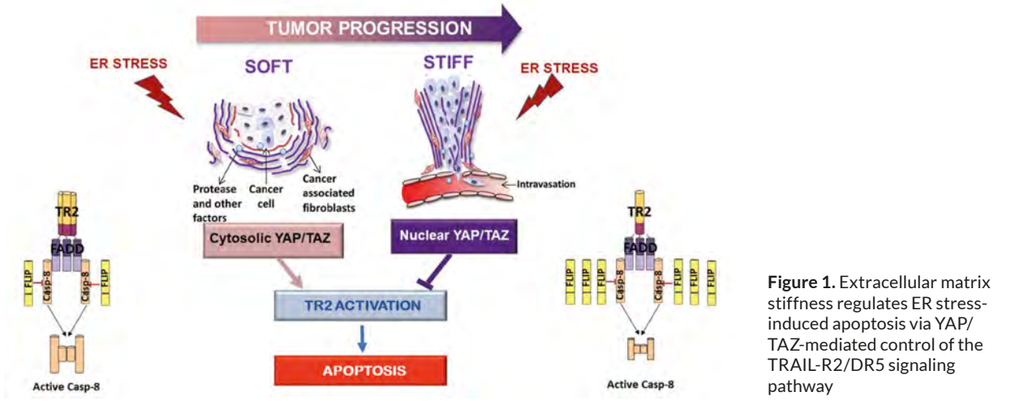


Figure 1. Extracellular matrix stiffness regulates ER stress-induced apoptosis via YAP/TAZ-mediated control of the TRAIL-R2/DR5 signaling pathway

chronic activation of the ER stress response is the induction of an apoptotic process, an essential question that remains unresolved is to know the mechanisms that allows tumor cells to remain viable in these conditions, thus enabling tumor progression. Mechanical signals emerging from extracellular matrix (ECM) rigidity and cell shape regulate the activity of transcriptional co-activators Yes-associated protein (YAP) and its paralog Transcriptional Coactivator with PDZ-binding motif (TAZ). However, the role of ECM rigidity and YAP/TAZ in tumor cell fate decisions under ER stress remains relatively unexplored. A major goal of our research is to decipher the molecular mechanisms underlying the decision between an adaptive protumoral response and cell death by apoptosis after ER stress generated during tumor growth. Within this general objective, we aim at defining the role of caspase-8 platforms and the impact of extracellular matrix stiffness and its downstream effectors of mechanotransduction YAP/TAZ in the fate of tumor cells undergoing ER stress.

Research Highlights

Our recent results suggest that the YAP/TAZ system plays an important role in the control of ER stress-induced cell death by mechanical signaling arising from ECM stiffness in tumor cells. Mechanistically, YAP/TAZ regulates apoptosis induced by ER stress in tumor cells by controlling the activation of the TRAIL-R2/DR5-mediated extrinsic apoptotic pathway through a dual mechanism. On the one hand, the YAP/TAZ system prevents intracellular

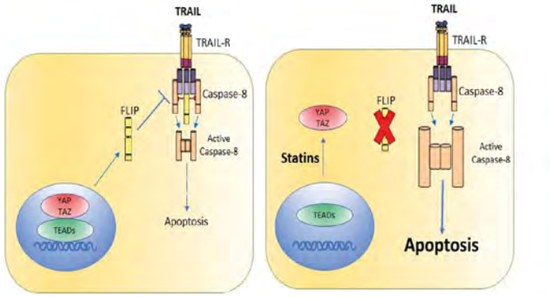


Figure 2. Inhibiting nuclear YAP/TAZ localization by statins promotes cFLIP down-regulation and TRAIL-induced apoptosis in tumor cells

TRAIL-R2/DR5 clustering in tumor cells. On the other hand, it inhibits cFLIP down-regulation in tumor cells experiencing ER stress. Moreover, while YAP/TAZ might have cytoplasmic functions, our findings with the tankyrase inhibitor XAV939 as well as the TEAD inhibitor further suggest a role of the nuclear YAP/TAZ-TEAD module in the regulation of ER stress-induced apoptosis. In addition, YAP/TAZ controls the expression of pro-inflammatory interleukin-8 (IL-8/CXCL8) in tumor cells undergoing ER stress by a TRAIL-R2/DR5/caspase-8-dependent mechanism. Although there may be other mechanisms involved in controlling cell death and inflammation in tumor cells facing environmental stress, our results support a model in which regulation of the subcellular localization and activity of the YAP/TAZ transcriptional co-activators would represent an important event in the microenvironmental control of cell fate decisions in tumor cells undergoing environmental stress.

Deciphering the mechanism responsible for TRAIL-R2/DR5 clustering in cells growing in soft ECM or depleted of YAP/TAZ is an issue that requires further investigation. In this respect, different studies have demonstrated an important role of the YAP/TAZ system in the transcriptional control of genes involved in cytoskeleton dynamics. One possible explanation for our observation of TRAIL-R2/DR5 oligomerization at intracellular membranes of the secretory pathway in tumor cells depleted of nuclear YAP/TAZ is the inhibition of vesicle trafficking required for protein transport from the Golgi to the plasma membrane as recently reported for other receptors. Alternatively, it has been reported that YAP/TAZ may have a transcriptional co-repressor function of different target genes, including TRAIL. In addition, ectopic expression of TRAIL has been shown to result in the intracellular retention of TRAIL receptors. Therefore, reducing the nuclear levels of YAP/TAZ either by growing the cells in soft ECM or by silencing their expression would lead to TRAIL up-regulation and TRAIL-induced intracellular oligomerization of TRAIL-R2/DR5, which may trigger apoptosis signaling and inflammatory cytokine production.

Our findings reveal that besides regulating TRAIL-R2/DR5 oligomerization, ECM stiffness is also modulating a YAP/TAZ-mediated signaling mechanism to control cFLIP expression in tumor cells. Along with the canonical role of cFLIP proteins controlling DISC-dependent caspase-8 activation at the plasma membrane, cFLIPL may be also present at the ER to inhibit caspase-8-mediated cleavage of ER-localized proteins. Our data also demonstrate that cFLIPL levels play an important role in tumor cell fate upon ER stress by inhibiting early activation of TRAIL-R2/DR5-activated apoptotic pathway thus granting the necessary conditions to mount an adaptive response that will restore proteostasis and support tumor progression. Therefore, our results underscore the importance of the increased ECM stiffness to maintain cFLIP levels and tumor cell viability in the adverse environmental conditions of the tumor microenvironment, through the activation of the YAP/TAZ-TEAD signaling module.

Different studies have reported that inhibition of the mevalonate pathway by statins may increase the sensitivity of tumor cells to tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), although the signaling mechanism leading to this sensitization remains largely unknown. We have investigated the role of the YAP (Yes-associated protein)/TAZ (transcriptional co-activator with PDZ-binding motif)-TEAD (TEA/ATTS domain) transcriptional complex in the metabolic control of TRAIL sensitivity by the mevalonate pathway. Our data reveal that depleting nuclear YAP/TAZ either by treatment with statins or by silencing YAP/TAZ expression with siRNAs synergizes with TRAIL in the activation of caspase-8 and apoptosis in tumor cells. Furthermore, blockage of TEAD activity either pharmacologically or with a genetically encoded inhibitor of the interaction of YAP1/TAZ with TEAD transcription factors, sensitizes tumor cells to TRAIL-induced apoptosis. Our results also show that the mevalonate pathway controls cellular FLICE-inhibitory protein (cFLIP) levels in tumor cells by restraining global protein synthesis. Collectively, our data suggest that a combined strategy of targeting TEAD activity and selectively activating the apoptotic machinery by agonists of apoptotic TRAIL receptors could be explored as a potential therapeutic approach in cancer treatment.

Grants

- 2021-2023: PY20_00754. Proyecto de Excelencia Junta de Andalucía.
- 2022-2025: PID2021-122226NB-I00. Ministerio de Ciencia e Innovación.

Publication Highlights

El Yousfi Y, Mora-Molina R, López-Rivas A and Yerbes R.* **2023**. Role of the YAP/TAZ-TEAD transcriptional complex in the metabolic control of TRAIL sensitivity by the mevalonate pathway in cancer cells. **Cells.** 12(19): 2370.

Principal Investigator

Dr. Rosa M. Ríos

Microtubule dynamics in health and disease

Group Leader

Current position

- Scientific researcher CSIC.

Group Members

Research Associates

- Laura Martínez (Assist Prof.,US).
- María P. Gavilán (Assist Prof.,US).

Postdoc

- Chiara Marcozzi.

Technicians

- Carmen García.
- Carmen Luque.

Former Members (2023-2024)

- **Technician:** María Montilla.

Research Activity

Overview

MT nucleation, the process initiating de novo MT formation, is tightly regulated to maintain cellular architecture and adapt to cellular changes. Centrosomes and the Golgi Apparatus (GA) function as the primary MT-organising centres (MTOCs) in most cells, coordinating their activities along the cell cycle and differentiation. Disrupting this balance halts cell cycle progression or impairs differentiation. At mitotic entry, GA MT nucleation is suppressed to ensure accurate

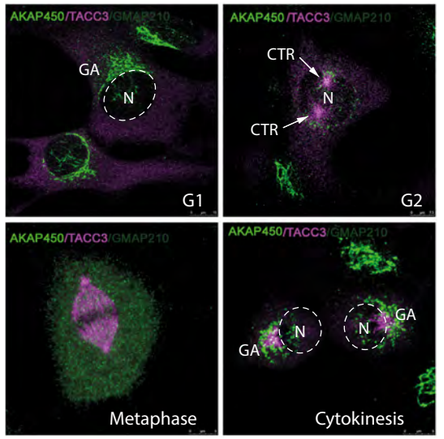


Figure 1. Dynamics of GA-bound AKAP450 throughout the cell cycle. Immunofluorescence images of hTERT-immortalized Retinal Pigment Epithelial (RPE-1) cells stained for AKAP450 (green) and the centrosomal marker TACC3 (red). AKAP450 dissociates from the GA at the G2/M transition, remains cytoplasmic during mitosis, and reassociates with Golgi membranes in telophase.

mitotic spindle assembly by centrosomes. Conversely, during differentiation, centrosomes become inactive or disappear, and the GA assumes MT network organization. Notably, recent studies have revealed that axon formation during neurogenesis relies on Golgi-dependent MT nucleation. Using proteomics, gene-editing, and advanced microscopy, we are investigating the molecular mechanisms regulating GA MT nucleation throughout the cell cycle, a previously underexplored area. Additionally, we are exploring whether defects in GA MT nucleation contribute to ELA and related syndromes, characterised by motoneuron axon degeneration. As a distinct yet related line of research, we are we are studying chemokine receptor and integrin dynamics during leukocyte migration.

Research Highlights

Inhibitory mechanisms of the MT nucleation activity of the Golgi Apparatus in interphase.

MT nucleation relies on the γ-tubulin ring complex (γTuRC), a conical structure of 14 γ-tubulin subunits held by γ-tubulin complex proteins (GCPs) and additional factors (Gao et al., 2024; doi: 10.1002/bies.202400117). γTuRCs are recruited to nucleation sites by various proteins collectively referred to as γTuRC receptors (Wu and Akmanova, 2017; doi: 10.1146/annurev-cellbio-100616-060615). In mammals, the two γ-tubulin isoforms γ-tub1 and γ-tub2 slightly differ at their C-terminus. Using a knock-in cell line with mCherry2/mini-Auxin Induced Degradation motif (mAID)-tagged γ-tub1, and γ-tub2 siRNA, we depleted each isoform individually or both. We found that γ-tub1 promotes MT nucleation at the GA, whereas, unexpectedly, γ-tub2 acts as a potent inhibitor, as its depletion strongly enhances GA-MT nucleation. Mechanistically, γ-tub2 reduced Golgi-associated γ-tub1 levels and competed with γ-tub1 for the MT nucleation activator CDK5Rap2 binding sites. Interestingly, γ-tub2 depletion increased γ receptor aggregation without altering their levels, suggesting γ-tub2 may regulate MT

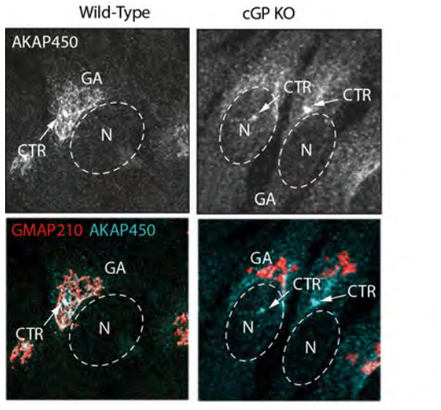


Figure 2. cGP is a receptor for AKAP450 at Golgi membranes. Immunofluorescence images of wild-type and cGP-knockout RPE-1 cells stained for AKAP450 (light blue) and the Golgi protein GMAP210 (red). In the absence of cGP, AKAP450 dissociates from the GA but remains at the centrosome, which, under these conditions, separates from the GA.

nucleation by limiting MT nucleation unit size on the Golgi. This study identifies γTuRC's first inhibitory role in MT nucleation.

Mechanisms underlying MT nucleation silencing during mitosis

AKAP450 is the key regulator of GA-MT nucleation (Rivero et al., 2009; doi: 10.1038/emboj.2009.47). It dissociates from the GA at G2/M and reassociates at telophase (Figure 1), making it a strong candidate for mediating mitotic silencing of GA-MT nucleation. To explore cell cycle-regulated interactions, we conducted comparative proteomic analyses of AKAP450-containing complexes from G1- and G2/M-synchronized cells (in collaboration with J. Choudhary, ICR, London). For this purpose, we generated an AKAP450-mAID-mCherry2 knock-in cell line and employed the FUCCI system to accurately determine the optimal timing for isolating cells in the G1 and G2/M phases. This approach identified most of the known AKAP450 interactors, confirming the reliability and the high quality of the experimental procedure. Additionally, it revealed several novel cell cycle-regulated interactions. Notably, an interaction between AKAP450 and a cis-Golgin protein (cGP) was prominently detected in G1-synchronized cell extracts but was absent in those from G2/M-synchronized cells. In the absence of cGP, AKAP450 dissociates from the GA (Figure 2), leading to a complete loss of MT nucleation. Moreover, STED microscopy revealed near-complete colocalization of AKAP450 and cGP on cis-Golgi membranes. The interaction was mapped to the N-terminal domain of AKAP450 (AK1b domain) and the C-terminal domain of cGP, where three mitotically phosphorylated residues were identified. Another cis-Golgi protein, GM130, was previously identified as an AKAP450 receptor at the GA (Hurtado et al., 2011; doi: 10.1083/jcb.201011014). Our findings show that in cells lacking GM130, both cGP and AKAP450 partially dissociate from the GA. Intriguingly, AK1b is targeted to the GA either in the absence of cGP or GM130 but fails to localize when both proteins are absent, suggesting that these proteins may function as redundant or complementary AKAP450 receptors. GM130 is also phosphorylated during mitosis by CDK1. Based on these observations, we hypothesize that GM130, cGP, and AKAP450 form a ternary complex on cis-Golgi membranes that dissociates upon mitotic entry due to the phosphorylation of GM130 and cGP. This dissociation may inhibit MT nucleation at the GA during mitosis.

Our proteomic analyses also revealed that the interaction between AKAP450 and the p150glued component of the dynein/dynactin complex is mitotically regulated. Since the dynein/dynactin complex is essential for successful MT nucleation, we are further investigating how mitotic phosphorylation contributes to the silencing of MT nucleation during mitosis.

Chemokine receptor and integrin dynamics during leukocyte migration.

Leukocyte movement involves adhesion molecules, chemokines, and receptors dynamically organized at the membrane. The CXCR4-CXCL12 axis and γ1-integrins are key for T cell migration. Using SPT-TIRF microscopy, we study CXCR4 and γ1-integrin dynamics in knock-in T cells under shear flow. CXCR4 mainly exists as monomers, with small dimer-nanoclusters sufficient for migration. We also investigate actin-associated proteins and receptor interactors regulating this organization.

Grants

- 2020-2023: 202080E095. Intramural Project. CSIC.
- 2022-2026: PID2022-141680NB-I00. Ministry of Science and Innovation.

Publication Highlights

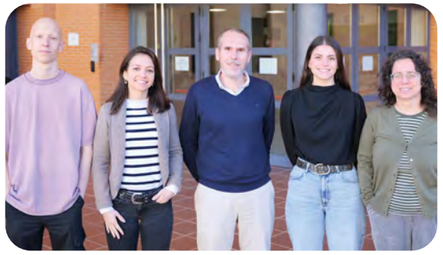
Medrano M, Contreras M, Caballero-Velázquez T, Martínez L, Bejarano-García JA, Calderón-Ruiz R, García-Calderón CB, Rosado IV, Pérez-Simón JA. **2024**. Cannabinoids induce cell death in leukaemic cells through Parthanatos and PARP-related metabolic disruptions. Br J **Cancer.** 130(9):1529.

Andrés-San Román JA, Gordillo-Vázquez C, Franco-Barranco D, Morato L, Fernández-Espartero CH, Baonza G, Tagua A, Vicente-Munuera P, Palacios AM, Gavilán MP, Martín-Belmonte F, Annese V, Gómez-Gálvez P, Arganda-Carreras I, Escudero LM. **2023**. CartoCell, a high-content pipeline for 3D image analysis, unveils cell morphology patterns in epithelia. **Cell Rep Methods**. (10):100597.

Principal Investigator

Dr. Fernando Monje-Casas

Cell division control
Group Leader



Current position

- Scientific researcher CSIC.

Group Members

Postdocs

- María Galindo Moreno.
- Ana María Rincón Romero (Assoc. Prof., US).

PhD Student

- Carmen Lázaga Gutiérrez de Rueda.

Technician

- Pablo Magán Osuna.

Former Members (2023-2024)

- **Postdoc:** Javier Manzano López.
- **PhD Student:** Alejandra Álvarez Llamas.
- **Master Students:** Irene Álvarez Mejías, Dario López Muñoz.
- **Technician:** Macarena Gómez Carmona.

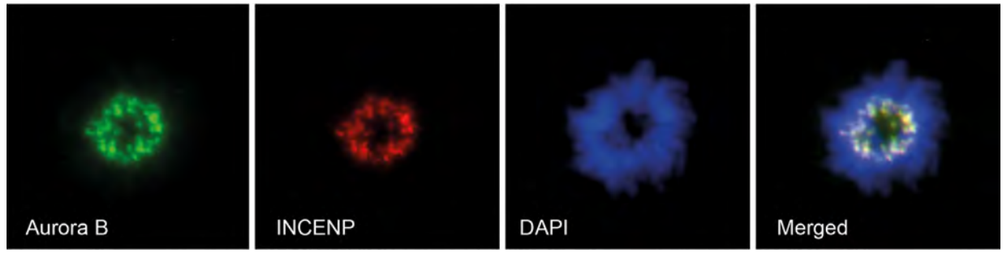


Figure 1: Localization of Aurora B (green) and INCENP (red), two components of the Chromosomal Passenger Complex, to the kinetochores of chromosomes during mitosis in a human RPE-1 cell. DNA (DAPI) is shown in blue and a merged imaged is also provided.

Research Activity

Overview

Our research aims to elucidate the mechanisms that control cell division and ensure the accurate distribution of the genetic material during this process. Errors in genome partitioning can lead to aneuploidy, a condition characterized by an abnormal number of chromosomes, which is a hallmark of cancer and many other genetic diseases. To prevent such errors, cells have developed checkpoints that verify DNA integrity and the fidelity of chromosome segregation during their division. Our goal is to better understand how these checkpoints are controlled and coordinated to regulate specific cell cycle transitions. Additionally, we are interested in evaluating how cells utilize the same machinery responsible for genome distribution to generate polarity during asymmetric divisions. Errors in establishing polarity during stem cell division can result in neurodegenerative disorders and premature aging. Therefore, advances in our understanding of asymmetric cell divisions are of significant social and economic importance.

Research Highlights

Maintaining genome integrity and a correct ploidy is essential for the survival and fitness of both unicellular and multicellular organisms. The spindle is a bipolar microtubule array that facilitates chromosome segregation. During mitosis, proper chromosomal attachment to spindle microtubules ensures that each daughter cell receives an equal set of chromosomes. A key mitotic event is the biorientation of chromosomes, which is

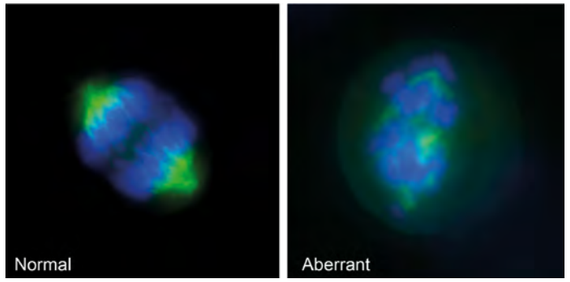


Figure 2: Image of a human RPE-1 cell displaying chromosome segregation defects as a consequence of an increase in Aurora B kinase activity (aberrant). A cell that is properly partitioning the DNA during mitosis (normal) is also shown for comparison. Microtubules are shown in green and the DNA (DAPI) in blue.

achieved when each sister chromatid from the same pair attaches to opposite spindle poles. Aurora B kinase plays a crucial role in this process. When biorientation is not correctly established, Aurora B phosphorylates outer kinetochore components causing kinetochore detachment from spindle microtubules and, consequently, activation of the spindle assembly checkpoint (SAC), which halts cell cycle progression in metaphase until all chromosomes are properly connected to the spindle. Besides this essential role, Aurora B also regulates DNA compaction, spindle stability, and cytokinesis. This kinase functions as part of the chromosomal passenger complex (CPC), which also includes inner centromere protein (INCENP), Borealin, and Survivin. INCENP activates Aurora B, while Borealin and Survivin facilitate complex targeting to the centromere, spindle midzone, and midbody. Defects in Aurora B activity cause chromosome segregation errors and aneuploidy. Notably, elevated Aurora B levels also originate aneuploidy and are commonly associated with poor prognosis in cancer, though the specific impact of its overexpression remains debated. Our group previously showed that overexpression of both Aurora B and INCENP homologs in *Saccharomyces cerevisiae* disrupts chromosome segregation and cell viability in a synergistic manner. During this period, we have extended our analyses by examining the effects of overexpressing different CPC components on cell cycle progression, genome stability, and the transcriptional landscape of human cells. Our findings could deep our understanding of tumorigenic processes linked to deregulated Aurora B expression.

Beyond chromosome segregation, the mitotic spindle has other critical functions in the cell. In asymmetric divisions, the spindle extends along the polarity axis to ensure an equitable genome distribution while preserving the differential inheritance of specific factors by mother and daughter cells. Its alignment with the polarity axis places the spindle in an optimal location to facilitate a distinct regulation of cellular processes in the mother and daughter cells, adding an extra level of complexity to their control. This differential regulation is frequently mediated by the asymmetric association of proteins with only one of both spindle microtubule-organizing centers (MTOCs), called centrosomes in higher eukaryotes and spindle pole bodies (SPBs) in *S. cerevisiae*. Recently, we discovered that autophagy constitutes one of the cellular processes differentially controlled in such a manner during the division of budding yeast. Specifically, in the 2023-2024 period we showed that the Bfa1/Bub2 complex, a mitotic exit inhibitor that asymmetrically localizes to SPBs, associates with the Nup159 nucleoporin during anaphase. This interaction is important for Nup159-mediated autophagy, potentially affecting the degradation of nuclear pore components differently in mother and daughter cells.

MTOCs duplicate early in the cell cycle, and the "old" and "new" MTOCs exhibit distinct characteristics that can influence asymmetric cell division. Indeed, our previous research demonstrated that non-random inheritance of SPBs is critical for maintaining the replicative lifespan in budding yeast. Proper SPB inheritance ensures the correct distribution of the Sir2 sirtuin, functional mitochondria, and protein aggregates, resetting the replicative lifespan in *S. cerevisiae* daughter cells. We also identified key regulators of non-random MTOC inheritance, such as Polo-like kinases, and explored their role in cellular aging. As a continuation of these studies, our recent research in both *S. cerevisiae* and human neuroblastoma cells finally aimed to uncover novel aspects of the molecular mechanisms underlying spindle MTOC distribution and their implications for disease, including tumorigenesis and developmental disorders.

Grants (starting or ending 2023-2024)

- 2020-2023: PID2019-105609GB-I00. Ministerio de Ciencia e Innovación.
- 2023-2026: PID2022-137413NB-I00. Ministerio de Ciencia e Innovación

Publication Highlights (2023-2024)

de Oya IG, Manzano-López J, Álvarez-Llamas A, Vázquez-Aroca MP, Cepeda-García C, Monje-Casas F. **2023**. Characterization of a novel interaction of the Nup159 nucleoporin with asymmetrically localized spindle pole body proteins and its link with autophagy. **PLoS Biology.** (8): e3002224.

Principal Investigator

Dr. Anabel Rojas

Pancreas and Liver Development and Disease

Group Leader

Current position

- Associate professor, University Pablo de Olavide (UPO).

Group Members

Master students

- María Sierra Jiménez.
- Lucía Navarro Santiago.

Technicians

- Irene Díaz Contreras.

Former Members (2023-2024)

- **PhD student:** Noelia Arroyo del Alba.
- **Technician:** Enrique Domínguez Mateos.

Figure 1

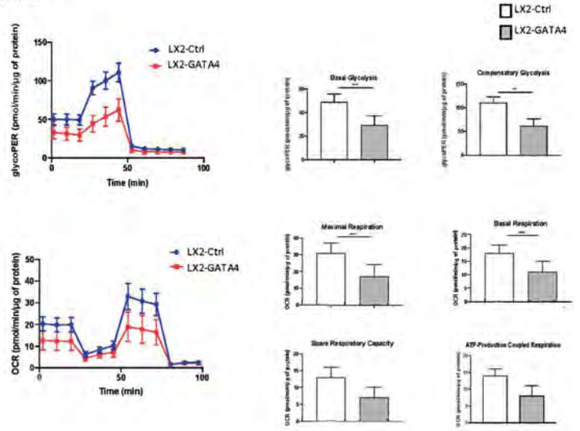


Figure 1. Analysis of oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) in activated hepatic stellate cells (LX2-ctrl) or GATA4-overexpresing hepatic stellate cells (LX2-GATA4).

Research Activity

Overview

Our main research line focus on the transcriptional control of hepatic fibrosis and hepatocellular carcinoma (HCC) in order to understand the molecular mechanisms driving disease progression and tumorigenesis. More specifically, we study the hepatic stellate cells (HSCs) biology as central players in liver fibrosis, and the transition into myofibroblast-like cells that secrete extracellular matrix components. In the tumor microenvironment, HSCs communicate with liver cancer cells through paracrine signals, including cytokines, growth factors, and extracellular vesicles, which profoundly impact tumor cell metabolism by promoting glycolysis, lipid metabolism reprogramming, and resistance to oxidative stress. These interactions foster tumor growth, angiogenesis, and therapy resistance. Transcriptional regulators such as nuclear receptors, epigenetic modifiers, and non-coding RNAs orchestrate HSC activation and their pro-tumorigenic signaling. Our research employs cell culture systems, mouse models of xenografts, and mouse model of liver cancer induction to unravel these pathways, aiming to identify novel therapeutic targets that inhibit fibrosis and disrupt the tumor-promoting microenvironment, ultimately improving precision treatments for HCC.

Figure 2

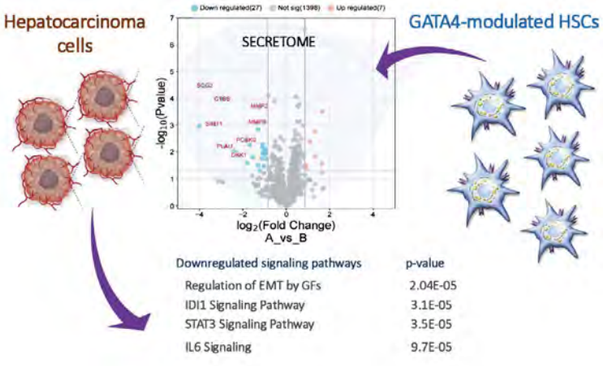


Figure 2. Transcriptomic analyses of hepatocarcinoma cells cocultured with activated hepatic stellate cells (LX2-Ctrl) or GATA4-overexpresing hepatic stellate cells (LX2-GATA4) and volcano plot showing differential proteins secreted by GATA4-overexpresing hepatic stellate cells (LX2-GATA4) compared with activated hepatic stellate cells.

Research Highlights

Our results show that GATA4 reprograms the metabolism of activated HSCs, effectively dampening their pro-fibrotic and pro-tumorigenic activity. Specifically, GATA4 reduces oxidative phosphorylation (OXPHOS) and glycolysis, two metabolic pathways that are typically upregulated in activated HSCs (Figure 1). This metabolic reprogramming diminishes the energy-intensive processes required for extracellular matrix production and tumor-supportive signaling, steering HSCs toward a more quiescent and less tumor-promoting state. By limiting the metabolic resources available for cancer cell support, GATA4-modulated HSCs disrupt the metabolic crosstalk that fuels tumor growth and survival.

Liver cancer cells in the presence of GATA4-modulated HSCs also exhibit significant transcriptomic changes, with a marked reduction in pathways associated with metastasis, tumor growth, and stress signaling (Figure 2). These pathways are critical for tumor progression, angiogenesis, and the adaptation of cancer cells to the hostile microenvironment. The altered transcriptional profile of liver cancer cells indicates that GATA4-modulated HSCs reshape the tumor microenvironment, creating conditions unfavorable for cancer cell proliferation and dissemination. A comprehensive analysis of proteins from GATA4-modulated HSCs secretome reveals a decrease in proteins that are typically upregulated in liver cancer and associated with poor prognosis. These include factors involved in matrix remodeling, growth factor signaling, and immune evasion, all of which contribute to a pro-tumorigenic niche. The downregulation of these proteins further supports the notion that GATA4 reprogramming not only inactivates HSCs but also neutralizes their capacity to sustain tumor-promoting processes.

Analyses of the exosomes derived from GATA4-modulated HSCs reveal a unique microRNA profile, with differential expression of specific microRNAs implicated in tumor suppression. These exosomal microRNAs may act as paracrine mediators, influencing liver cancer cells by silencing oncogenic pathways and enhancing anti-tumor responses. The altered cargo of these exosomes suggests a broader role for GATA4 in modulating intercellular communication within the tumor microenvironment, providing a novel avenue for therapeutic intervention.

The tumor-suppressive effects of GATA4-modulated HSCs were further corroborated in xenograft experiments. Liver tumor cells co-implanted with GATA4-modulated HSCs demonstrated significantly reduced proliferation compared to tumor cells co-implanted with control HSCs (Figure 3). This in vivo evidence highlights the functional impact of GATA4-mediated HSC inactivation on tumor growth and reinforces the translational potential of targeting GATA4 as a therapeutic strategy.

Figure 3

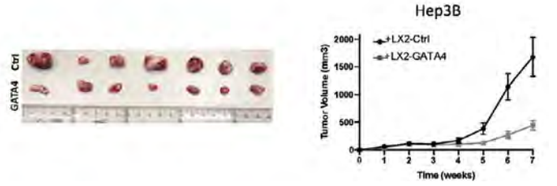


Figure 3. Xenograft tumor formation in nude mice using hepatocellular carcinoma cells (Hep3B) coupled with activated hepatic stellate cells (LX2-Ctrl) or GATA4-overexpresing hepatic stellate cells (LX2-GATA4).

Grants

- 2021-2023: PID2020-114656RB-100. Ministerio de Ciencia e Innovación.
- 2024-2026: PID-2023-147826OB-I00. Ministerio de Ciencia e Innovación.

Publication Highlights

Bruxel MA, da Silva FN, da Silva RA, Zimath PL, Rojas A, Moreira ELG, Quesada I, Rafacho A. **2023**. Preconception exposure to malathion and glucose homeostasis in rats: Effects on dams during pregnancy and post-term periods, and on their progeny. **Environ Pollut.** 316(Pt 2):120633.

Principal Investigator

Dr. Román González-Prieto

Ubiquitin (-like) signalling & proteomics

Emerging Group Leader

Current position

- Distinguished Researcher (EMERGIA program). University of Seville (US).

Group Members

Postdoc

- Carmen Espejo Serrano.

PhD Students

- Lourdes González Vinceiro.
- Emily Soto Hidalgo.

Master Student/Erasmus +

- Sergio Rodríguez Heras.

Former Members (2023-2024)

- **PhD student:** Daniel Salas Lloret.

Research Activity

Overview

The stability of our genomes depends on a plethora of proteins which are able to scaffold, replicate, repair and regulate the expression of the DNA, among other functions. Protein function is regulated by different Post Transalational Modifications, including the modification of by ubiquitin and other ubiquitin-like modifiers, which is performed by an enzymatic cascade consisting of E1, E2 and E3 enzymes. E3 enzymes confer substrate specificity for ubiquitin(-like) modifiers, and determining which E3 modifies which substrate is the next challenge in ubiquitin(-like) proteomics. In the research group, we have developed novel mass spectrometry-based proteomics approaches that enable the identification of E3-specific substrates which we combine with molecular and cellular biology techniques to understand the function and relevance of E3 enzymes and their substrates in the biology of the genome, including DNA damage repair and tolerance mechanisms and genome organization.

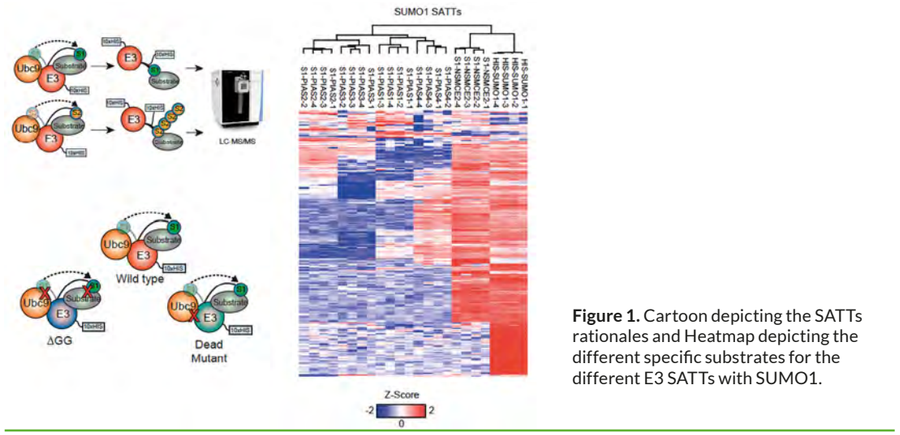


Figure 1. Cartoon depicting the SATTs rationales and Heatmap depicting the different specific substrates for the different E3 SATTs with SUMO1.

Research Highlights

Identification of the E3-specific SUMO proteome

We adapted our TULIP2 methodology to identify substrates for SUMO E3s. In contrast to ubiquitin, the number of E3 enzymes for SUMO is more limited, thus, we performed a proteome-wide search identifying specific substrates for 8 different E3s for SUMO1 and SUMO2/3 and providing an online tool to browse the preferential E3 for a given substrate of interest.

We found that, in contrast to previous hypothesis in the field, the SUMO E3 enzymes are relatively specific in their substrates and the specific SUMO E3 enzymes are involved in novel pathways within cell physiology.

Novel insight in the role of the ubiquitin E3 activity of the BRCA1/BARD1 heterodimer

Deficiencies in BRCA1/BARD1 are the main cause of hereditary breast and ovarian cancer. BRCA1/BARD1 are tumor-suppressor genes that participate in the Homologous Recombination (HR) DNA Repair pathway, and which possess ubiquitin E3 ligase activity. However, the role of the ubiquitin-ligase activity in cancer predisposition and HR remains controversial. Many efforts have been performed to identify BRCA1/BARD1

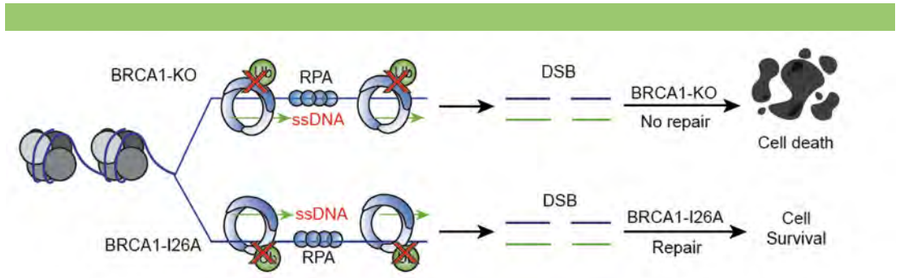


Figure 2. Model for the relevance of BRCA1/BARD1 E3 activity for DNA replication, repair and cell survival.

ubiquitination substrates, all of them based in indirect evidence. Here, we employed the TULIP2 methodology (Salas-Lloret et al. 2019, see below), enabling the direct identification of E3-specific substrates by mass spectrometry-based proteomics and identified PCNA as a mayor target of BRCA1/BARD1 for ubiquitination in unchallenged conditions. Deficiencies in BRCA1/BARD1 E3 ligase activity phenocopies ubiquitin deficient PCNA mutants. Our data enables us to conclude that ubiquitin E3 ligase activity of BRCA1/BARD1 is not required for DNA Double Strand Break repair by HR, but to promote continuous DNA synthesis in normal growth conditions via PCNA ubiquitination.

Grants (starting or ending 2023-2024)

- CNS2022-135216. Agencia Estatal de Investigación.
- PID2021-122361NAI00. Agencia Estatal de Investigación.
- EMERGIA20_00276. Junta de Andalucía.

Publication Highlights

Salas-Lloret D, Garcia-Rodriguez N, Soto-Hidalgo E, Gonzalez-Vinceiro L, Espejo-Serrano C, Giebel L, Mateos-Martin ML, de Ru AH, van Veelen PA, Huertas P et al. **2024**. BRCA1/BARD1 ubiquitinates PCNA in unperturbed conditions to promote continuous DNA synthesis. **Nature communications.** 15: 4292.

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Nguyen BA, Singh V, Afrin S, Yakubovska A, Wang L, Ahmed Y, Pedretti R, Fernandez-Ramirez MDC, Singh P, Pekala M et al. **2024**. Structural polymorphism of amyloid fibrils in ATTR amyloidosis revealed by cryo-electron microscopy. **Nature communications.** 15: 581.

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Salas-Lloret D, Jansen NS, Nagamalleswari E, van der Meulen C, Gracheva E, de Ru AH, Otte HAM, van Veelen PA, Pichler A, Goedhart J et al. **2023**. SUMO-activated target traps (SATTs) enable the identification of a comprehensive E3-specific SUMO proteome. **Sci Adv.** 9: eadh2073

Yalcin Z, Koot D, Bezstarosti K, Salas-Lloret D, Bleijerveld OB, Boersma V, Falcone M, Gonzalez-Prieto R, Altelaar M, Demmers JAA et al. **2023**. Ubiquitinome Profiling Reveals in Vivo UBE2D3 Targets and Implicates UBE2D3 in Protein Quality Control. **Molecular & cellular proteomics**. 22(6):100548

Principal Investigator

Dr. Patricia Altea-Manzano

Metabolic Regulation and Signaling in Cancer

Emerging Group Leader

Current position

- Ramon y Cajal Researcher. CSIC.

Group Members

Postdocs
- Jose Martín Gómez.
- Bruna Martins García.

PhD students
- Álvaro Ruiz Tabas.
- Gwennan Delyth Ward.
- Cristina Álvarez Álvarez.

Lab technician
- María Gálvez Jiménez.

Master student
- José Casquero Blanco.

Former Members (2023-2024)

- **Lab manager and technician:** Víctor García Cabrera.

Research Activity

Overview

Our laboratory is dedicated to understanding the mechanisms that enable metastasizing cancer cells to adapt to changing environments and drive cancer progression. We focus on two main areas: the role of metabolites as signaling drivers and the impact of environmental factors on metastatic niches. By studying how specific nutrients and metabolites influence tumor cell behavior, we aim to uncover novel regulatory pathways that promote metastasis. Our research has demonstrated that metabolites like palmitate can act beyond traditional energy roles to drive post-translational

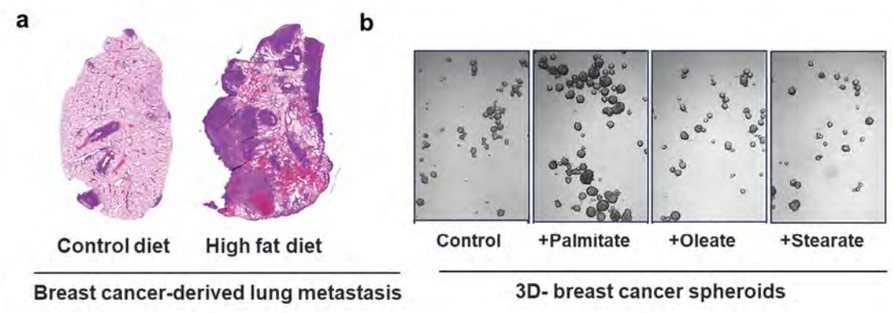

Figure 1. Effect of the fatty acid palmitate in breast cancer metastasis. (a) Lung metastases (H&E staining) increase in mice fed with high-fat diet. (b) Breast cancer spheroid growth is enhanced upon supplementation of palmitate compared to other fatty acids.

modifications (e.g., S-palmitoylation), crucial for metastatic progression. Additionally, we investigate how factors such as diet reshape the metabolic composition of distant organs, creating conditions that favor metastasis. By combining multi-omics approaches and in vivo models, our ultimate goal is to identify actionable therapeutic targets to prevent and treat metastatic cancers.

Research Highlights

Widespread metastases are the primary cause of cancer-related deaths mainly due to the lack of effective treatments to arrest metastatic progression to date. Unlike primary tumors, specific somatic mutations (normally targeted by anti-cancer drugs) triggering the metastatic process cannot be readily identified. Instead, recent evidence supports that metastatic cells dynamically tune their phenotypic states by adapting their epigenetic, transcriptional, and post-transcriptional landscapes in response to changing conditions in the metastatic cascade.

One of the non-genetic adaptations of cells after leaving primary tumors is metabolism. Recent evidence supports the presence of dynamic changes in the metabolism of metastatic cells that contribute to their ability to successfully transition through the different environments of the metastatic

cascade. In this regard, once established at the distant site, many metastases display different metabolic traits compared to the tumors from which they originate.

This metabolic reprogramming observed in metastatic cells is influenced by both intrinsic factors of cancer cells (such as tissue of origin and mutations) and extrinsic factors related to the distant organ (including resident cells, the immune system, and nutrient availability). Metastatic cells demonstrate the ability to utilize nutrients from the new organ for survival and growth. For instance, several metastatic organs such as the lung, liver, and lymph nodes present a lipid-rich microenvironment. In the lung, the pulmonary surfactant in the alveolar structures contains palmitate-containing lipids. We have demonstrated that metastatic breast cancer cells use the lung surfactant as a source of the palmitate, which is crucial for the activation of specific pro-metastatic signaling via the acetylation of the nuclear factor-κB (NF-κB). These findings were published in 2023 by Dr. Altea-Manzano as her second work on the metabolic regulation of metastasis. Moreover, our work highlights the unconventional signaling roles of metabolites, advancing our understanding of how metabolic adaptations fuel cancer dissemination and colonization in distant organs.

In 2024, Dr. Altea-Manzano transitioned to an independent research role, securing a Junior Group Leader position at CABIMER. This was made possible through highly competitive funding awards, including an ERC Starting Grant and the Ramón y Cajal Program. These prestigious grants have laid the foundation for the establishment of the Metabolic Regulation and Signaling in Cancer Lab. As a continuation of Dr. Altea-Manzano´s work, the lab has found that metastatic cells carry unique dependencies on palmitoylation dynamics and those are dependent on the organ environment. Moreover, we are studying how the organ of metastasis changes due to diet priming, and whether these changes can boost metastasis formation.

Additionally, Dr. Altea-Manzano's research contributions have been widely recognized, earning four prestigious national awards in 2024, including the XII Losada Villasante Award and the National Research Award "Gabriella Morreale." This acknowledgment underscores the scientific impact and potential of the lab's ongoing work in unraveling the complexities of cancer metabolism and metastasis.

Grants (starting or ending 2023-2024)

- 2024-2028: ERC Starting Grant 2023. European Commission.
- 2024-2026: Proyecto Intramural 2024. Consejo Superior de Investigaciones Científicas.
- 2024-2029: Ramón y Cajal Program 2022. European Commission-Spanish Research Agency.

Publication Highlights

Vandekeere A*, Karraz SE*, Altea-Manzano P# & Fendt S-M#. **2024**. Metabolic rewiring during metastasis: the interplay between environment and host. **Annual Review of Cancer Biology.** Vol. 8, pp. 269–290.

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Integrative Pathophysiology and Therapies

Integrative Pathophysiology and Therapies department

Research in the department focuses on the multi-level study of the molecular mechanisms that control a wide variety of diseases. For such a holistic approach, we use multiple approaches, from cell lines and human primary cells, to experimental animal models and human samples. Groups in the department have a focus on the identification of therapies that include both the search for drugs, cell and gene therapies, as well as the identification of biomarkers as tools for the clinical diagnosis and prognosis of these diseases. All this implies that our research is necessarily translational in nature using both basic and preclinical models and patient / donor samples. We aim to identify key factors, mechanisms of action and therapeutic targets focused on the treatment of diseases related to metabolic and immune stress, neuropathies and other degenerative diseases, often associated to aging, as retinopathies.

In particular, the research activity of this department is aimed at either finding drugs and therapeutic targets or identify biomarkers with prognostic value associated with healthy aging, cell survival, regeneration, optimal function of organs and women reproductive stages such as menopause to treat and better understand different pathologies such as atherosclerosis, diabetes, liver fibrosis, epilepsy, degenerative diseases, such as Alzheimer and amytrophic lateral sclerosis and degenerative pathologies of the retina.

HEAD OF DEPARTMENT

Dr. Inés Pineda-Torra

RESEARCH GROUPS

1. **Pancreatic Islets and Stem Cells**
   Dr. Franz Martin
2. **Pancreatic Islet Development & Regeneration**
   Dr. Benoit Gauthier
3. **Cell Therapy for Neuropathologies**
   Dr. Manuel Álvarez-Dolado
4. **Cellular and Molecular Neuroimmunology**
   Dr. David Pozo
5. **Immune Signalling in Neurodegenerative diseases**
   Dr. Cintia Roodveldt
6. **Metabolic Interventions for Successful Aging**
   Dr. Alejandro Martín-Montalvo
7. **Retinal neurodegeneration and advanced therapies**
   Dr. Francisco Javier Díaz-Corrales
8. **Stem Cells and Translational Neurology**
   Dr. Vivian Capilla-González
9. **Metabolism, Immunology and Cardiovascular Risk**
   Dr. Inés Pineda-Torra

Principal Investigator

Dr. Inés Pineda Torra

Metabolism, Immunology & Cardiovascular Risk

Group Leader

Current position

- Distinguished Scientist. Fundación Pública Andaluza Progreso y Salud (FPS).
- Since December 2023 Scientific Lead of the AVANTE consortium.
- Since April 2024 Scientific & Technical Lead of the HARMONi consortium.
- Head of the Integrative Pathophysiology and Therapies Department.

Group Members

Postdoctorals:

- Jesus Roca Garcia.
- Marta Rojas Torres.
- Cristina Molina Lopez.
- Angela Rubio Tenor.

Technicians

- Yolanda Aguilera García.
- Nuria Mellado Damas Sanz.
- Alejandro Gonzalez Mendoza.

MSc student

- Alicia Santamaría Quiles.

Former Members (2023-2024)

- **Technician:** Miguel Calero.
- **Postdoctoral:** Carlos Jimenez Cortejana.

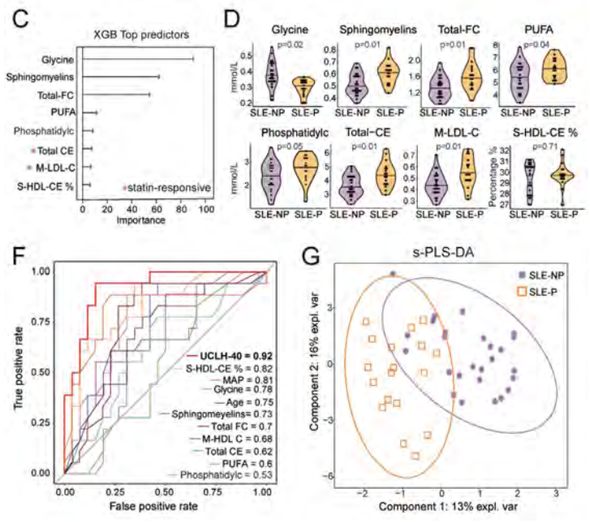

Figure 1. Subclinical plaque in women with atherosclerosis can be predicted using a combined serum metabolite / clinical trait signature identified using machine learning models. (A) AUC-ROC and confusion matrix for predicted group membership (true = green, false = red) for XGB model in SLE-P vs SLE-NP. **(B)** Violin plots for top features in XGB model with statistical significance indicated. **(C)** AUC-ROC curves for the UCLH-40 signature and top individual features from XGB model (A) predicting SLE-P vs SLE-NP classification. **(D)** sPLS-DA model using UCLH-40 signature to classify SLE-P from SLE-NP.

Research Activity

Overview

Cardiovascular disease (CVD) remains the leading cause of mortality worldwide, and the main pathology underlying ischemic CVD is atherosclerosis, which results from dysregulation and build-up of lipids alongside various immune responses in the vascular wall. My group aims to understand how lipids modulate systemic and intracellular metabolic and immune pathways and how that affects CVD risk and development. Previous work in London focused at understanding the cardiovascular risk present in women with autoimmune diseases such as SLE. Now we are aiming to understand the elevated cardiovascular risk in post-menopausal women During this period we aimed to a) uncover novel modes of crosstalk between lipid metabolism and immunity, b) understand the regulation of lipid metabolism at the level of gene expression, mainly mediated by the Liver X Receptor (LXR) in immune cells such as monocytes, c) elucidate the impact of sex hormones in circulating metabolites and immune responses, and d) understand the mechanisms underlying the

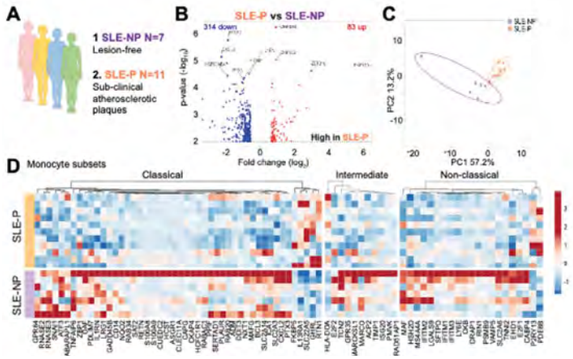

Figure 2. A. Graphic depicting SLE cohort: SLE-no plaque (SLE-NP) N=7, SLE-Plaque (SLE-P) N=11, samples obtained for CD14+ monocyte bulk RNA-seq. **B.** Volcano plot of differentially expressed genes (DEGs) in SLE-P vs SLE-NP by log2fold change (>1.5 or <-1.5 fold change) and p-value<0.01, regulation change indicated by colour with downregulated in blue and upregulated in red. **C.** Principal component analysis using 50-most significant DEGs from panel A. **D.** Heatmap of DEGs in SLE-P vs SLE-NP (1.5 or <-1.5 FC and p-value <0.01) expressed by monocyte subsets: classical, intermediate, and non-classical monocytes (Wont et al., 2011).

increased cardiovascular risk in women with autoimmune disorders such as systemic lupus erythematosus (SLE). We used human and immune cells.

Research Highlights

1. Establish cohorts of women pre- (pre-M) and post-menopause (post-M).

We have gathered a cohort of healthy women with no previous history of CVD (Gr-SLE), in collaboration with colleagues from UCL-London n= 53). In addition, we have recruited a cohort of healthy women (n=40) with low cardiovascular risk (CR) at pre-menopausal (PRE-M) or post-menopausal (POST-M_LR) stage and a group of post-menopausal women (n=40) with higher cardiovascular risk (POST-M_HR). Women donors were newly recruited through the Andalusian Biobank (BSSPA). Specific exlusion criteria include undergoing hormonal treatment, any autoimmune disease or other life-threatening disease, and recent vaccination (last 3 weeks) to prevent alterations in the immune system. From each donor we have received serum to perform the metabolomic and proteomic analyses, RNA from whole blood to perform the transcriptomic analysis, and blood to isolate PBMCs, monocytes and lymphocytes to determine their transcriptomic profiles, changes in membrane lipid composition and immune function. Furthermore, as part of the HARMONi and AVANTE consortia we have recruited men (n=66) and women (n=266) to perform venous and capillary blood analyses as above (except from cell isolation).

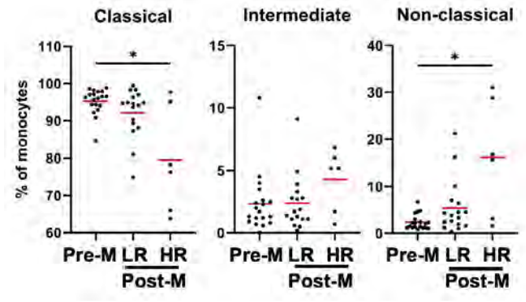

Figure 3. Monocyte changes with menopause and cardiovascular risk. Distribution of monocyte subtype populations between donor groups. Flow cytometry was used to identify monocytes from isolated PBMCs of pre- (Pre-M) and post menopausal (Post-M) women with low or high cardiovascular risk (Low/High-R). CD14+ and CD16+ markers were used for monocyte subtype classification. Cumulative data showing total monocyte percentage and monocyte subtype distribution between classical, intermediate and non-classical populations. Bars show median values. Two-way ANOVA with Tukey's test; *P<0.05.

2. Serum metabolomic profiles of women and machine learning studies.

We have profiled women with SLE and no previous history of CVD (Figs. 1). These women were assessed for the presence (SLE-P, n=18) or absence (SLE-NP, n=26) of subclinical atherosclerosis using vascular ultrasound for carotid/femoral intima-media thickness. Serum metabolomics using an NMR platform was performed and analysed using machine learning (ML) pipelines. Univariate logistic regression analysis of the lipid-focused metabolomics identified 69 statistically significant metabolites distinct between SLE-P and SLE-NP patients after controlling for various clinical traits. Using various ML models, an improved atherosclerosis-risk predictive signature was developed comprising 35-metabolites/5-clinical traits (UCLH-40) that classified SLE-P patients with high accuracy (AUC=0.92) (Fig 1A, B) and outperformed CVD-risk assessment tools and lipid profiles measured in routine care. This 'atherosclerosis-risk signature' performed better than the individual metabolites at predicting the presence of atherosclerotic plaque (Fig.1C) and was able to classify the patients from each group (Fig.1D). Furthermore, this signature was validated in a second independent adult female SLE cohort (n=98) that predicted plaque status with moderate accuracy (AUC=0.79) using the top performing XBG model. The signature was then refined into a 5-feature subclinical plaque-predictive score, which not only stratified the combined SLE-P/SLE-NP cohorts (n=142) (AUC=0.84), but also identified distinct high and low sub-clinical atherosclerosis-risk subgroups in a 'real-world' setting of unscanned adult SLE patients (n=38) and finally, predicted 3-year atherosclerosis

progression in female post-pubertal patients with juvenile-onset SLE (n=36) (AUC=0.79). We are now performing similar analyses aiming to dissect differences between women pre-M and post-M and with low and high CVD risk. This work has been submitted for publication to the Journal of the American Heart Association (JAHA).

3. Transcriptomic profiles in blood.

We profiled the women with SLE and no previous history of CVD. Global gene expression signatures were compared using whole blood RNA-seq in SLE-P (n=12) and SLE-NP (n=8) patients. Differential gene expression analysis identified 284 DEGs. Gene set enrichment analysis (GSEA) was conducted on the DEGs to interrogate pathways that could be perturbed in SLE and atherosclerosis. Inflammatory and immunomodulatory pathways were downregulated in patients with subclinical plaque. Whereas genes associated with myeloid and erythrocyte processes and several pathways implicated in metabolism were upregulated in SLE-P patients. Metabolic pathway enrichment analysis identified enrichment in the citrate cycle (TCA cycle) and bile acid biosynthesis, in the genes downregulated in SLE-P (not shown). Conversely, glutathione biosynthesis, glycolysis and glucogenesis were all upregulated (not shown). We are now performing similar analyses with the other cohorts we have.

4.Global female monocyte gene expression and monocyte subset profiles.

Monocyte transcriptomic profiles were compared between patients with SLE (n=18) and age-matched healthy controls (HC, n=9). Overall, 654 downregulated and 715 upregulated genes were differentially expressed. This gene set discriminates SLE patients from HC and highlights the difference between SLE-NP and SLE-P patients. Enriched GO processes showed involvement of immunomodulatory mechanisms, key in SLE. We have observed also important differences between the SLE-P and SLE-NP subgroups (Fig.2) In addition, we have examined the monocyte counts (which have been previously linked to circulating cholesterol levels) between pre- and post-menopausal woman. Most differences in monocyte subsets were observed in Post-M women with high CR (Fig. 3). We are now performing global analyses of differential gene expression in all groups of women.

Grants

- 2024-2029: Axa Research Fund.
- 2022-2024: CPP2022-010039. Agencia Estatal de Investigación, AEI. Proyectos colaboración Publico Privada.
- 2023: PMPTA23/00023. Instituto de Salud Carlos III.
- 2022-2025: 126077OB-I00. Ministerio de Ciencia e Innovación.

Publication Highlights

Martin-Gutierrez L, Waddington KE, Maggio A, Coelewij L, Oppong A, Yang N, Adriani M, Nytrova P, Farrell R, Pineda-Torra I, Jury EC. **2024.** Dysregulated Lipid Metabolism Networks Modulate T-cell Function in People with Relapsing Remitting Multiple Sclerosis. **Clinical and Experimental Immunology**. 217(2):204-218.

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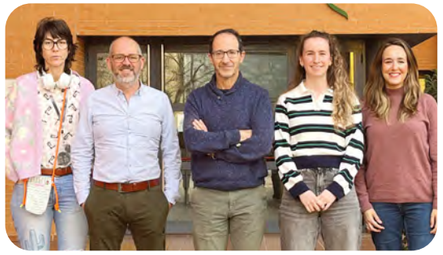
Jury EC, Peng J, Pineda-Torra I, Ciurtin C, Robinson GA. **2023**. Systemic lupus erythematosus patients have unique changes in serum metabolic profiles across age associated with cardiometabolic risk. **Rheumatology**. 63(10):2741-2753.

Principal Investigator

Dr. Franz Martin

Nutrition and metabolic diseases

Group Leader



Current position

- Principal Investigator of CIBER of Diabetes and Associated Metabolic Diseases (CIBERDEM).
- Full Professor at University Pablo de Olavide (UPO).

Group Members

Senior Researchers

- Blanca Escudero.
- Mª Ángeles Ortega.

PhD Student

- Lucía López.

Technicians

- Raquel Araujo.
- Antonio Cárdenas.
- José Moral.

Research Activity

Overview

Our primary research line is to study the role of nutrients, foods and diets in the pathogenesis of obesity, type 2 diabetes (T2DM) and metabolic dysfunction- associated fatty liver disease (MAFLD). Mainly, we are involved in understanding the mechanisms of actions by which Western hypercaloric high-fat and high-carbohydrate diets promote the onset of these metabolic diseases. We focus on this issue from a system physiology approach.

Research Highlights

Our main research highlights are:

1. In a C57BL/6 mice model of alcohol-associated liver disease (ALD) and knockout for the methylation-controlled J protein (MCJ), a protein that acts as an endogenous negative regulator of mitochondrial respiration, we found that MCJ was a mediator for ALD

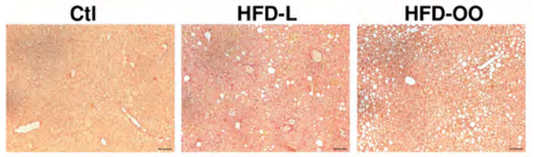


Figure 1. Images showing the liver fibrosis of offsprings from mothers that were fed, during pregnancy and lactation, with control diet (Ctl), lard high-fat diet (HFD-L) and extra-virgin olive oil high fat diet (HFD-EVOO). The offspring were fed during three months with HFD-L.

progression. Whole-body deficiency of MCJ was detrimental during ALD because it exacerbated the systemic effects of alcohol abuse through altered intestinal permeability, increased endotoxemia, and dysregulation of pancreatic function. In addition, liver-specific MCJ silencing prevented main ALD hallmarks, that is, mitochondrial dysfunction, steatosis, inflammation, and oxidative stress, as it restores the NAD + /NADH ratio and SIRT1 function, hence preventing de novo lipogenesis and improving lipid oxidation.

2. Extracts obtained from persimmon skin, using sequential high-pressure and high vacuum technologies, followed by fermentation with natural microbiota present in persimmon, were rich in a wide variety of bioactive compounds. These extracts favored the growth of key gut bacteria (R. champanellensis, F. prausnitzii and B. thetaiotaomicron,) for maintaining intestinal and metabolic health while having antibacterial activity against harmful bacteria (E. coli). Metabolization of the extracts by the bacteria mentioned above generated short-chain fatty acids (SCFA) that improved the integrity of the intestinal mucosa. Furthermore, incubation of the extracts with stool samples from healthy human donors increased SCFA production, as well as an increase in the diversity and abundance of bacterial families (Lachnospiraceae), which are emerging as new probiotics for the treatment of diseases such as DM2 and MAFLD.

3. When mothers were fed, during pregnancy and lactation with extra-virgin olive oil (EVOO), their offspring were protected against the appearance of T2DM and MAFLD, after the intake of hypercaloric Western diets. This protection was due to decreased expression of genes involved in inflammation, TLR4 signaling pathway, and PPAR-□ pathway in adipose tissue and liver of mothers and their offspring. Moreover, in the liver of mothers and offsprings, the overexpression of miR-19b-3p, miR-381, miR-136, miR-582, and miR-6406 were involved in the observed protection.

4. In a nutritional intervention study with adult obese patients with T2DM and MAFLD, after 3 months of a Mediterranean hypocaloric diet-based nutritional intervention, patients improved total cholesterol, insulin, HOMA-IR, and HbA1c. Still, they reverted at follow-up (another 3 months). However, transaminases

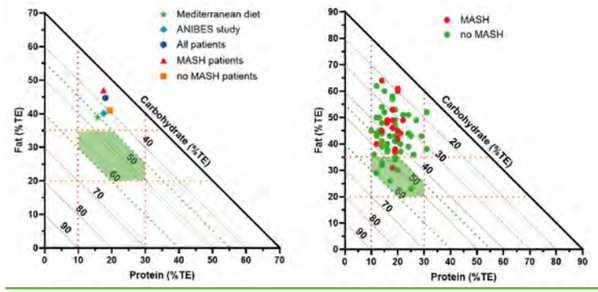


Figure 2. Nutritional geometry framework of patients with obesity, insulin resistance and MAFLD.

levels and liver stiffness improved after 3 months and remained stable at follow-up. A weight loss of ≥3% was enough to confer all these benefits. Patients who achieved ≥3% weight loss increased the consumption of vegetables, fruits, olive oil, fish/seafood, legumes, and nuts. They also decreased sweet consumption. These patients also improved their adherence to the Mediterranean diet, which was maintained at follow-up.

Grants

- 2021-2024: PID2020-116731RB-C21. Ministerio de Ciencia e Innovación.
- 2022-2023: UPO-1380683. FEDER Andalucía.

Publication Highlights

Montero-Vallejo R, Maya-Miles D, Ampuero J, Martín F, Romero-Gómez M, Gallego-Durán R. **2024**. Novel insights into metabolic-associated steatotic liver disease preclinical models. **Liver Int.** 44: 644-662.

Gallego-Durán R, Ampuero J, Maya-Miles D, Pastor-Ramírez H, Montero-Vallejo R, Rivera-Esteban J, Álvarez-Amor L, Pareja MJ, Rico MC, Millán R, Robles-Frías MJ, Aller R, Rojas Á, Muñoz-Hernández R, Gil-Gómez A, Gato S, García-Lozano M, Arias-Loste MT, Abad J, Calleja JL, Andrade RJ, Crespo J, González-Rodríguez Á, García-Monzón C, Andreola F, Pericás JM, Jalan R, Martín-Bermudo F, Romero-Gómez M. **2024**. Fibroblast growth factor 21 is a hepatokine involved in MASLD progression. **United European Gastroenterol J.** 12: 1056-1068.

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Cobo-Vuilleumier N, Lorenzo PI, Martin Vazquez E, López Noriega L, Nano R, Piemonti L, Martín F, Gauthier BR. **2024**. Enhancing human islet xenotransplant survival and function in diabetic immunocompetent mice through LRH-1/NR5A2 pharmacological activation. **Front Immunol.** 15: 1470881.

Cobo-Vuilleumier N, Rodríguez-Fernandez S, López-Noriega L, Lorenzo PI, Franco JM, Lachaud CC, Vazquez EM, Legido RA, Dorronsoro A, López-Férnandez-Sobrino R, Fernández-Santos B, Serrano CE, Salas-Lloret D, van Overbeek N, Ramos-Rodriguez M, Mateo-Rodríguez C, Hidalgo L, Marin-Canas S, Nano R, Arroba AI, Caro AC, Vertegaal AC, Montalvo AM, Martín F, Aguilar-Diosdado M, Piemonti L, Pasquali L, Prieto RG, Sánchez MIG, Eizirik DL, Martínez-Brocca MA, Vives-Pi M, Gauthier BR. **2024**. LRH-1/NR5A2 targets mitochondrial dynamics to reprogram type 1 diabetes macrophages and dendritic cells into an immune tolerance phenotype. **Clin Transl Med.** 14: e70134.

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Berná G, López-Bermudo L, Escudero-López B, Martín F. **2023**. We are what we eat: The role of lipids in metabolic diseases. **Adv Food Nutr Res.** 105: 173-219.

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Principal Investigator

Dr. Benoit R. Gauthier

Pancreatic Islet Development and Regeneration Unit

Group Leader

Current position

- Principle Investigator in Diabetes, Junta de Andalucía-Consejería de Salud y Consumo.
- Member of CIBERDEM.

Group Members

Lab Manager
- Petra Isabel Lorenzo Ovejero.

Senior Researcher
- Nadia Cobo Vuilleumier.

Postdocs
- Christian Lachaud.
- Jaime Muños Franco.

PhD student
- Elena García Díaz.

Technicians
- Beatriz María Fernández Santos.
- Pedro Antonio Soriano González.

Former Members (2023-2024)

- **Postdocs:** Valentine Comaills, Akaitz, Dorronsoro González y Livia López Noriega.
- **PhD Students:** María Eugenia Martín Vázquez García.
- **Technicians:** Cristina Cerrada Romero y Daniel De Llano Teixeira.

Research Activity

Overview

Diabetes Mellitus (DM) is characterized by hyperglycemia, which arises from either impaired insulin secretion by pancreatic islet beta cells or resistance to the glucose-lowering effects of insulin. Insufficient insulin production results from beta cell dysfunction, as observed in Type 2 DM and gestational DM, or from immune-mediated beta cell destruction in Type 1 DM (T1D). Our research aims to develop novel drug therapies by identifying and characterizing diabetes-associated genes that regulate beta-cell expansion, survival, and function. We also investigate how the expression of these genes in other tissues, such as immune cells, influences islet function and survival through complex organ crosstalk. In parallel, we explore cell therapies as a complementary strategy to restore beta cell mass and function. To achieve these goals, we employ comprehensive genetic, molecular, and cellular approaches, integrating omics technologies with human and mouse cell lines, mouse models, primary human tissues, and iPSC-derived islet-like organoids. Our long-term objective is to pioneer advanced pharmacological and regenerative therapies for DM. This effort is strengthened by an extensive national and international collaborative network, involving partnerships with hospitals, research institutes, pharmaceutical companies, and private foundations.

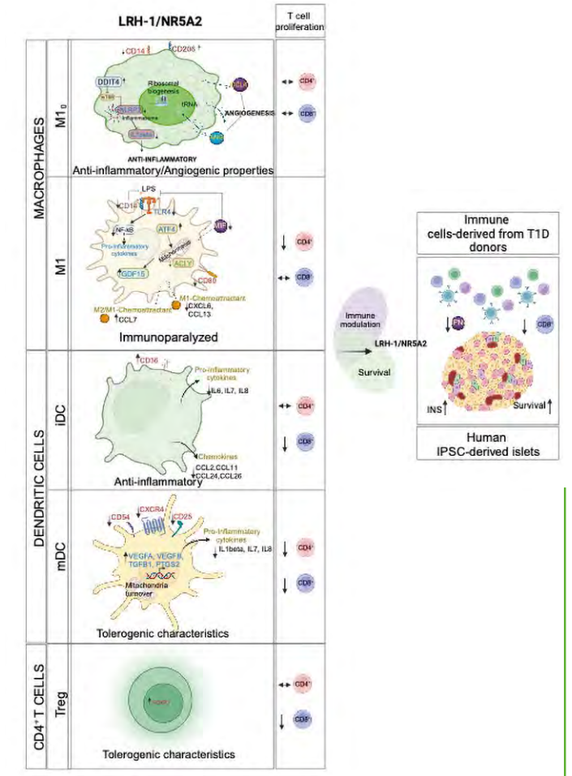

Figure 1. Cellular and molecular mechanism of action mediated by the pharmacological effect of LRH-1/NR5A2 in human inmune cells. Proposed model of genetic and inmune cell-tailored reprogramming induced by LRH-1/NR5A2 activation and its impact on T-effector cell proliferation. From Cobo-Vuilleumier Clin. Transl.Med. 2024.

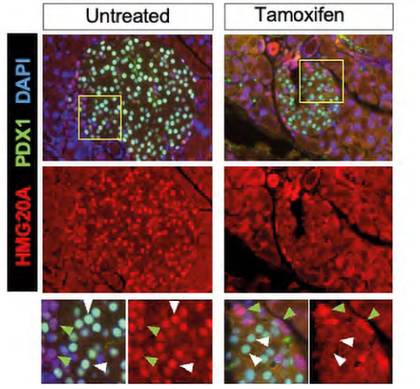

Figure 2. Inducible deletion of HMG20A in beta cells in mice: Representative immunofluorescent images showing especific HMG20A depletion in beta cells (PDX1+cells) after Tamoxifen treatment.

Research Highlights

The main highlights for the period 2023-24 are:

1. LRH-1/NR5A2 emerges as a promising pharmacological target for an immuno-regenerative therapy in T1D

We previously demonstrated that activation of the nuclear receptor LRH-1/NR5A2 with BL001, a small-molecule agonist developed in-house, can reverse hyperglycemia in mouse models of T1D by attenuating the autoimmune attack and promoting beta cell survival and regeneration, a process mimicking wound healing (Martín-Vázquez, et al., Int. J. Biol. Sci., 2023). Over the past 2 years, we have investigated whether LRH-1/NR5A2 activation could also induce immune tolerization in human subjects with long-standing T1D and enhance islet survival. LRH-1/NR5A2 activation led to the genetic and immunometabolic reprogramming of T1D immune cells, characterized by reduced pro-inflammatory markers and cytokine secretion, increased mitohormesis in pro-inflammatory M1 macrophages, and enhanced mitochondrial turnover in mature dendritic cells. These changes shifted the immune landscape from a pro-inflammatory to an anti-inflammatory/tolerogenic state, effectively inhibiting cytotoxic T-cell proliferation. BL001 treatment also expanded regulatory T-cells and Th2 cells within peripheral blood mononuclear cells (PBMCs). Importantly, these molecular and cellular changes were specific to immune cells from individuals with T1D, as immune cells from healthy individuals responded differently to the drug. Additionally, BL001 mitigated PBMC-induced apoptosis and preserved insulin expression in human iPSC-derived islet organoids (Cobo-Vuilleumier et al., Clin. Transl. Med. 2024). In parallel we showed that transplanted human islets under the kidney capsule of immune competent diabetic mice treated with BL001 had lower blood glucose levels, improved survival, and preserved beta cell mass with detectable human C-peptide, while vehicle-treated mice showed reduced insulin-expressing cells (Cobo-Vuilleumier et al., Front. Immunol., 2024). Taken together, our findings indicate that LRH-1/NR5A2 activation promotes and anti-inflammatory environment favoring human islet survival, engraftment, and function (Figure 1). We are currently evaluating the immunomodulatory effects of BL001 on blood cells from children with recent-onset T1D, utilizing cutting-edge CyTOF technology to generate highly multiplexed, single cell data from minimal cell suspension samples. In collaboration with the Institute for Chemical Research, we are also developing enzyme-responsive Zr-based metal-organic frameworks to enable controlled delivery of BL001 (Carillo-Carrion et al., ACS Appl. Mater. Interfaces, 2023).

2. Umbilical cord mesenchymal stromal cell transplantation as an immunomodulatory therapy for T1D

We have also investigated the effects of umbilical cord mesenchymal stromal cells (UC-MSCs) transplantation on delaying the onset of hyperglycemia in the RIP-B7.1 mouse model of experimental autoimmune diabetes. We demonstrated that intraperitoneal administration of UC-MSC significantly reduced insulitis and immune infiltration in pancreatic tissues, correlating with lower blood glucose levels and delayed diabetes onset. This effect was driven by the ability of UC-MSCs to induce immunosuppressive responses, including the recruitment of myeloid-derived suppressor cells, regulatory T cells, and anti-inflammatory cytokine production in pancreatic lymph nodes and spleen. Our findings highlighted the potential of UC-MSC as a promising immunomodulatory therapy for T1D by mitigating autoimmune attack and preserving beta cell function (Lachaud, et al., Front. Cell Dev. Biol., 2023).

3. Generation of a conditional, tissue-specific knockout mouse model to uncover HMG20A-regulated mechanisms in metabolic adaptation and Type 2 Diabetes Mellitus (T2D)

Our previous work demonstrated that the 'Metabesity' factor HMG20A regulates islet beta cell functional maturity and astrocyte polarization as part of a gluco-adaptive and neuro-protective response to physiological stress, such as obesity and diabetes. We further showed that treatment of obese mice with ORY1001, an LSD1/CoREST inhibitor that mimics the effects of HMG20A, normalized glucose intolerance. These findings highlight the role of HMG20A in coordinating adaptive organ responses to metabolic stress and support the potential of ORY1001 as a novel therapeutic approach for T2D. To further investigate the cellular and molecular mechanisms regulated by HMG20A, we have successfully generated a conditional, tissue-specific HMG20A knockout mouse model over the past 2 years (Figure 2).

Grants

- 2024-2027: DiabetesCERO. Foundation.
- 2024-2027: DEM2024/PIM03. CIBERDEM/ISCIII.
- 2022-2026: 3-SRA-2023-1307-S-B. Juvenile Diabetes Research Foundation.
- 2022-2025: PID2021-123083NB-I00. Ministerio de Ciencia, Innovación y Universidades.
- 2023-2026: PRE2022-105389. Ministerio de Ciencia, Innovación y Universidades.
- 2020-2025: Vencer el Cáncer.

Publication Highlights

Cobo-Vuilleumier N, Rodriguez-Fernandez S, Lopez-Noriega L, Lorenzo PI, Franco JM, Lachaud CC, Vazquez EM, Legido RA, Dorronsoro A, Lopez-Fernandez-Sobrino R, Fernandez-Santos B, Serrano CE, Salas-Lloret D, van Overbeek N, Ramos-Rodriguez M, Mateo-Rodriguez C, Hidalgo L, Marin-Canas S, Nano R, Arroba AI, Caro AC, Vertegaal AC, Montalvo AM, Martin F, Aguilar-Diosdado M, Piemonti L, Pasquali L, Prieto RG, Sanchez MIG, Eizirik DL, Martinez-Brocca MA, Vives-Pi M, Gauthier BR. **2024**. LRH-1/NR5A2 targets mitochondrial dynamics to reprogram type 1 diabetes macrophages and dendritic cells into an immune tolerance phenotype. 2024. **Clin Transl Med.** 14:e70134.

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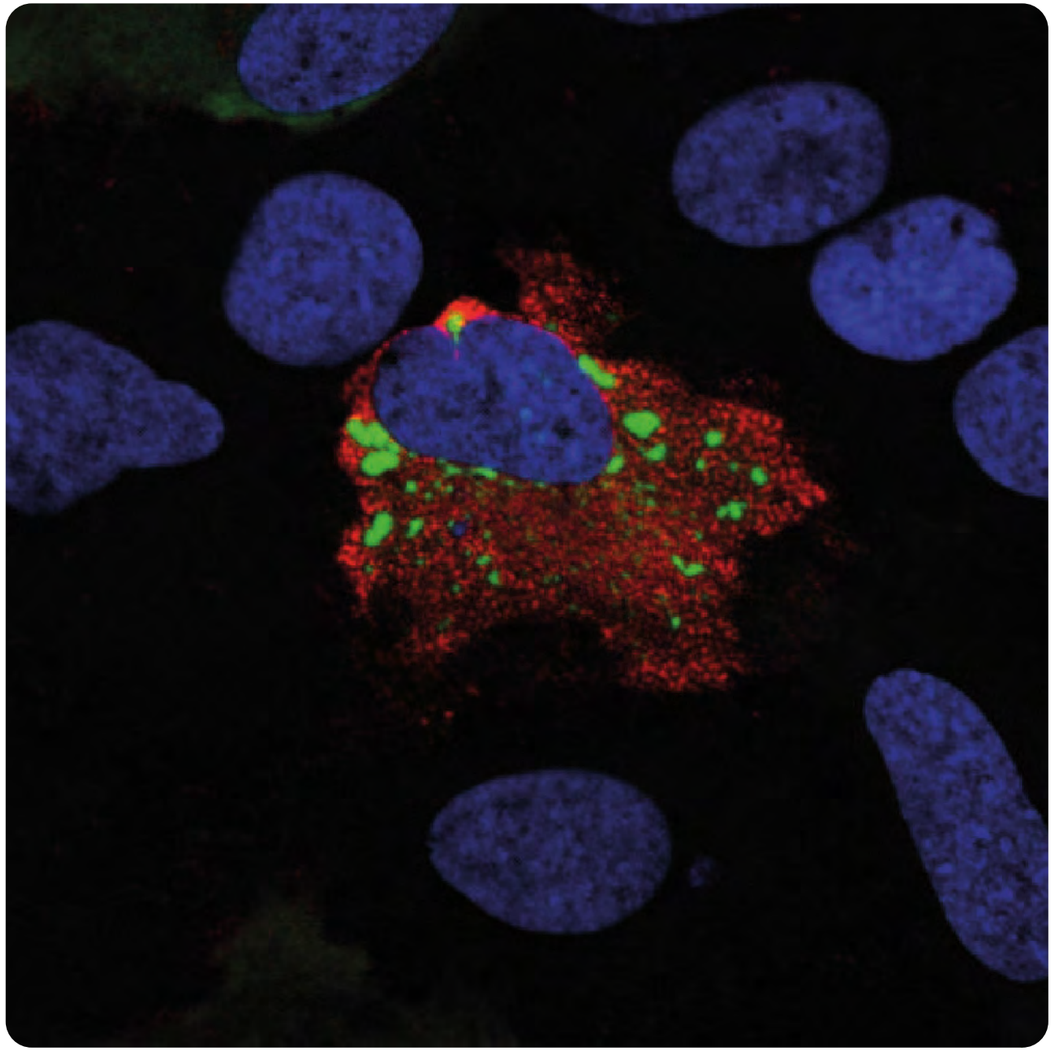
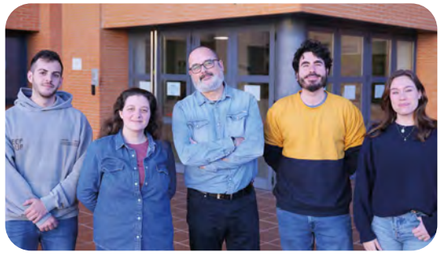


Principal Investigator

Dr. Manuel Álvarez-Dolado

Cell-Based Therapies for Neuropathologies

Group Leader



Current position

- Tenured Scientist CSIC-CABIMER.

Group Members

Postdoc

- Maurizio Riga.

PhD Students

- Mª Mercedes Pérez Fernández.
- Benito Domínguez Velasco.
- Mar Navarro Alonso.
- Silvestre Ruano Rodríguez.

Technician

- Maite Puentes Lérida.

Former Members (2023-2024)

- **Master Students:** Sara de la Torre Checa y Carmen Ángel Gómez.
- **Grade students:** María Dolores Delgado Egea, Zayra Delgado Izquierdo, Carmen Ortiz Salguero.

Research Activity

Overview

We develop basic and translational research projeacts to better understand and treat diseases that affect the nervous system, with special interest in those related to GABAergic interneuron deficits such as infantile epilepsy (Dravet and Stxbp1 syndromes), or mental disorders (depression, anxiety, schizophrenia).

We perform pre-clinical assays in mouse models of disease to verify the potential benefit of innovative therapeutic strategies. These include GABAergic interneuron transplants at perinatal and adult stages of the disease, or transcranial magnetic stimulation of the brain, both leading to reduce seizure frequency and revert cognitive and behavioural alterations.

At basic level, we also work deciphering the role of the sodium voltage-gated channel Nav1.1 in the function of GABAergic interneurons in the prefrontal cortex (PFC). This is relevant to better understand the symptoms of Dravet

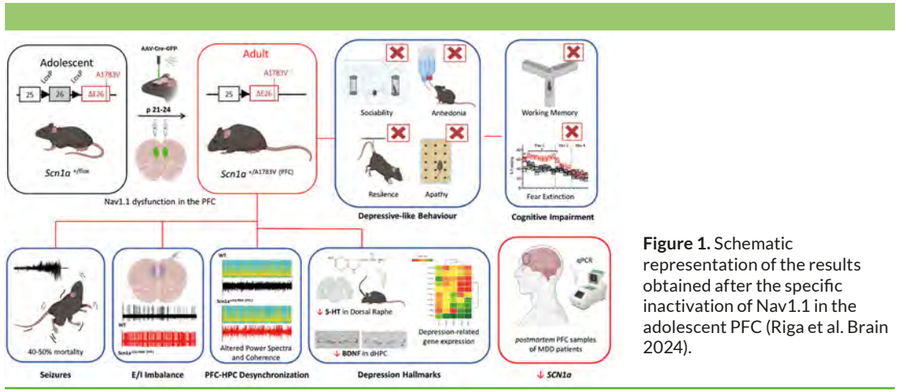


Figure 1. Schematic representation of the results obtained after the specific inactivation of Nav1.1 in the adolescent PFC (Riga et al. Brain 2024).

syndrome (DS) and also the etiology of some mental disorders, such as depression.

Finally, we are also interested in studying the pathophysiology of another severe epileptic encephalopathy, the Stxbp1 Syndrome. We are performing phenotype/genotype correlations and analysing omic data with the help of artificial intelligence methods.

Research Highlights

Inactivation of Nav1.1 channel in the prefrontal cortex leads to epilepsy, cognitive impairment, and a depressive-like phenotype.

Dysfunction of Nav1.1 channel cause DS, and it has been implicated in the cognitive deficits observed in Alzheimer´s patients. We studied the role of Nav1.1 in the PFC to delineate the implication of this brain area in the origin of some DS symptoms and the putative relationship of this channel with the etiology of mental disorders. We selected the PFC because its well-known role in cognitive and superior brain functions. In addition, altered development of this region during adolescence is related to depression and schizophrenia onset.

Dysfunction of Nav1.1 activity in the PFC of adolescent mice enhanced the local excitation/inhibition ratio, resulting in epileptic activity, cognitive deficits and depressive-like behaviour in adulthood, along with a gene expression profile linked to major depressive disorder (MDD). Additionally, it reduced extracellular serotonin concentration in the dorsal raphe nucleus and brain-derived neurotrophic factor expression in the hippocampus, two MDD-related brain areas beyond the PFC. Epileptic seizures, alike to DS, and depression were two processes that emerged in parallel, and independently of each other, as consequence of Nav1.1 dysfunction. Finally, we found reduced expression levels of *SCN1A*, the gene encoding Nav1.1, in post-

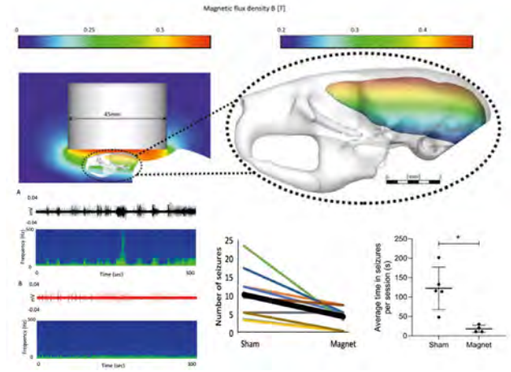


Figure 2. Contours of the magnetic field applied in the mouse brain. Recording of epileptiform brain activity in DS mice (A, black) and its downmodulation after tSMS application (B, red). tSMS reduced the frequency of seizures, their duration, and severity in DS mice.

mortem PFC samples from human MDD subjects (Fig. 1).

Collectively, our results provided a novel mechanistic framework linking Nav1.1 dysfunction in the PFC to the pathogenesis of epileptic and depressive disorders.

Innovative therapeutic strategies for infantile epileptic encephalopathies.

DS is a severe epileptic encephalopathy characterized by recurrent seizures and highly resistant to antiepileptic medication. In collaboration with the NEUROcom group from the Universidade da Coruña, we explored transcranial static magnetic stimulation (tSMS) as a non-invasive neuromodulatory treatment in a mouse model of DS. The application of tSMS significantly reduced seizure frequency, with remaining seizures being shorter and less intense compared to control conditions (Fig. 2). tSMS is a simple, affordable, and non-invasive safe technique easily applicable to humans. Our findings suggest that tSMS could be a promising and complementary nonpharmacological treatment for DS.

We are also working in a cell-based therapeutic approach with GABAergic interneuron precursors derived from the medial ganglionic eminence. We are transplanting these cells in mouse models of Dravet and Stxbp1 syndromes, where we observed an anticonvulsivant activity of the precursors together with restoration of normal brain rhythms and improvement of behavioural and cognitive deficits. In Stxbp1 deficient mice the rescue was almost total when transplants were performed perinatally (P3-5). We observed a significant partial restoration in transplanted adult mice, as well. In DS model we only observed significant improvements when the transplanted progenitors over-expressed the

Nav1.1 channel. The results strongly suggest that naïve or genetically-modified GABAergic neuronal precursors are a promising source of cells for regenerative medicine to treat these infantile epileptic conditions.

Genotype/Phenotype correlations and omic analysis for Stxbp1 Syndrome

Stxbp1 Syndrome is characterized by medically refractory seizures, brain activity alterations, ataxia, autism, and severe psychomotor delay. Diagnosis is poorly developed, and mutation subtypes are not well correlated with clinical manifestations or the response to medication. This leads to deficient genetic counseling and delays in the implementation of correct treatments, what significantly impacts the prognosis of these children. In collaboration with the Spanish Stxbp1 association and Dr. FJ Esteban from Jaen University, we are analyzing genomic, transcriptomic, metabolomic, and clinical data from Stxbp1 subjects by applying computational and artificial intelligence methods. With this we search to identify biomarkers for clinical diagnosis, implement a personalized medicine, and a better understanding of this pathology. The initial results indicate a strong polygenic risk in the origin of the diverse symptomatology associated to this syndrome.

Grants

- 2021-2024: Ministerio de Ciencia e Innovación (PID2021-127044OB-I00).
- 2024-2027: Junta de Andalucía. Consejería de Salud y Consumo. Proyectos de investigación e innovación de colaboración público-privada (PIP-0113-2024).
- 2021-2023: Fundación Alicia Koplowitz.
- 2020-2024: Technological contract CSIC - Stxbp1 Syndrome Association.
- 2021-2023: Apoyo Dravet Association.

Publication Highlights

Riga MS, Pérez-Fernández M, Miquel-Rio L, Paz V, Campa L, Martínez-Losa M, Esteban FJ, Callado LF, Meana J, Artigas F, Bortolozzi A, Álvarez-Dolado M. **2024.** Scn1a haploinsufficiency in the prefrontal cortex leads to cognitive impairment and depressive phenotype. **Brain.** 147(12):4169.

Moshtaghion SM, Caballano-Infantes E, Plaza Reyes Á, Valdés-Sánchez L, Fernández PG, de la Cerda B, Riga MS, Álvarez-Dolado M, Peñalver P, Morales JC, Díaz-Corrales FJ. **2024.** Piceid Octanoate Protects Retinal Cells against Oxidative Damage by Regulating the Sirtuin 1/Poly-ADP-Ribose Polymerase 1 Axis In Vitro and in rd10 Mice. **Antioxidants** (Basel). 13(2):201.

Rivadulla C, Pardo-Vazquez JL, de Labra C, Aguilar J, Suarez E, Paz C, Álvarez-Dolado M, Cudeiro J. **2023**. Transcranial static magnetic stimulation reduces seizures in a mouse model of Dravet syndrome. **Exp Neurol.** 370:114581.

Principal Investigator

Dr. David Pozo

Cellular and Molecular Neuroimmunology

Group Leader

Current position

- Associate Professor of Biochemistry and Molecular Biology. University of Seville (US) Medical School.

Group Members

PhD students

- Lucía Silvera-Carrasco.
- Carlos Gómez-Navas.

Technician

- Daniel Tejada Moreno.

Former Members (2023-2024)

PhD student

- Victoria Areal-Quecuty.

Postdoc

- Zaira González-Sánchez.

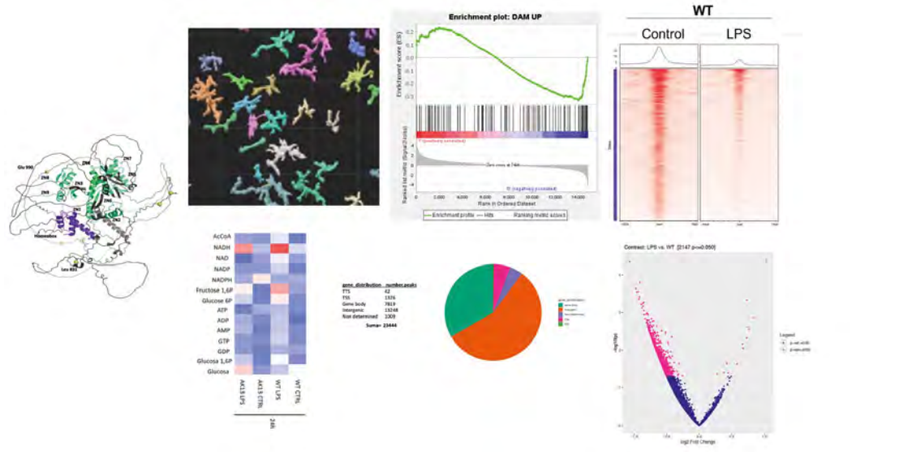


Figure 1. Activity-Dependedent Neuroprotective Protein (ADNP) is a master regulator of microglia specialization. Molecular characterization includes RNAseq, CHIPseq, metabolomics and proteomics analysis and signalling pathways under ADNP regulation. ADNP nuclear levels are characterized by Stelaris 3D confocal microscopy in Amyotrophic Lateral Sclerosis (ALS).

Research Activity

Overview

The Cellular and Molecular Neuroimnunology Laboratory (CMNL) of the University of Seville at CABIMER is focused on understanding molecular and cellular mechanisms that regulate immune homeostasis and contribute to neuronal dysfunction and death. Microglia are the primary immunocompetent cells in the brain playing a critical role during physiological and pathological conditions. The study of the mechanisms by which microglia influence brain processes are essential for developing targeted therapeutic strategies and for the maintaining of brain homeostasis. In this sense, the characterization of endogenous molecules with both neuroprotective and immunoregulatory properties is of special interest not only in terms of new therapeutic strategies, but particularly taking into consideration the increasing role of immune mediators in central nervous system plasticity. We identified neuropeptide

activity-dependent neuroprotective protein (ADNP) and NAP-derived peptide as new neuroimmunodulators by using different models (acute brain inflammation, septic shock, MS, ALS and Adnp haploinsuffiency mice) disclosing an emerging role in brain immune homeostasis. Limited bioavailability is often a bottleneck for neuropeptide translational research. We have developed new smart delivery platforms to enhance neuropeptide drugability and also to target glial cells for nanoparticle-mediated immune reprogramming. Out interest in translational research is to understand the inflammatory component in pathological cell–cell communication tissue crosstalk in the Austism Spectrum Disorder (ASD) and Amyotrophic Lateral Sclerosis (ALS).

The active research lines are as follows:

a. Role of the neuroprotein protein ADNP as a master regulator in promoting microglial cell specialization. Focus on autism spectrum disorder (ASD) and Amyotrophic Lateral Sclerosis (ALS).
b. Exploring in vivo/in vitro ADNP-mediated effects by using human engrafted stem cell-derived microglia in ALS mice brain
c. Modulation of innate and adaptive immunity by endogenous neuropeptides in neurodegeneration.
d. Nanoparticles for controlled and targeted drug delivery: improving the drugability of neuropeptides and smart reprogramming of glia in neurodegenerative diseases. Biomaterial-based modulation of the immune system: engineering immunomodulatory nanomaterials.
e. Energy metabolism as modifier of the immune response: tissue crosstalk in Amyotrophic Lateral Sclerosis (ALS) and neurodegeneration.

Grants

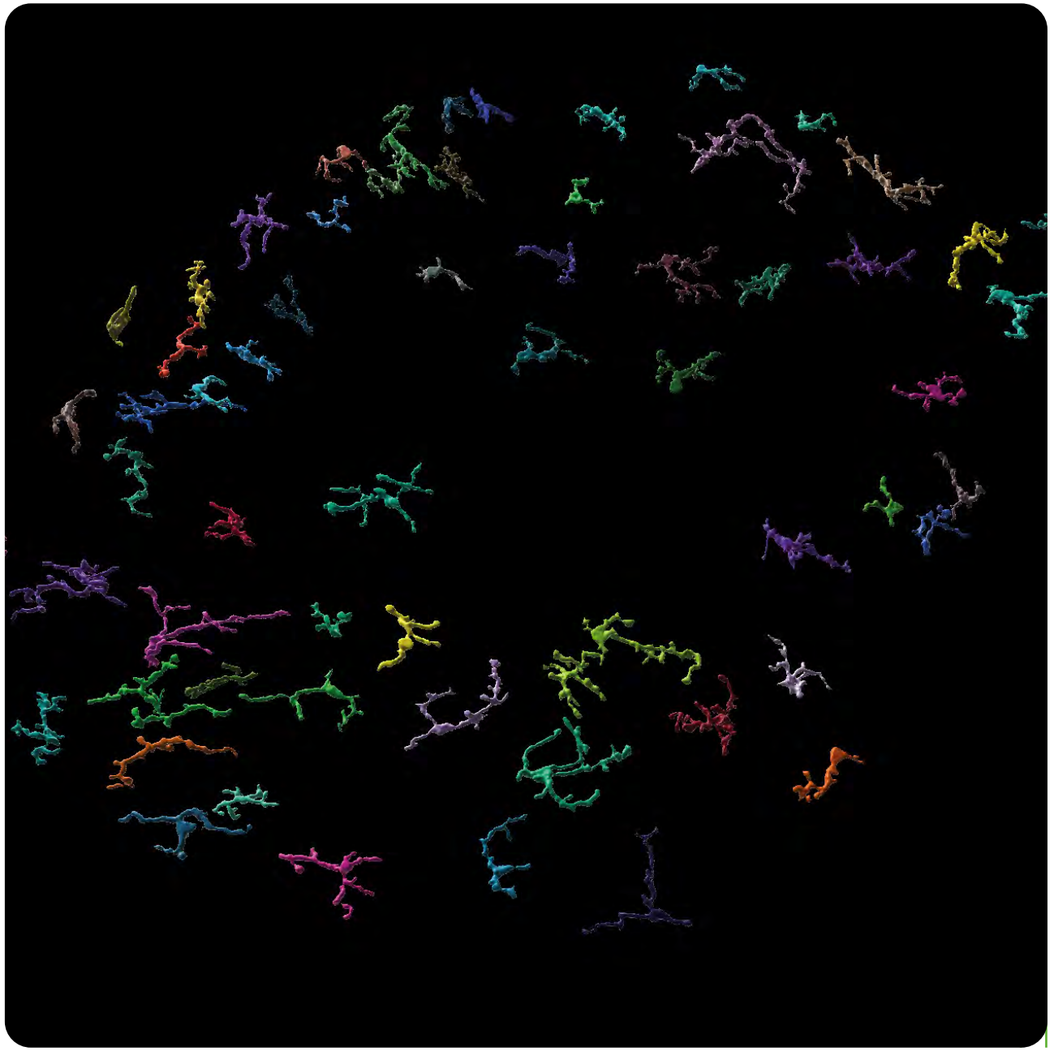
- 2022-2025: PI-0232-2022. Consejería de Salud y Familias. Junta de Andalucía.
- 2019-2023: RTI2018-098432-B-I00. Retos-Investigación, Plan Nacional, Ministry of Science.

Publication Highlights

Silva-Hucha S, Fernández de Sevilla ME, Humphreys KM, Benson FE, Franco JM, Pozo D, Pastor AM, Morcuende S. **2024**. VEGF expression disparities in brainstem motor neurons of the SOD1G93A ALS model: Correlations with neuronal vulnerability. **Neurotherapeutics**. 21(3):e00340.

Aranda-Abreu GE, Carreón-Rodriguez A, Zuñiga S, Pozo D. **2024**. SARS-CoV-2 in neurodegenerative diseases. **Front Neurosci**. 16:18:1360234

Pérez-Cabello JA, Silvera-Carrasco L Franco JM, Capilla-González V, Armaos A, Gómez-Lima M, García-García R, Yap XW, Leal-Lasarte M, Lall D, Baloh RH, Martínez S, Miyata Y, Tartaglia GG, Sawarkar R, García-Domínguez M, Pozo D, Roodveldt C. **2023**. MAPK/MAK/MRK overlapping kinase (MOK) controls microglial inflammatory/type-I IFN responses via Brd4 and is involved in ALS. **PNAS USA**. 120(28):e2302143120.



Principal Investigator

Dr. Alejandro Martín-Montalvo

Metabolic Interventions for Healthy Aging

Group Leader

Current position

- Tenured Scientist CSIC.

Group Members

Postdocs

- Raúl López-Fernández.
- Almudena Ruiz García.

PhD Students

- Inmaculada Pino Pérez.
- María Camacho Cabrera.

Technicians

- María Rivero Lobo.
- María del Carmen Vilches Pérez.

Former Members (2023-2024)

- **Postdoc:** Isabel Espadas Villanueva.
- **PhD Students:** María Ángeles Cáliz Molina, Alejandro Sola García, Ryan Conesa Bakkali.

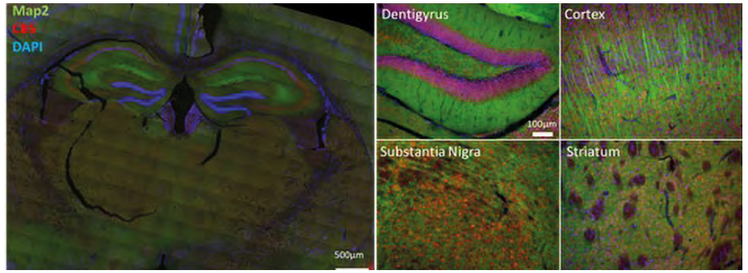


Figure 1. Immunohistochemistry in the brain. Map2 is used as neuronal marker. The Cystathionine-β-synthase (CBS) is stained in red. Nuclei are stained with Dapi in blue.

Research Activity

Overview

Life expectancy is steadily increasing; however, the quality of life in the elderly often remains suboptimal, with approximately 50% of individuals over 80 years of age experiencing some form of dependence. Given the high prevalence of age-related pathologies—such as sarcopenia, diabetes, neurodegenerative diseases, and cancer—there is an urgent need for therapies that not only prevent and treat these conditions but also promote active, healthy aging. This represents one of the most ambitious goals in modern healthcare.

Our aim is to understand the molecular mechanisms driving the aging process and to explore the potential of novel geroprotective strategies for promoting healthy aging. To achieve this, we employ a variety of approaches, ranging from cell cultures to in vivo studies using laboratory mice. In addition, we analyze human samples to determine whether the molecular mechanisms identified in our research are relevant to human health.

Research Highlights

The Use of Geroprotectors for Healthy Aging

We are currently exploring innovative geroprotective strategies aimed at promoting healthy aging and preventing disorders that impair quality of life and independence in the elderly. At the mechanistic level, our focus is to understand the role of two key biological processes relevant to aging and age-related diseases:

1. The Modulation of Acetyl-CoA Metabolism in Healthspan and Lifespan

ATP-citrate lyase (ACLY) is a cytosolic enzyme widely distributed across various

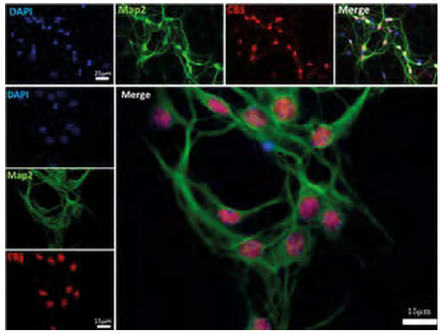


Figure 2. Immunocytochemistry in neuronal cultures. Map2 is used as neuronal marker. The Cystathionine-β-synthase (CBS) is stained in red. Nuclei are stained with Dapi in blue.

human and animal tissues. A major function of ACLY is the production of acetyl-CoA (Ac-CoA), a crucial molecule for the biosynthesis of cholesterol and fatty acids. ACLY utilizes cytosolic citrate—an inhibitor of glycolysis—to generate Ac-CoA. Consequently, ACLY activity can influence not only lipid synthesis but also glycolytic processes. Elevated levels of nuclear and cytosolic Ac-CoA promote protein acetylation, a post-translational modification that regulates protein activity, interactions, and cellular localization. Inhibition of ACLY may, in principle, reduce Ac-CoA levels in the cytosol and nucleus, potentially leading to the inhibition of lipogenesis and the promotion of the deacetylated state of "acetylatable" proteins, which mimics an energy-depleted state. This could, in turn, trigger effects similar to those induced by increased sirtuin activity or calorie restriction.

We are investigating the physiological effects of pharmacologically inhibiting ACLY, the key enzyme responsible for Ac-CoA generation. Our studies have shown that wild-type mice, whether fed a standard or high-fat diet supplemented with ACLY inhibitors, exhibit certain improvements in physical health, metabolic homeostasis, and longevity. Mechanistic studies have focused on the effects of ACLY inhibition on immunomodulatory processes and tissue regeneration. Our research is also uncovering the potential of ACLY inhibitors in promoting muscle regeneration. Moreover, human biopsy analysis has revealed that phospho-active ACLY levels are crucial for human health, possibly serving as a marker for end-stage liver disease.

2. The Modulation of Cysteine Post-Translational Modifications in Healthspan and Lifespan

Hydrogen sulfide (H_2S), a gasotransmitter, can diffuse freely through cell membranes and induce intracellular signaling responses. H2S is generated via both non-enzymatic mechanisms using sulfur donors and enzymatic processes involving cystathionine β-lyase (CGL), cystathionine β-synthase (CBS), and β-mercaptopyruvate sulfurtransferase (MST). H_2S modulates the activity of a variety of proteins, including membrane ion channels, structural proteins, enzymes, and transcription factors. One of the primary mechanisms through which H_2S affects protein function is by post-translational modification of cysteines through S-sulfhydration. Although the role of S-sulfhydration in regulating protein activity, stability, and localization is an emerging area of study, the precise mechanisms by which H_2S mediates this modification remain unclear. Thermodynamically, the direct interaction of H_2S with unmodified cysteine to induce S-sulfhydration is not straightforward. It is hypothesized that S-sulfhydration might occur on cysteines that are already modified, such as through oxidation events like nitrosylation or sulfenylation. The chemistry of this novel post-translational modification warrants further investigation.

We are currently studying the potential cellular and physiological benefits of modulating cysteine post-translational modifications, with a particular focus on enhancing cysteine persulfidation. Our ongoing longevity studies in rodents, including both wild-type mice and experimental models of age-related diseases, aim to evaluate improvements in physical health, neurocognitive function, and metabolic homeostasis across different stages of life. On a mechanistic level, we are advancing our understanding of sulfur metabolism using transcriptomic and proteomic approaches. In a translational context, we are assessing the relevance of cysteine post-translational modifications in human health.

Grants

- 2022-2025. PID2021-123965OB-I00. Ministerio de Ciencia e Innovación.
- 2022-2023 202220I059. CSIC.
- 2024-2027. Código: PI-0035-2024. Consejería de Salud y Familias de Andalucía
- 2024-2027. Código: CPP2023-010925. Ministerio de Ciencia e Innovación. Universidades.

Publication Highlights

Espadas I, Cáliz-Molina MÁ, López-Fernández-Sobrino R, Panadero-Morón C, Sola-García A, Soriano-Navarro M, Martínez-Force E, Venegas-Calerón M, Salas JJ, Martín F, Gauthier BR, Alfaro-Cervelló C, Martí-Aguado D, Capilla-González V, Martín-Montalvo A. **2024**. Hydroxycitrate delays early mortality in mice and promotes muscle regeneration while inducing a rich hepatic energetic status. **Aging Cell**. 23(9):e14205.

Hine C, Ponti AK, Cáliz-Molina MÁ, Martín-Montalvo A. **2024**. H_2S serves as the immunoregulatory essence of apoptotic cell death. **Cell Metab.** 36(1):3-5.

Sola-García A, Cáliz-Molina MÁ, Espadas I, Petr M, Panadero-Morón C, González-Morán D, Martín-Vázquez ME, Narbona-Pérez ÁJ, López-Noriega L, Martínez-Corrales G, López-Fernández-Sobrino R, Carmona-Marin LM, Martínez-Force E, Yanes O, Vinaixa M, López-López D, Reyes JC, Dopazo J, Martín F, Gauthier BR, Scheibye-Knudsen M, Capilla-González V, Martín-Montalvo A. **2023**. Metabolic reprogramming by Acly inhibition using SB-204990 alters glucoregulation and modulates molecular mechanisms associated with aging. **Commun Biol.** 6(1):250.

Principal Investigator

Dr. Cintia Roodveldt

Immune Signalling in Neurodegenerative Proteinopathies

Group Leader

Current position

- Associate Professor of Biochemistry and Molecular Biology. University of Seville (US).

Group Members

PhD Students

- Raquel García-García.
- Sabine M. Vernon.

Former Members (2023-2024)

- Jesús A. Pérez-Cabello.
- Daniel Tejada Moreno.

Research Activity

Overview

Neurodegenerative diseases, including Parkinson's, Alzheimer's, and Amyotrophic Lateral Sclerosis (ALS), are incurable and increasingly prevalent disorders characterized by progressive neuronal loss, the deposition of abnormal protein aggregates in the CNS, and the buildup of neuroinflammation. The underlying pathogenic mechanisms of these highly complex pathologies are still insufficiently understood, but it is currently thought that dysregulated immune responses involving exacerbated microglial activation and peripheral immune imbalance, play a major role in pathogenesis (Roodveldt et al., 2024).

The aim of our research is to identify novel molecular mechanisms, signaling pathways and therapeutic targets related to immune dysregulation in neurodegenerative diseases, particularly ALS and Parkinson.

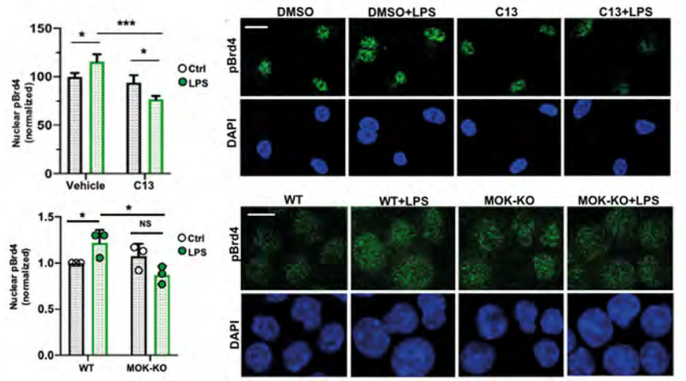


Figure 1. MOK regulates phospho-Brd4 levels in microglia under inflammatory conditions. Quantification of nuclear Ser492-phospho-Brd4 levels by confocal IF with primary microglial cells pretreated with 10 µM MOK-inhibitor (top), or with MOK-KO or wild-type (WT) SIM-A9 cells (bottom), stimulated or not with 1 µg/mL LPS for 4h. Images are from one out of three independent experiments (N=3). Scale bar: 10 µm.

The active research lines are as follows:

a. Molecular mechanisms of immune responses in neurodegenerative proteinopathies, including ALS and Parkinson.
b. Role and therapeutic potential of signaling kinases in microglial responses and neuroinflammation in ALS.
c. Molecular mechanisms and transcriptional dynamics of immune cells in neurodegenerative diseases.

Research Highlights

Amyotrophic lateral sclerosis (ALS) remains a devastating and incurable disease. Several studies have shown that microglia, the main immunocompetent cells in the CNS, become activated and neurotoxic, thereby contributing to motor neuron loss and disease onset and progression. The mechanisms driving microglia neurotoxicity in ALS and other neurodegenerative diseases remain incompletely understood. Our research work recently revealed the signaling kinase MOK (MAPK/MAK/MRK overlapping kinase), as a protein that mediates microglial inflammatory and type-IFN responses through a mechanism that directly implicates the epigenetic reader BRD4 (Pérez-Cabello, PNAS 2023). Given that signaling kinases are known to be central immune mediators whose functions may be dysregulated in neuroinflammation-associated neurodegenerative diseases (Roodveldt et al., 2024; García-García et al., 2021) we sought to dissect the signaling pathways regulated by MOK in ALS-linked microglial responses. Apart from identifying Brd4 as the first downstream target reported for MOK, we showed that MOK regulates both Brd4's phosphorylated levels and chromatin-binding functions in the cell

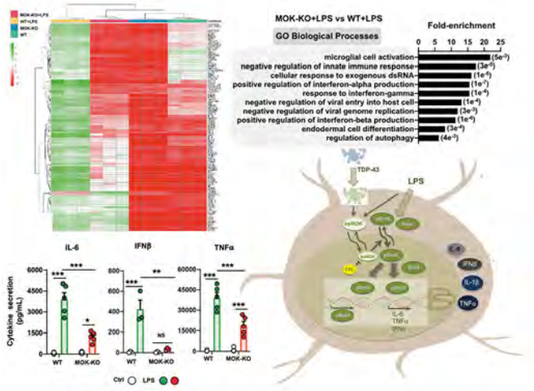


Figure 2. LMOK regulates microglial activation and inflammatory and type-I IFN responses in cell culture. Heatmap showing unsupervised clustering analysis for the top protein-coding genes based on relative expression levels (upper left, N=3). GO term enrichment analysis of total DEGs for MOK-KO+LPS vs. WT+LPS. Shown are the top hits based on the P-value (in parentheses) with at least three up-/down-regulated genes (upper right). Quantification of IL-6, IFNβ and TNFα levels by ELISA in supernatants from WT and MOK-KO SIM-A9 cells stimulated with 1 µg/mL LPS for 5h (bottom, left; N=4/5). Schematic representation of the role proposed for MOK kinase in microglial inflammatory responses (bottom, right).

nucleus (Figure 1; Pérez-Cabello et al., 2023). By applying proteomics and transcriptomics analyses, we revealed a number of key immune pathways that are activated upon immune stimulation in a MOK-dependent manner (Figure 2).Remarkably, we also found that MOK is altered in spinal cord tissue from ALS patients and mouse models, particularly in microglial cells (Pérez-Cabello et al., 2023). Overall, our results support a role for MOK in the pathogenic mechanisms of ALS and contributes to the search for novel and effective therapeutic targets against ALS and other neurodegenerative diseases.

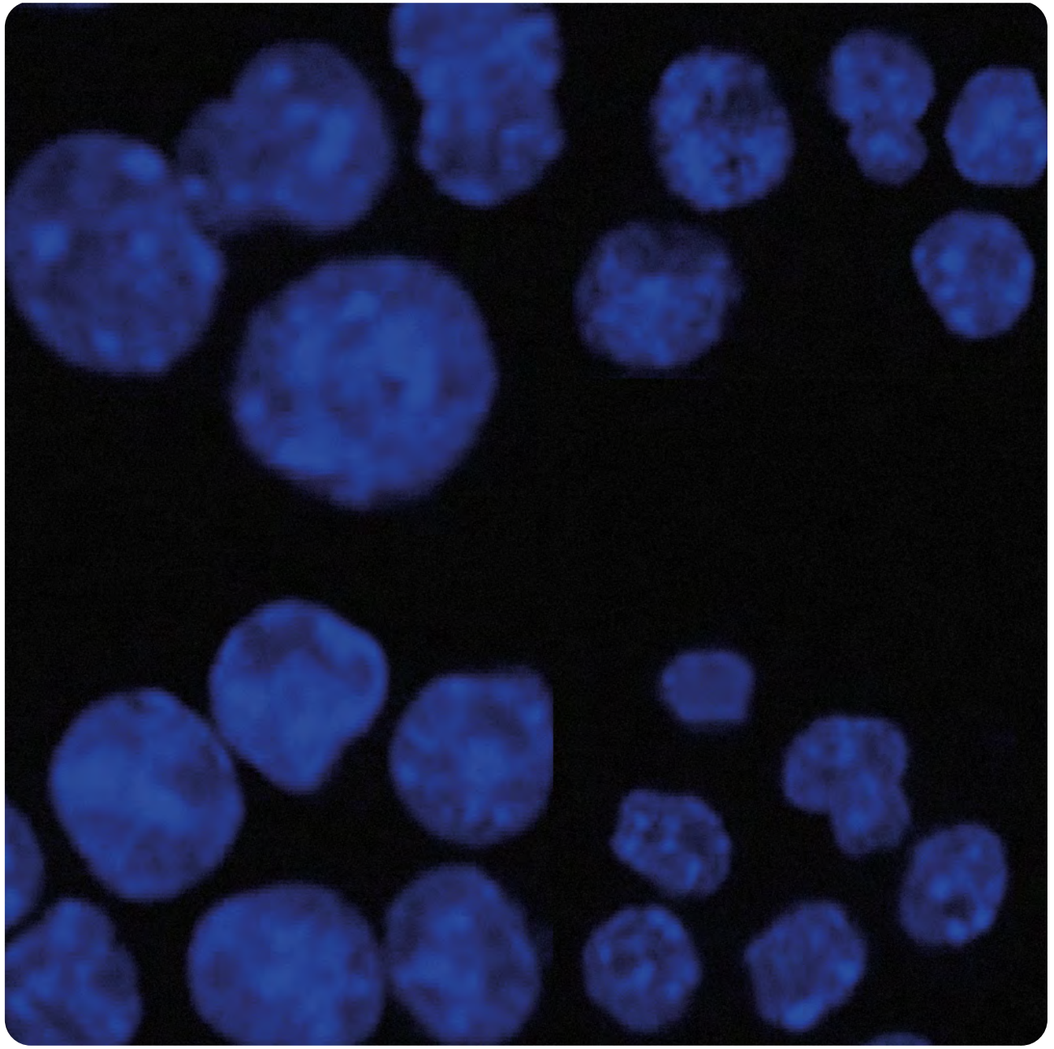
Grants

- 2023-2026: PID2022-140838OB-I00. Plan Nacional, Ministry of Science.
- 2019-2023: RTI2018-098432-B-I00. Plan Nacional, Ministry of Science.

Publication Highlights

Roodveldt C, Bernardino L, Oztop-Cakmak O, Dragic M, Espolin Fladmark K, Ertan S, Pita C, Ciglar L, Busra A, Garraux G, Williams-Gray C, Pacheco R, Romero-Ramos M. **2024**. Immunity in Parkinson's disease: what we know so far. **Brain.** 147(10):3306-3324.

Pérez-Cabello JA, Silvera-Carrasco L Franco JM, Capilla-González V, Armaos A, Gómez-Lima M, García-García R, Yap XW, Leal-Lasarte M, Lall D, Baloh RH, Martínez S, Miyata Y, Tartaglia GG, Sawarkar R, García-Domínguez M, Pozo D, Roodveldt C. **2023.** MAPK/MAK/MRK overlapping kinase (MOK) controls microglial inflammatory/type-I IFN responses via Brd4 and is involved in ALS. **PNAS USA**. 120(28):e2302143120.



Principal Investigator

Dr. Francisco Javier Díaz-Corrales

Retinal neurodegeneration and advanced therapies

Group Leader

Current position

- Staff Scientist/Nicolás Monardes Program. Fundación Pública Andaluza Progreso y Salud (FPS).

Group Members

Senior Researcher

- Berta De la Cerda Haynes.

Postdocs

- Estefanía Caballanos Infante.
- Álvaro Plaza Reyes.

PhD student

- Mohamad Mehdi Moshtaghion.

Technicians

- María Lourdes Valdés Sánchez.
- Marta Torres Valcárcel.
- Adoración Montero Sánchez.

Former Members (2023-2024)

- **Master students:** Laurie Clauzon, Leonié Broutel.
- **Technician:** Andrea Garrido Gomes, Sofía Jiménez Gavira.

Research Activity

Overview

Our laboratory is dedicated to developing advanced therapies and innovative drugs to combat degenerative retinal diseases and ocular pathologies that lead to blindness, such as retinitis pigmentosa, age-related macular degeneration (AMD), and cataracts. Additionally, we focus on studying the molecular mechanisms underlying retinal cell death and identifying novel biomarkers for these diseases, which enable personalized medicine and improve the clinical translation of our findings, and establishing advanced 3D in vitro models of disease to gain deeper knowledge of ocular pathophysiology.

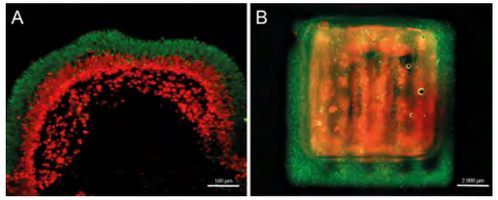


Figure 1. Retinal organoid generated from induced pluripotent stem cells (iPSCs) showing the expression of the OTX2 gene in green and PAX6 in red in two different retinal cell layers (A). Image showing a 3D bio-printed artificial retina containing two layers of human cell lines derived from retinal pigment epithelium (RPE) (red) and photoreceptors (green) (B). Bio-printed retinas were generated using a 3D-Bioplotter system (EnvisionTEC, Germany) at the TPM Tecnopolo Mario Veronesi, Mirandola, Modena, Italy. Retinal organoids and bio-printed retinas are useful models for assessing the safety and efficacy of gene therapy or therapeutic molecules.

Retinitis pigmentosa is a group of hereditary disorders characterized by progressive vision loss due to photoreceptor degeneration. AMD, the leading cause of blindness in the elderly, results from the deterioration of the central retina, impairing sharp vision required for tasks like reading and driving. Cataracts, in contrast, involve the clouding of the eye's lens, causing significant vision impairment and eventual blindness if untreated. Leveraging cutting-edge technologies and a strong commitment to translational research, we aim to prevent and treat vision loss, enhance patient quality of life, and advance ocular regenerative medicine.

Research Highlights

During the 2023-2024 period, our research has advanced significantly in the field of retinal neurodegeneration and advanced therapies. Key achievements include:

1) iPSC-Based Platforms: We optimized induced pluripotent stem cells (iPSC) differentiation protocols into retinal pigment epithelium (RPE) and retinal organoids (Fig. 1A), providing robust in vitro models for retinal diseases. Using CRISPR-Cas9, we also generated isogenic iPSC lines by correcting disease-causing mutations, and created reporter lines carrying fluorescent tags to isolate specific retinal cell types from 3D

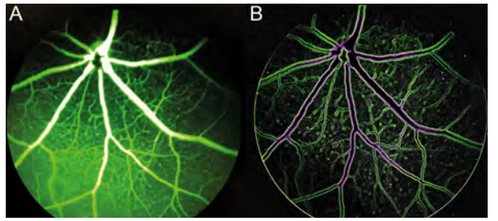


Figure 2. Images show the fundus of a C57BL/6J mouse 5 minutes after subcutaneous injection of fluorescein (A), and the reconstruction with an edge-enhancement filter to assess the distribution of retinal veins and capillaries (B). The images were acquired using a Micron III microscope (Phoenix Research Laboratories, Inc.), a high-resolution system with a CCD camera. This system is particularly useful for evaluating the expression of fluorescent proteins in gene therapy studies.represent 25 or 50 µm.

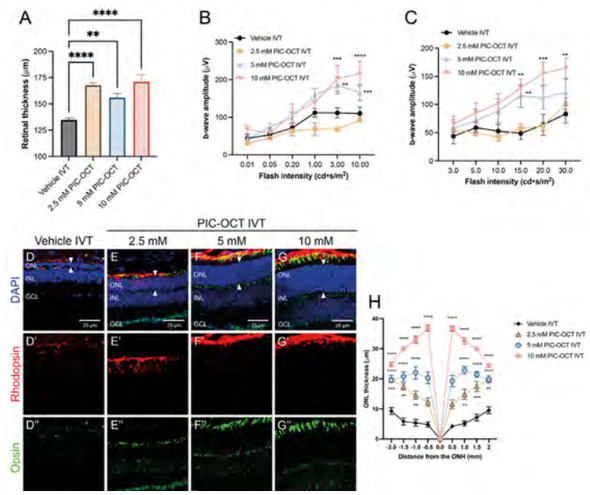


Figure 3. Effect of PIC-OCT treatment on rd10 mice. The rd10 retinitis pigmentosa mouse model was treated with intravitreal (IVT) injections of 2.5-, 5- and 10-mM PIC-OCT. Quantification of retinal thickness by optical coherence tomography (OCT) (A). Electroretinogram (ERG) quantification of b-wave amplitude under dark-adapted conditions (B) and light-adapted conditions (C). Immunostaining of mouse retinas (D–G") with anti-rhodopsin (red; D'–G') and anti-opsin (green; D"–G") antibodies. Quantification of outer nuclear layer (ONL) thickness (H). $^{**}p < 0.01$; $^{***}p < 0.001$; $^{****}p < 0.0001$. (Valdes et al., Pharmaceuticals 2024, 17, 1482).

cultures, refining both disease modeling and therapeutic strategies.

2) Regenerative Medicine for AMD: We engineered and tested scaffolds for RPE transplants aimed at treating AMD. These transplants are being preclinically evaluated and hold great promise for restoring vision in patients with advanced retinal damage.

3) Bioprinted Retinal Constructs: Significant progress was made in generating 3D bioprinted retinal patches (Fig. 1B), a breakthrough in regenerative strategies with the potential to enable personalized treatments for complex retinal pathologies.

4) Gene Therapy and Nanoparticle Delivery Systems: We tested innovative nanoparticles for delivering therapeutic genes targeting hereditary retinal diseases. Current studies focus on the genes CRB1, PRPF31, and PANK2, offering a promising approach to precision medicine. The efficiency and safety will be evaluated in mouse models (Fig. 2).

5) PIC-OCT, a Therapeutic Breakthrough: One of our major achievements was the discovery and characterization of Piceid Octanoate (PIC-OCT), a compound that protects retinal cells from oxidative stress by activating Sirtuin 1, a key regulator of cellular stress responses, and inhibiting parthanatos, a cell death pathway associated with retinal degeneration. This dual action significantly delayed photoreceptor degeneration in a retinitis pigmentosa mouse model (Fig. 3), highlighting its potential as a novel therapeutic agent.

6) Mechanisms Underlying EYS Mutations in Retinitis Pigmentosa: We explored the cellular mechanisms disrupted by EYS gene mutations, which are linked to retinitis pigmentosa. Our findings revealed that these mutations impair essential functions in RPE cells and photoreceptors, including ciliogenesis, autophagy, and epithelial-mesenchymal transition (EMT), providing valuable insights into the pathology and potential therapeutic targets of EYS-associated retinitis pigmentosa.
7) Biomarkers for AMD: By analyzing tear fluid, we identified vascular endothelial growth factor (VEGF) as a non-invasive biomarker capable of monitoring disease progression and assessing treatment efficacy in AMD. This innovation represents a significant advancement for personalized medicine.

8) Lens Opacity Prevention: We patented a new molecule that prevents lens opacity, offering a novel solution for treating cataracts and improving the management of this prevalent condition.

9) Development of evaluation systems for retinal models: In collaboration with the Engineer School of U. of Seville, we are preparing and testing a Lab-on-chip to measure the functionality of retinal explants and retinal organoids using microelectrode arrays to study models of human disease.

10) Collaborations and Spin-Off Activities: Our research benefits from strong collaborations with clinicians at Macarena Hospital's Ophthalmology Department and patient associations, ensuring clinical relevance. We also maintain active partnerships with Harvard University, the Karolinska Institute, the University of Seville, and the University of Modena, further enhancing the global impact of our work. Additionally, international collaborations include student exchanges with Okayama University (Japan) and the CPE Institute of Lyon (France). Finally, our spin-off company, LIMNOPHARMA, continues to bridge the gap between laboratory discoveries and therapeutic applications. These achievements underscore our commitment to pioneering therapies for retinal and ocular diseases, advancing translational research, and fostering innovation through collaboration.

Grants

- 2024-2026: PI23/00662. Instituto de Salud Carlos III (ISCIII).
- 2024-2026: CNS2023-144370 Agencia Estatal de Investigación (AEI).
- 2024-2025: DTS23/00023. Instituto de Salud Carlos III (ISCIII).
- 2023-2025: FUNDALUCE 2022.
- 2023-2025: PI-0044-2022.Consejería de Salud y Consumo.
- 2021-2023: ONCE.
- 2022-2023: DTS21/00086. Instituto de Salud Carlos III (ISCIII).
- 2021-2023: PI20/00043. Instituto de Salud Carlos III (ISCIII).

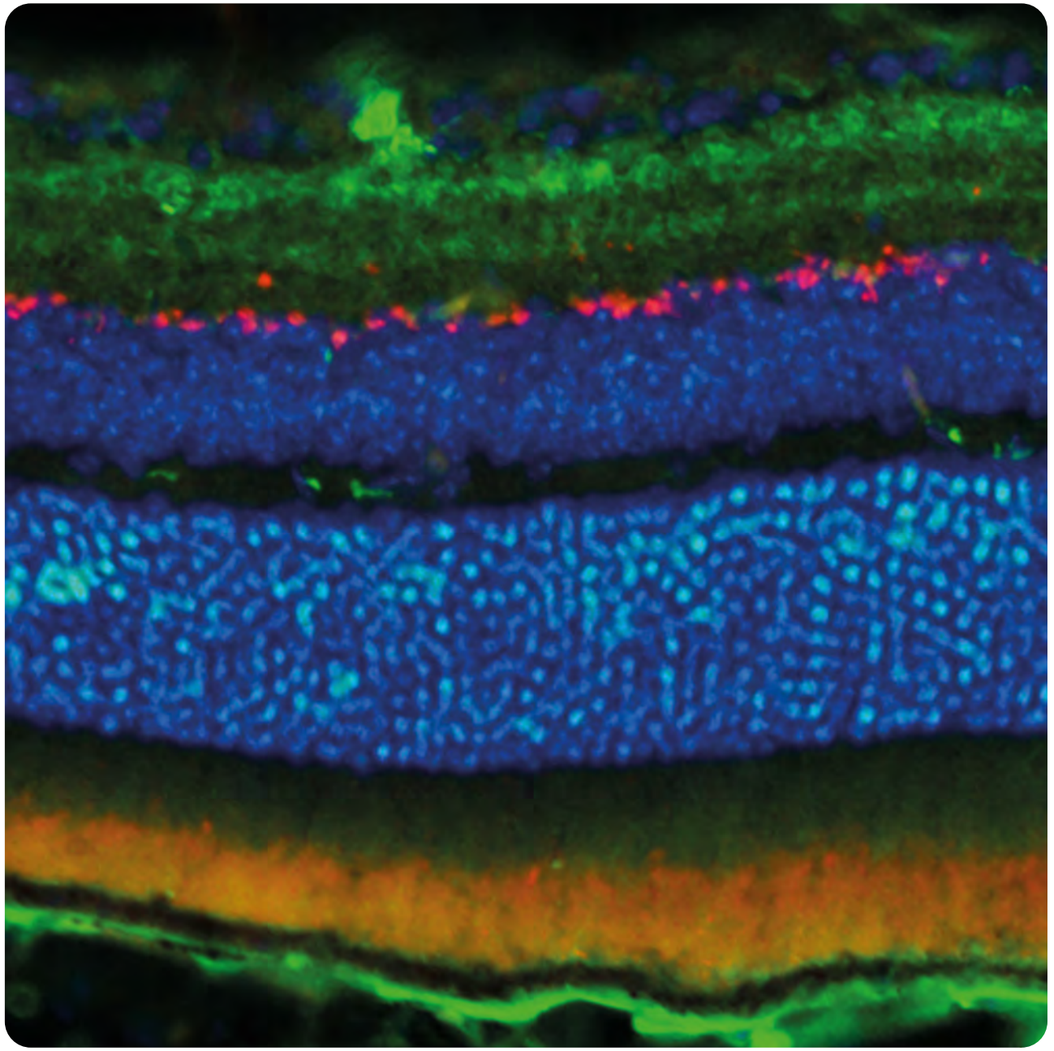
Integrative Pathophysiology and Therapies

Publication Highlights

Moshtaghion SM, Caballano-Infantes E, Plaza Reyes Á, Valdés-Sánchez L, Fernández PG, de la Cerda B, Riga MS, Álvarez-Dolado M, Peñalver P, Morales JC, Díaz-Corrales FJ. **2024.** Piceid Octanoate Protects Retinal Cells against Oxidative Damage by Regulating the Sirtuin 1/Poly-ADP-Ribose Polymerase 1 Axis In Vitro and in rd10 Mice. **Antioxidants** (Basel) .13(2):201.

Valdés-Sánchez L, Moshtaghion SM, Caballano-Infantes E, Peñalver P, Rodríguez-Ruiz R, González-Alfonso JL, Plou FJ, Desmet T, Morales JC, Díaz-Corrales FJ. **2024.** Synthesis and Evaluation of Glucosyl-, Acyl- and Silyl- Resveratrol Derivatives as Retinoprotective Agents: Piceid Octanoate Notably Delays Photoreceptor Degeneration in a Retinitis Pigmentosa Mouse Model. **Pharmaceuticals** (Basel). 17(11):1482.

Caballano Infantes E, Clauzon L, de la Cerda Haynes B, Díaz-Corrales F. **2024.** Generation of the human iPSC line ESi132-A from a patient with retinitis pigmentosa caused by a mutation in the PRPF31 gene. **Stem Cell Res.** 7;82:103623.



Principal Investigator

Dr. Vivian Capilla González

Stem Cells and Translational Neurology

Emerging Group Leader

Current position

- Staff Scientist/Miguel Servet Program. Fundación Pública Andaluza Progreso y Salud (FPS).
- Since 2025: Ramón y Cajal Program CSIC.

Group Members

Postdoctoral

- Dr. Jesús María Sierra Párraga.

PhD Students

- Laura Olmedo Moreno.
- Concepción Panadero Morón.

Technician

- Rubén Bueno Fernández.

Former Members (2023-2024)

- **PhD Student:** Caroline Stockwell Perea.
- **Technicians:** Carlos Pinto Perea, Maria Norma Adán Castro, Laura López Mangas, Carmen Burgos Cazorla, Laura Ruz Servián, Paula Juárez Blázquez, Carmen Baliña Sánchez.

Research Activity

Overview

Latest advances in diagnosis and treatments have improved survival rates of people suffering brain tumors. However, adverse effects of cancer therapies are still affecting the health of many patients that survive cancer. For this reason, researchers are focusing on the development of new strategies to minimize the sequelae of oncological treatments and to promote a healthy cancer-free life. In this context, cell-based therapy has emerged as a promising alternative in regenerative medicine.

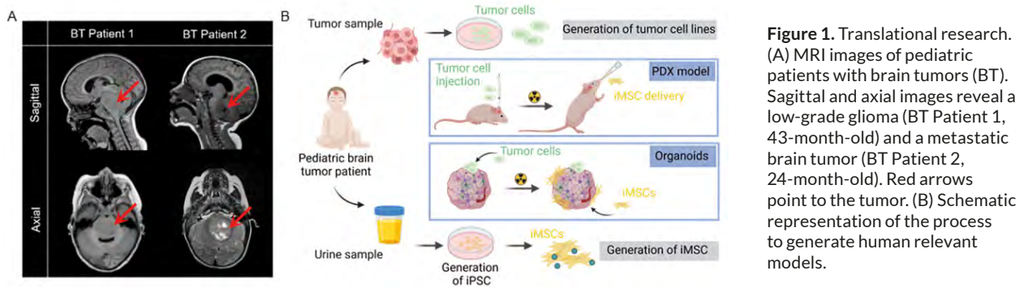


Figure 1. Translational research. (A) MRI images of pediatric patients with brain tumors (BT). Sagittal and axial images reveal a low-grade glioma (BT Patient 1, 43-month-old) and a metastatic brain tumor (BT Patient 2, 24-month-old). Red arrows point to the tumor. (B) Schematic representation of the process to generate human relevant models.

Our group brings over 15 years of experience in stem cell research. Currently, we are interested in investigating the neuroprotective effects of stem cells against radiation, but also the potential anti-cancer properties of cell-based therapies for brain tumors.

Research Highlights

Cancer continues to pose a significant global health challenge, with an estimated 19.3 million new cases and 10 million cancer deaths reported in 2020. Nevertheless, advances in early detection and treatment have led to an increasing number of cancer survivors. This shift has drawn growing attention to the long-term health and quality-of-life challenges faced by patients after cancer treatment.

Radiotherapy is among the most common cancer treatments, with approximately 50% of all patients receiving it at some point during their treatment. Despite its effectiveness, this therapy often leads to both short- and long-term side effects that can significantly impact patients' quality of life. Radiation exposure can cause progressive and sometimes irreversible functional impairments, including difficulties with learning and memory, reduced processing speed, attention deficits, and other cognitive challenges. These effects are particularly pronounced in pediatric patients, whose developing systems are more vulnerable, emphasizing the urgent need for innovative strategies to mitigate these adverse outcomes while ensuring treatment efficacy.

Our scientific program investigates Advanced Therapies to improve cancer treatments with the ultimate goal of enhancing the quality of life for cancer patients. We have been pioneers in demonstrating the therapeutic potential of intranasally delivered mesenchymal stem cells (MSCs) to mitigate radiation-induced brain damage. In a first work, we described that MSC treatment improved motor coordination, odor discrimination ability, and cognitive performance in whole-brain irradiated mice. In addition, we made significant progress in

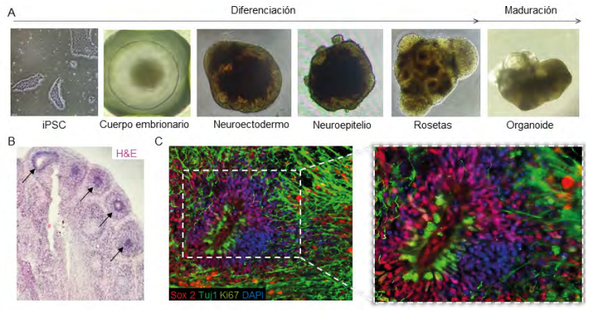


Figure 2. iPSC research. (A) Process of cerebral organoid development from patient-derived iPSC. (B) Hematoxylin and Eosin staining of a mature cerebral organoid showing complex morphology with heterogeneous regions (arrows). (C) Immunofluorescence images of a mature cerebral organoid showing expression of the neuronal progenitor markers SOX2, the proliferation marker Ki67, and the immature neuron marker Tuj1.

understanding the mechanisms underlying the therapeutic effects of MSCs. Our findings indicated that MSC administration reduces neuroinflammation, protects against oxidative stress, and prevents neural cell loss in irradiated brains, conferring a protective effect that prevent neurological decline after radiation. Omic analyses identified pathways and molecules involved in neuroregeneration and recovery that were modulated by MSCs in the irradiated brain, including the damage-induced c-AMP response element-binding (CREB) signaling. In addition to demonstrating their efficacy, we also provided evidences of the safety of MSC intranasal delivery in juvenile mice, showing that this strategy is a promising non-invasive therapy for treating radiation-induced brain injuries.

Building on these findings, we are now focusing on translational research to refine and expand this therapeutic approach. Using patient-derived models, including induced pluripotent stem cells (iPSCs), brain organoids, and PDX mice, we aim to deepen our understanding of how MSCs act in human relevant models. Importantly, we are investigating how MSCs interact not only with the damaged brain tissues, but with cancer cells. This information will allow us to explore the dual functionality of MSCs as both neuroprotective agents and modulators of the tumor microenvironment. By dissecting the molecular interactions between MSCs and tumor cells, we aim to harness their oncostatic properties to develop a bifunctional therapy that not only prevents radiation-induced side effects but also suppresses tumor progression. Our research integrates advanced techniques such as transcriptomics and proteomics to gain a deeper understanding of these processes and optimize MSC-based therapies. These advances are paving the way for personalized, non-invasive therapeutic strategies to improve cancer treatments outcomes.

Grants

- 2024-2026: CPP2023-010925. Ministerio de Ciencia, Innovación y Universidades
- 2024-2026: PI23/00315. Instituto de Salud Carlos III
- 2023-2024: RED2022-134081-T. Ministerio Ciencia e Innovación.
- 2022-2024: 2022/02876-0. Fundação de Amparo à Pesquisa do Estado de São Paulo.
- 2022-2023: PY20/00481. PAIDI. Junta de Andalucía.
- 2021-2023: PI20/00341. Instituto de Salud Carlos III
- 2020-2024: CP19/00046. Instituto de Salud Carlos III.
- 2020-active: Asociación Pablo Ugarte (Proyecto +VIDA).

Publication Highlights

Oliveira GP, Limaa MA, Pereira G, (...) Rocha FV. **2024**. Palladium(II) and Platinum(II) Thiophene-Based Thiosemicarbazones: Synthesis, Properties, and Anticancer Studies. **Journal of Molecular Structure**. 1322;1,2025;140306.

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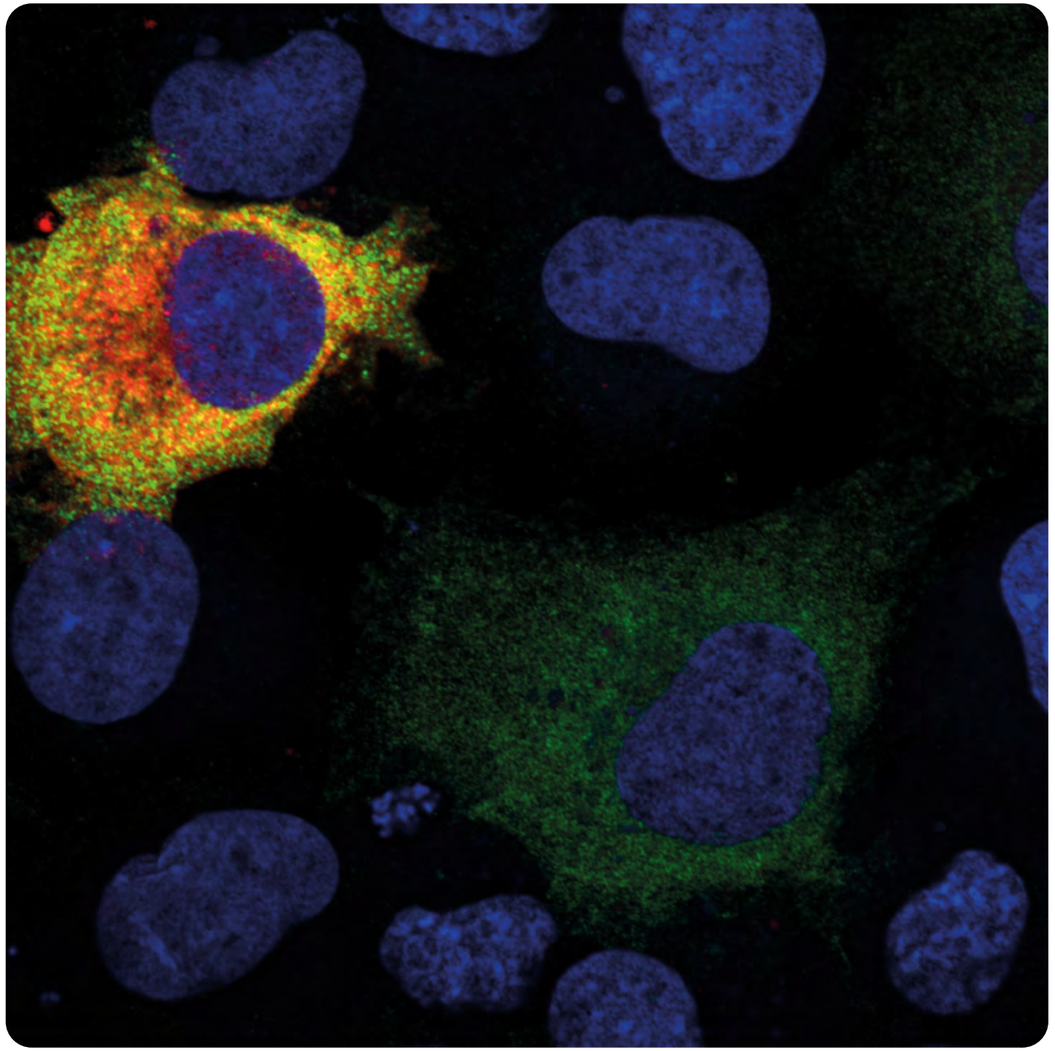
Scientific Core Services

SCIENTIFIC CORE SERVICES

- Genomics
- Biological Resources
- Microscopy
- Bioinformatics
- Citometry and Sorter
- Cell Culture
- Model Organism
- Histology
- Washing and Sterilization
- Biological Safety

MANAGEMENT UNITS



Pilar Cebolla
Manager

Cabimer in Numbers

It is with satisfaction that I present the Manager's Report for the 2023-2024 scientific period at CABIMER. As the Manager, my primary responsibility is to ensure that our scientific community receives the necessary support to focus on research and innovation while minimizing administrative or operational distractions.

The successful management of an annual budget of approximately 3.5 million euros, supported by partnerships with the Spanish National Research Council (CSIC), the University of Seville, the University "Pablo de Olavide," the Andalusian Ministry of Health and Consumer Affairs, and the Ministry of University, Research, and Innovation, has allowed us to enhance our core services. Additionally, revenue from our advanced Genomic platform has contributed to covering operational costs, including technical services, IT support, security, and maintenance.

In terms of research project funding, by the end of 2024, we were managing 120 projects with a total volume of €19.8 million, including prestigious grants such as an ERC Starting Grant and an AXA Research Grant.

By the end of the period, we have obtained €3.2 million to finance new equipment, that will enhace our scientific capabilities of the image system and allow for the establishment of the Proteomics unit at CABIMER.

We have successfully increased the participation of sponsoring companies in events, seminars, and the III CABIMER International Workshop. We are also very proud that, after a search for sponsors to establish the CABIMER Award for the work of young researchers, we secured the support of the Biomol Foundation. Thanks to this collaboration, two editions of the award have been held in 2023 and 2024.

Our commitment to excellence is reflected in the dedication of our technical and general services staff, who have successfully navigated rising costs and increased administrative complexity while maintaining high-quality support for research, as well as our unwavering dedication to transparency and accountability.

In 2024, the Andalusian Health Institute Act was enacted, leading to the eventual integration of personnel and researchers from the Fundación Pública Andaluza Progreso y Salud. Consequently, in November 2024, the change in CABIMER's managing entity was approved. Starting in 2025, management will be overseen by the "Fundación Andaluza para la Gestión de la Investigación en Salud de Sevilla" (FISEVI), ensuring a smooth transition and continuity in providing solid support in the effective management of research.

We have also continued our communication efforts, reaching over 1,000 visitors, including high school students, patient associations, and institutional representatives, through guided tours and participation in 86 outreach events and conferences.

With 208 personnel, including 29 Group Leaders and 11 senior researchers by the end of 2024, CABIMER continues to be a hub for young talent and gender equality in science. Despite progress in increasing female representation among group leaders to 31%, there is still work to be done in this area.

Looking ahead, our goals include achieving recognition as a National Excellence Research Centre, increasing private funding, enhancing collaboration with companies and clinicians and implementing the services of the new and advanced proteomics unit to support both internal and external users.

I would like to close extending my sincere gratitude to the Director, Vice-Director, and all scientific, technical, and support staff for their dedication and hard work, which have been instrumental in achieving CABIMER's objectives and advancing scientific research.

Genomics

Scientific Coordinator

- Dr. Cristina González-Aguilera.

Technicians

- Dr. Eloísa Andúja.
- Dr. María Ceballos Chávez.
- Dr. Mónica Pérez.
- Dr. Dolores Pérez de Camino.
- Victoria Jiménez.

Since its establishment in 2007, the CABIMER Genomics Core Facility has been dedicated to supporting researchers both within and outside the institute by providing cutting-edge resources and services tailored to high-throughput functional genomics. Next-generation sequencing (NGS) and microarray technologies have evolved to become critical tools for comprehensive studies in transcriptomics, epigenetics, and genomics.

The Core Facility works with three Illumina-based NGS platforms: iSeq100, NextSeq500, and NOVASeq6000. Adding these sequencers is the 10x Genomics Chromium Controller, a microfluidics system designed for single-cell partitioning and barcoding, enabling advanced single-cell analyses. Recently, the incorporation of the BD Rhapsody™ HT Xpress system (Becton Dickinson Biosciences) has further expanded the facility's single-cell capabilities through its innovative cell capture and barcoding technology.

Standardized protocols developed by the Core Facility include a broad range of applications, such as whole-genome sequencing, ChIP-Seq, DRIP-Seq, MNase-Seq, RNA-Seq, scRNA-Seq, and scATAC-Seq, among others. These protocols are validated to a variety of eukaryotic species and research objectives. In addition, the facility offers expert guidance in experimental design and data analysis to ensure robust and reproducible outcomes.

The ability to process large sample volumes with precision and reproducibility allows researchers to quicken their studies, whether targeting extensive gene sets or specific genomic elements. This capability stems from the integration of high-performance technologies and the expertise of a skilled professional team. By continuously optimizing its infrastructure, the Core Facility ensures that it meets the demands of cutting-edge research.

CABIMER's Genomics Core Facility has become an indispensable asset, not only for in-house researchers but also for academic institutions, research centers and hospitals across Andalusia and Spain. Its services have been essential in advancing scientific studies, particularly in healthcare-related fields. Recognized as a leading public platform for genome-wide sequencing, the facility plays a key role in driving innovation and discovery. It takes immense pride in being a trusted partner in the research journeys of numerous laboratories and institutions.

Bioinformatics

Scientific Coordinator

- Dr. Daniel Rico.

Technician

- Dr. Eugenia Soler.

The Bioinformatics Unit at CABIMER, operational since June 2023, has made key strides in optimizing resources and enhancing research quality.

The Unit manages the Genomics Unit's computational server, eliminating the need for costly licenses like Illumina BaseSpace and ensuring local data storage for improved security. It also manages the CABIMER HPC cluster, using Docker technology to ensure reproducibility and portability of analysis workflows. Training is provided to users on Docker and the SLURM job scheduling system.

Additionally, the Unit has expanded the center's capabilities through exploratory analysis of emerging technologies, such as meRIP-seq, chrRNA-seq, and long-read DNA replication sequencing. It also offers bioinformatics training and promotes collaboration among research groups.

Scientific Core Services: Biological Resources

Biological Resources

Scientific Coordinator

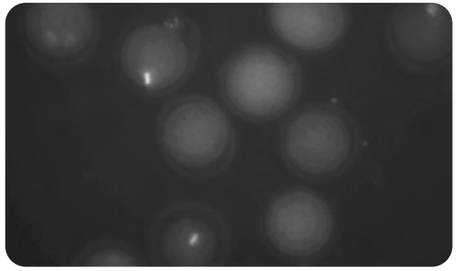
- Dr. Luis Sánchez Palazón.

Veterinarian

- Dr. Itziar Benito Latasa de Aranibar.

Technicians

- María Gálvez Jiménez (until September 2024) .
- Miriam González Fernández.
- Joaquín González Magro (from November 2024).
- Flora Guerrero Iglesias (until January 2024).
- María Reina Manuel (until December 2024).
- Noemí Solis Palomo (from June 2023).
- Rosario Segarra Bermúdez.
- Gabriela Vázquez Salas (from October 2024).



Enucleation of mouse oocytes

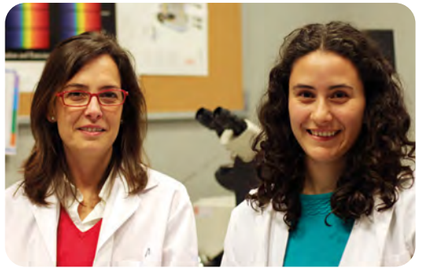
Researchers in Cabimer are using mouse models in a variety of ways, from basic research into disease mechanisms to translational research. The Biological Resources Unit enables animal experimentation in Cabimer providing the necessary resources under conditions required by national and EU legislation (Spanish RD 53/2013 and EU Directive 2010/63) for the protection of animals used for scientific purposes. The mission is to provide for the care, health and well-being of animals as well as to provide specialized techniques and equipment for research.

The unit has capacity for some 6000 mice maintained in Specific Pathogen Free (SPF) condition, a health status monitored through a comprehensive health surveillance programme. Animal biosafety level 1 and 2 are available in our facility. The number of research groups using the animal facility has increased during 2023-2024 and we are now operating close to full capacity. Providing a sufficient number of well-trained and committed staff is a very important factor ensuring high standards of animal care.

Laboratory space and equipment is available for in vivo imaging, metabolic monitoring, inhalational anesthetic behavioural test, stereotaxic surgery, electroretinography, optical coherence tomography and transgenesis, including microinjection of DNA into zygotes and rederivation of transgenic lines by embryo transfer.

Scientific Core Services: Microscopy

Microscopy



Scientific Coordinator

- Dr. Pablo Huertas Sánchez.

Technicians

- Dr. Paloma Domínguez Giménez.
- Dr. Clara B. García Calderón.

The Microscopy Facility at CABIMER provides technical support to both CABIMER and external scientists, helping them at every step of the way from sample preparation to data interpretation in all types of Microscopy approaches with fixed or live biological samples. The Microscopy Unit is distinguished by its versatility, stemming from the diversity of biological models employed at the institute. This flexibility enables the unit to adapt to new challenges and develop creative strategies. A non comprehensive list of the equipment can be found at http://www.cabimer.es/web3/unidades-apoyo/microscopia/#equipamiento.

The unit makes a constant effort to increase its portfolio of equipment and applications. Overall, during this period we have enhanced our facility's image acquisition capabilities and analysis, for both live and fixed samples. These upgrades facilitated by the latest advancements in software and camera technology, has led to significant improvements in image quality and resolution. Furthermore, we have streamlined our analysis workflow, enabling researchers to undertake more in-depth data analysis, perform automatic segmentation, detect objects, add annotations, track object motion, generate volume renderings and utilize AI tools. The result is analysis-ready for presentation and publication, contributing to our commitment to excellence in research and innovation.

For the last period, the facility has been able to maintain its staff resources by sharing a member of staff with the Cytometry Facility and the Microscopy Facility. This arrangement has enabled both facilities to respond more quickly to our researchers' queries and to maintain greater control over all microscopes, as well as facilitating the resolution of incidents.

Cytometry and Sorter

Scientific Coordinator

- Dr. Vivian Capilla-González .

Technicians

- Dr. María José Quintero Carrasco.
- Dr. Clara B. García Calderón.

Flow cytometry is a powerful tool that measures functional and structural characteristics of heterogeneous mixtures of cells. Measurements are performed in liquid samples of single cells, which flow one at a time through a stream focused to a laser beam at rates up to several thousand particles per second. Both scattered light and fluorescence emitted by the cells are collected, filtered, digitized and sent to a computer for analysis. The main applications of flow cytometry include immunophenotyping, cell cycle progression, cell death and protein expression, among many others. Through the cell sorting technology, flow cytometry also allows the physical isolation of distinct populations of cells of interest for further downstream applications, including cell culture, RNA or protein analysis and single cell cloning.

The Flow Cytometry Core Facility of CABIMER is equipped with two BD FACSCalibur Analyzers to perform routine flow cytometry measurements and one BD LSRFortessa X-20 instrument with four laser beams to cover advanced multi-color flow cytometry experiments. This last equipment allows the simultaneous measurement of up to 16 fluorescence parameters, in addition to the analysis of cell size and complexity. It also includes a loader to automate sample acquisition in 96- and 384-well plates. In addition, the Facility has also a BD FACSAriaIIIu Cell Sorter that provides researchers the capability to analyze and sort cells by differences in physiology, metabolism, morphology and other characteristics. This sorter can separate up to 4 defined populations simultaneously, as well as perform single cell cloning and index sorting. Importantly, the Facility provides offline data analysis software to help researchers perform analysis and representation of their data quickly and effectively. In addition, three FlowJo and one FCS Express software licenses are available for the users to improve final interpretation of the results generated in the unit.

The specialized professionals of the Flow Cytometry Core Facility are responsible for the training and advice of internal and external researchers to develop efficient and reliable flow cytometry assays. The main goal of the Facility is to offer the highest quality services to advance fundamental and applied science.

Cell Culture

Scientific Coordinator

- Dr. Andrés J. López-Contreras.

Technicians

- Dr. M. Mercedes Dana Jiménez.

The Cell Culture Core Facility at CABIMER consists of multiple specialized areas dedicated to primary and cell line cultures. Seven rooms are allocated to established cell lines, one room to non-human primary cultures, one biosafety level II room for infecting cells with viruses, and one room to carry out human primary cultures.

The Facility supports researchers by facilitating access and training to essential equipment, and providing key reagents for cell culture, including serum, trypsin, antibiotics, glutamine, and PBS.

The Facility is equipped with numerous normoxic and hypoxic incubators, safety cabinets, centrifuges, electroporation systems and microscopes. As more specialized equipment, the Cell Culture contains a cell analyzer xCELLigence® RTCA DP to quantify cell proliferation and morphology changes in a real-time manner, an ultracentrifuge for isolation of viral vectors and three automated cell counters CellDrop™ DeNovix (these instruments enable the fastest cell counts, viability assessment, and GFP transfection efficiency measurements across the widest range of cell density, cell type and application).

Recently, our infrastructure has been improved with the acquisition of two new cryopreservation freezers Custom BioGenic Systems V1500-AB, with patented jacketed technology, which offer safe dry and isothermal storage for samples without liquid nitrogen contact; a new transfection system Invitrogen™ Neon™, which offers an innovative electroporation method that generates a more uniform electric field for a significant increase in transfection efficiency and cell viability; an ultra-low temperature (ULT) freezer Eppendorf CryoCube F101h (for storing viral samples) and two new TELSTAR Biological Safety Cabinets Class II.

The Facility makes continuous efforts to accommodate the growing number of users by optimizing and redistributing available space or incorporating new areas to meet evolving research needs.

Model Organism

Scientific Coordinator

- Dr. Tatiana García Muse.

Technician

- Cristina Hernández.

Scientific Core Services: Model Organism

Scientific CABIMER's objectives encompass both the advance in the knowledge of the molecular mechanisms responsible for genetic disorders and cancer and the development of new cellular therapies to address efficiently these diseases. Consistent with these general aims, CABIMER offers a large number of facilities to develop high-quality research based on cell lines and mice. Additionally, CABIMER's research requires the use of different model organisms at two levels:

a. *Organisms used as general research tools* (required for most research groups). They include the bacteria *Escherichia coli*, which is required for genetic engineering, ectopic expression of recombinant proteins for purification, and in vivo assays of gene expression.
b. *Organisms used as living models* by specific research groups to understand the molecular causes of genetic instability and defects in cell cycle progression as two major features of cancer and many genetic disorders. These organisms include the yeast *Saccharomyces cerevisiae* and the nematode *Caenorhabditis elegans*.

The main objective of this Service is to provide specific facilities for convenient research with these model organisms. More specifically, this Service is aimed at:

1. Organization, maintenance and handling of specific cell collections.
2. Preparation of specific and general solutions and buffers.
3. Preparation of media for the growth of different model organisms.
4. Growth and collection of high volumes of cell cultures for protein purifications.
5. Preparation of competent cells for transformation and electroporation.

Histology

Scientific Coordinator

- Dr. Anabel Rojas González.

Technician

- Dr. Daniel Rodríguez Martínez.

Scientific Core Services: Histology

Histology, as a branch of the morphological sciences, is a very relevant discipline that allows to understand the shape and structure of tissues, and the characterization of abnormalities at the cellular level. In order to meet the demands of researchers, CABIMER has built a highly specialized histology service, which includes tumor tissue characterisation, embryo histology, and animal pathology. The samples gathered for analysis are handled with the highest quality standards and cutting-edge technology, allowing us to provide a full range of histology services to our research community as well as the neighboring academic and private sectors.

The Histology Core Facility was created in May 2010 as an internal service and since then it has observed an important increment in the demand of the offered services. In last years, we have extended our techniques to different species, including invertebrates, becoming an important support for other academic and research institutions.

The Histology facility provides guidance, protocols and equipment allowing fixation techniques, intracardiac perfusion, sectioning of tissues and classical staining for easy viewing of samples. Specific protocols will be provided on demand and upon availability.

The Histology unit offers methods for the histological analysis of human and animal biological samples. Some of the procedures accessible in this service include the preparation of paraffin embedded samples in the automatic processor of tissue, which streamlines the work of the researches in terms of samples manipulation and protocol time. Histological slices of paraffin blocks and frozen tissues can be generated using an automated microtome and a cryostat, respectively. A vibratome is used for floating samples. Histology Core Facility also provides Tissues Microarrays (TMA), allowing researchers to investigate a large number of tissue samples integrated on a single histologic slide.

The Histology core Facility offers the complete processing of organoids from their inclusion in Histogel™ before inclusion in paraffin for microtome sections, or cryopreservation of organoids before inclusion in OCT for frozen sections in a cryostat. The Histology Core also offers staining techniques for various cellular structures. Performing different sample stains, including hematoxylin and eosin, Masson trichrome, Nissls/Cresyl Violet

Lab Material and Sterilization Unit

Scientific Coordinator

- Prof. Ralf Erik Wellinger.

Technicians

- Mª Jose Figueroa.
- Mª Dolores Carrión.

The Lab Material and Sterilization Unit is a basic and fundamental support service that serves all research units of CABIMER. This Unit is responsible for the collection, processing, washing, sterilization and distribution of all the laboratory material as well as the sterilization of growth medium and stock solutions (glassware, plastic and consumables). Special trained personnel handle the processing of the biological waste generated by the research groups as well as by other support units, meeting all safety regulations for Biohazaraus material.

To carry out this work, the Unit is in continuous contact with the different research groups and associated support units, to offer them an optimal service and to rapidly adapt to newly arising demands.

Due to the incorporation of new and the expansion of existing research groups as well as the generation of new services, the Unit was forced to adapt and to provide a more personalized service mainly focusing on the needs of each research group. Accordingly, the demand for glassware, plastic material and consumables increased by more than 50%, since each research group works with different types of materials that must be adequately processed. This adaptation required that the equipment of the Unit (autoclaves, thermo-disinfector, etc.) now operates full time to provide maximal service.

To ensure utmost quality of the Sterilization Unit, all management and working procedures undergo regular controls and are executed in accordance with standards outlined in bio-safety regulations.

Biological Safety

Scientific Coordinator

- Dr. José Carlos Reyes.

Technician

- Juan Carlos Ostos.

The Biosafety service provides guidance and advice on all aspects of biological safety at CABIMER, including protection against biological agents, chemicals and radiations. CABIMER is authorized to work with non-encapsulated as well as with encapsulated radioactivity sources and have two different radioisotopes laboratories equipped with all required means of shielding, containment and detection of ionizing radiation. At the disposal of authorized users there is also a biological irradiator BioBeam 8000 that allows the study, among other applications, of the repair of genetic damage in different experimental models. The Service manages, together with the Cell Culture Unit, two Biosafety level 2 laboratories (BSL2) equipped to work with biological agents of level 2 such as lentiviral or retroviral vectors. The proper management of biosanitary, toxic and radioactive waste generated in a research center like CABIMER is considered a cornerstone in risks prevention. Improvements in working protocols with chemical or biological agents, information on the risks of each scientific activity, and increase the level to training to researchers, are the main goals of the unit in the last few years. In this context, the continuous incorporation of researcher groups, has led to an increase in management and waste generation until reach a production of 31 Tm in 2023-2024.

Management Units

Manager
- Pilar Cebolla

Executive Assistant
- Berta Ferrer

Human Resources
- Irene González

Labor Risk Prevention
- Juan Carlos Ostos

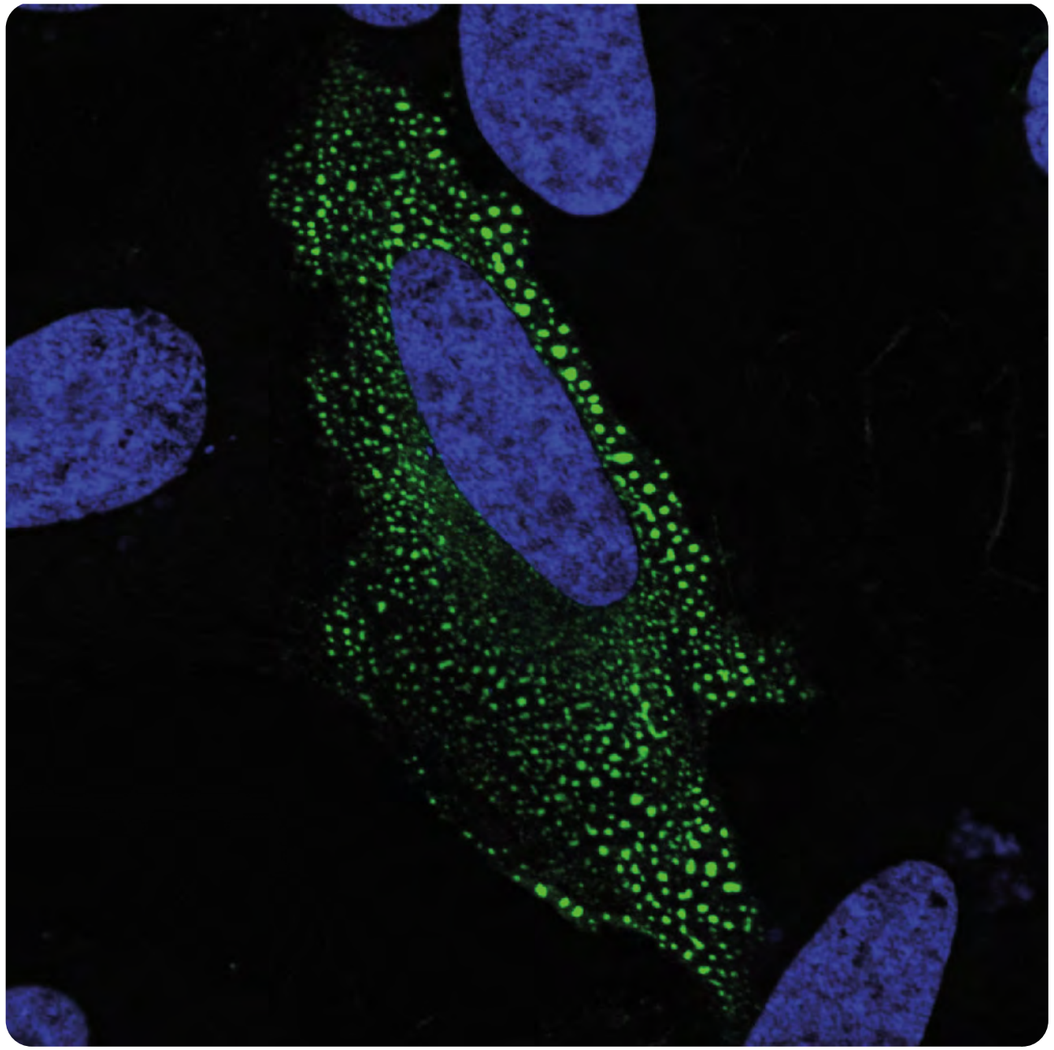
Project and economic Management
- Carmen Ramos
- Inmaculada Uclés
- Marta Sahuquillo
- Esperanza Muñoz

Purchasing and supplies
- Francisco J. Dorantes
- María Isabel Tovaruela
- Jennifer Chiguano
- Lucía Díaz
- Lorena Espinar

Communication and Diffusion
- Jose Carlos Arroyo

IT Service
- Arturo Fernández
- Antonio Manuel Eslava

Maintenance
- Rafael León



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Communication and Diffusion: Book Chapter and Patent

Book Chapter

2024

- Altea-Manzano P, Fendt SM, Vera-Ramirez L. ^{13}C Tracer Analysis and Metabolomics in Dormant Cancer Cells. **Methods Mol Biol.** 2811:195-206

Patent

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Doctoral Theses

2023

Cristina Guillén Mendoza
Insights into RNA-mediated genoma instability by altering aly expresión and the use of new drug" Thesis Supervisors: Prof. Andrés Aguilera López and Dra. Rosa Mª Luna Varo. Universidad de Sevilla

Andrea Móo Bajo
"Bases moleculares del síndrome de aicardi-goutières" Thesis Supervisors: Dr. Pablo Huertas Sánchez and Dr. Sonia Jimeno González. Universidad de Sevilla

Mª Eugenia Martín-Vázquez García
Targeting organogénesis anf Beta Cell survival: role of the LRH1/NR5A2-PTGS2/COX2 signalling axis in ppancreatic islet physiology and pathophysiology". Thesis Supervisors: Dr. Benoit Gauthier and Dr. Anabel Rojas. Universidad Pablo de Olavide

Laura Basurto Cayuela
Inhibición de la actividad ATPasa del complejo SWI/SNF: cambios cromatínicos y de la expresión génica. Thesis Supervisor: Dr. Jose Carlos Reyes and Dr. Mario García-Domínguez. Universidad de Sevilla

Rosa Camarillo Daza
Non-canonical DNA structures in Double Strand Break repair. Thesis Supervisors: Dr. Pablo Huertas and Dr. Sonia Jimeno. Universidad de Sevilla

Alejandra Álvarez
"Análisis de la posible asociación específica de moléculas de ARN a los centros organizadores de microtúbulos del huso en Saccharomyces cerevisiae ". Thesis supervisor: Dr. Fernando Monje Casas. Universidad de Sevilla

Guillermo Rodríguez Real
"Unraveling the connection between centrosomes and the DNA damage response". Thesis supervisors: Dr. Pablo Huertas Sánchez and Dr. Fernando Romero Balestra

2024

Elena Gómez Marín
"Caracterización Molecular y Funciona del complejo PHF14HMG20A". Thesis supervisor: Dr. Jose Carlos Reyes. Universidad Pablo de Olavide

Cristina González Garrido
"Efecto sobre la Recombinación Homóloga de la distribución de las histonas parentales y la localización de los obstáculos de replicación en las cadenas nacientes" . Thesis supervisor: Dr. Félix Prado Velasco. Universidad de Sevilla

Jose Javier marqueta García
"DNA break prevention and repair: role of RNA factors and histone deacetylases". Thesis supervisors: Prof. Dr. Andrés Aguilera López and Dr. Belén Gómez González. Universidad de Sevilla

Federica Bruno
"Effect of Chromatin Replication on RNA Polymerase II Activity and RNA synthesis". Thesis supervisor: Dr. Cristina González Aguilera. Universidad de Sevilla

Jesús Ángel Pérez Cabello
Mecanismos modulatorios de MOK en la regulación de la respuesta inflamatoria de la microglía en ELA". Thesis supervisors: Dr. Cintia Roodveldt and Dr. David Pozo Pérez. Universidad de Sevilla

María Eugenia Soler Oliva
Role of Fact and other chromatin-related in the origino of genetic instability". Thesis surpervisors: Prof. Dr. Aguilera López and Dr. Helene Gaillard. Universidad de Sevilla

Seminar Speakers

2023

January 2023

"Modeling liver fibrosis with induced pluripotent stem cells (iPSC)" January 20th. Pau Sancho Bru. IDIBAPS, Barcelona, Spain

"Dual functional role of Gasdermin B in breast cancer" January 27th. Gema Moreno Bueno. IIB, Madrid, Spain

February 2023

"Targeted protein degradation: a novel paradigm in drug development" February 03rd. Cristina Mayor-Ruiz. Institute for Research in Biomedicine-IRB, Barcelona, Spain

"How does nucleolar stress lead to ageing in mammals? Undestanding neurogeneration from a ribosome perspective" February 17th. Oskar Fernández Capetillo.CNIO, Madrid, Spain

"SNAREopathies and STXBP1 syndrome disease mechanisms" June 24th. Matthijs Verhage. CNCR - Vrije Universiteit Amsterdam, Amsterdam, Netherlands

March 2023

"SLX4: playing with nucleases, helicases and beyond" March 03rd. Pierre Henri Gaillard CRCM - Marseille, Marseille, FrancePierre Henri Gaillard. CRCM – Marseille, France

"Replication fork remodelling in cancer and stem cells" March 10th. Massimo Lopes. ETH Zurich – CH

"Controlling nucleases and helicases that determine our genetic make-up" March 17th. Joao Matos. Max Perutz Labs, Vienna, Austria

"Unravelling the genetic pathways underlying ageing in mice" March 24th.Colin Selman. Institute of Biodiversity, Animal Health and Comparative Medicine, University of Glasgow, Glasgow, United Kingdom

April 2023

"Advancing knowledge in the biology of the oval cell and its role in liver pathology". April 14th.Angela Martinez Valverde. Instituto de Investigaciones Biomédicas Alberto Sols

May 2023

"SUMO rules!" May 5th. Andrea Pichler. Max Planck Institute of Immunobiology and Epigenetics, Freiburg, Germany

"New non-canonical functions of GPCR-related signalling nodes" May 12th. Cati Ribas. CBMSO, Madrid, Spain

"Exploring the role of Polycomb and MLL complexes on chromatin topology, stem cell differentiation, and cancer" May 19th. Luciano di Croce. Centre for Genomic Regulation (CRG)-ICREA, Barcelona, Spain

"Causes and Consequences of Replication Stress" May 29th. Karlene Cimprich. Stanford University, Stanford, USA

June 2023

"New mechanisms and models in ALS" June 2nd. Abraham Acevedo. Hospital Universitario de Canarias, Tenerife, Spain

"Molecular mechanisms in transcription-coupled DNA repair" June 16th. Martijn Luijsterburg. Leiden University Medical Center, Leiden, Netherlands

"The importance of Post-translational control in liver disease" June 30th. Malu Martínez-Chantar. CICbioGUNE, Bilbao, Spain

July 2023

"Dual functional role of Gasdermin B in breast cancer" July 7th. Gema Moreno Bueno. IIBM, Madrid, Spain

October 2023

"Blocking DNA synthesis at origins and at forks: insights into CDC7 kinase targeting drugs using functional genomics approaches" October 6th. Corrado Santocanale. Centre for Chromosome Biology, School of Natural Sciences, National University of Ireland Galway, Galway, Ireland

"Causes and Consequences of Replication Stress" October 19th. Karlene Cimprich. Stanford University, Stanford, USA

"Disease-associated nuclease-helicase DNA2 is an essential gatekeeper to recombination at stalled DNA replication forks from yeast to human" October 27th. Uli Rass. Genome Damage and Stability Centre. CRUK. University of Sussex,Sussex, UK

November 2023

"Avoiding defects in embryonic development by repairing DNA?" November 10th. Gerry Crossan. MRC Laboratory of Molecular Biology, Cambridge UK

"Metabolic supramolecular drugs for modulating inflammation" November 24th. Giuseppe Battaglia. ICREA, Barcelona,Spain

December 2023

"Epigenomic Control of Macrophage-Driven Inflammation" December 1st. Inez Rogatsky. Hospital for Special Surgery & Weill Cornell Medicine, New York, USA

2024

January 2024

"Drug-repurposing for mitochondrial metabolism inhibition in leukaemia" January 19th. Vignir Helgason. Wolfson Wohl Cancer Research Centre, School of Cancer Sciences, University of Glasgow, Glasgow, UK

"Deciphering the 4D epigenomic landscape to understand normal and malignant haematopoiese" January 26th. Biola M Javierre. Josep Carreras Leukaemia Research Institute, Barcelona, Spain

February 2024

"The emotional circuitry affected in Parkinson's disease: Role of serotonin system"February 9th. Analía Borltolozzi. Instituto de Investigaciones Biomédicas de Barcelona (IibB), Barcelona, Spain

"Mechanisms of neuronal diversification and evolution" February 16th. Nuria Flames. Instituto de Biomedicina de Valencia (IBV-CSIC), Valencia, Spain

March 2024

"Transcriptional control of adipogenesis-regulating the master switch" March 1st. Susanne Mandrup. University of Southern Denmark, Denmark

"Role of the ubiquitination system in genome stability" March 8th. Lorenza Penengo. , Institute of Molecular Cancer Research, University of Zurich, Zurich, Switzerland

"Cold temperature delays aging and proteostasis collapse" March 15th. David Vilchez. Cologne Excellence Cluster for Cellular Stress Responses in Aging-Associated Diseases (CECAD), University of Cologne, Cologne, Germany

April 2024

"What do Chromatin Modifications do? Epigenome Editing to dissect Context-dependent Function." April 26th. Jamie Hackett. EMBL Rome, Rome, Italy

May 2024

"TDP-43 roles in axon guidance and its sefulness as biomarker." May 17th. Emanuele Buratti. International Centre for Genetic Engineering and Biotechnology (ICGEB) Trieste, Italy

June 2024

"Microtubules as mediators of stress responses and repair." June 7th. Susana Godinho. Barts Cancer Institute, Centre for Cancer cell and Molecular Biology. CRUK. Queen Mary, University of London, UK

"Uncovering Small Ubiquitin-like Modifier signal transduction networks in a proteome-wide manner." June 14th. Alfred Vertegaal. Leiden University Medical Center, Leiden, The Netherlands

"Myelination: the brain beyond neurons in health and disease." June 21st. Fernando de Castro. Instituto Cajal (CSIC) Madrid, Spain

October 2024

"New insights in the control and timing of Translesion Synthesis in yeast." October 4th. Vincent Pagès. Cancer Research Centre of Marseille, France

"PIAS2b-dsRNAi for Mitotic Catastrophe in Anaplastic Carcinomas." October 25th. Clara Álvarez. CIMUS - Universidad de Santiago de Compostela, Spain

November 2024

"Genomes in 3D: implications for development and evolution." November 8th. Darío Lupiañez. CABD, Sevilla, Spain

"DNA's Cosmic Weapon: The Entangled Power of WASp and Fanconi Anemia in Replication." November 21st. Yatin Vyas. Penn State College of Medicine and Penn State Health Children's Hospital Pennsylvania, USA

"Control of proteostasis in development and rare diseases." November 29th. Rosa Barrio. CIC-Biogune, Spain

December 2024

"From Beats to Metabolism: Unveiling the Heart's Metabolic Power." November 29th. Guadalupe Sabio. CNIO, Madrid, Spain

Workshops, Retreats & Seminars

International Workshop

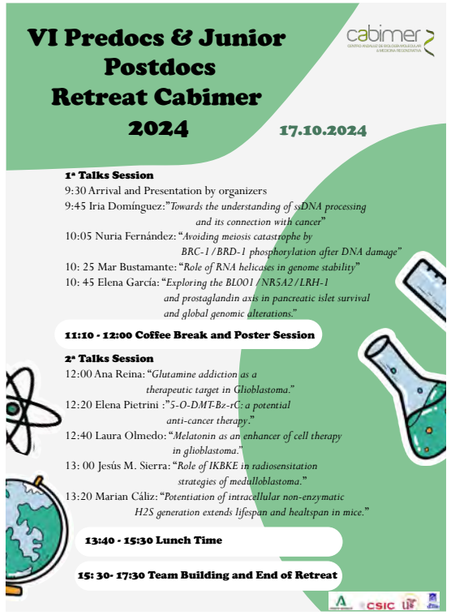
Workshops, Retreats & Seminars

Internal Workshops

Workshops, Retreats & Seminars

Seminars

Predoc and Postdoc Retreats



Awards & Events

CABIMER Award granted by the AECC (2023)

Awards

biomol

I Edición Premios Cabimer- Biomol

29 de Noviembre 2023

Fundación Instituto biomol

II Edición Premios CABIMER - Fundación Biomol

Salón de Actos de CABIMER, Avd. Américo Vespucio 24

30 de Octubre, 2024.

asociación española contra el cáncer

Premios Contra el Cáncer 2024

12 de marzo de 2024

Ayuda postdoctoral AECC (Dra. Paula Aguilera)

aseica

Premios ASEICA 2024

5 de noviembre de 2024

Premios ASEICA al Talento Investigador Joven (Dra. Patricia Altea Manzano)

fede

X Edición de premios FEDE

5 de octubre de 2024

Reconocimiento a la Investigación Pública (Dr. Benoit Gauthier)

Premios Losada Villasante

XII Premios Losada Villasante

8 de octubre de 2024

Categoría de Investigación Científica (Dra. Patricia Altea Manzano)

Science Week 2023

Science Fair 2023

XXXV Night Race

Science Fair 2024

Science Week 2024

International Day of Women and Girls in Science 11th February 2023 & 2024

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Dr. Susan M. Gasser
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Dr. Marisol Soengas
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Madrid (Spain)

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Dr. Vivek Malhotra (ended 2024)
Centre de Regulació Genòmica-CRG
Barcelona (Spain)

Dr. Ramón Gomis (ended 2024)
The August Pi i Sunyer Biomedical Research Institute (IDIBAPS). Barcelona (Spain)

Dr. Pier Paolo Di Fiore (ended 2024)
IFOM- FIRC Institute of Molecular Oncology.
Milan (Italy)

Where we are

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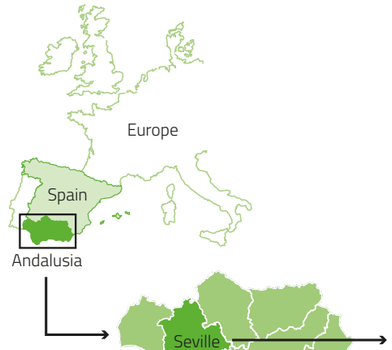

GPS Coordinates:
Latitude: 37.4112 or 37° 24'6720 N
Longitude: -6.0066 or 6° 0'3960 W


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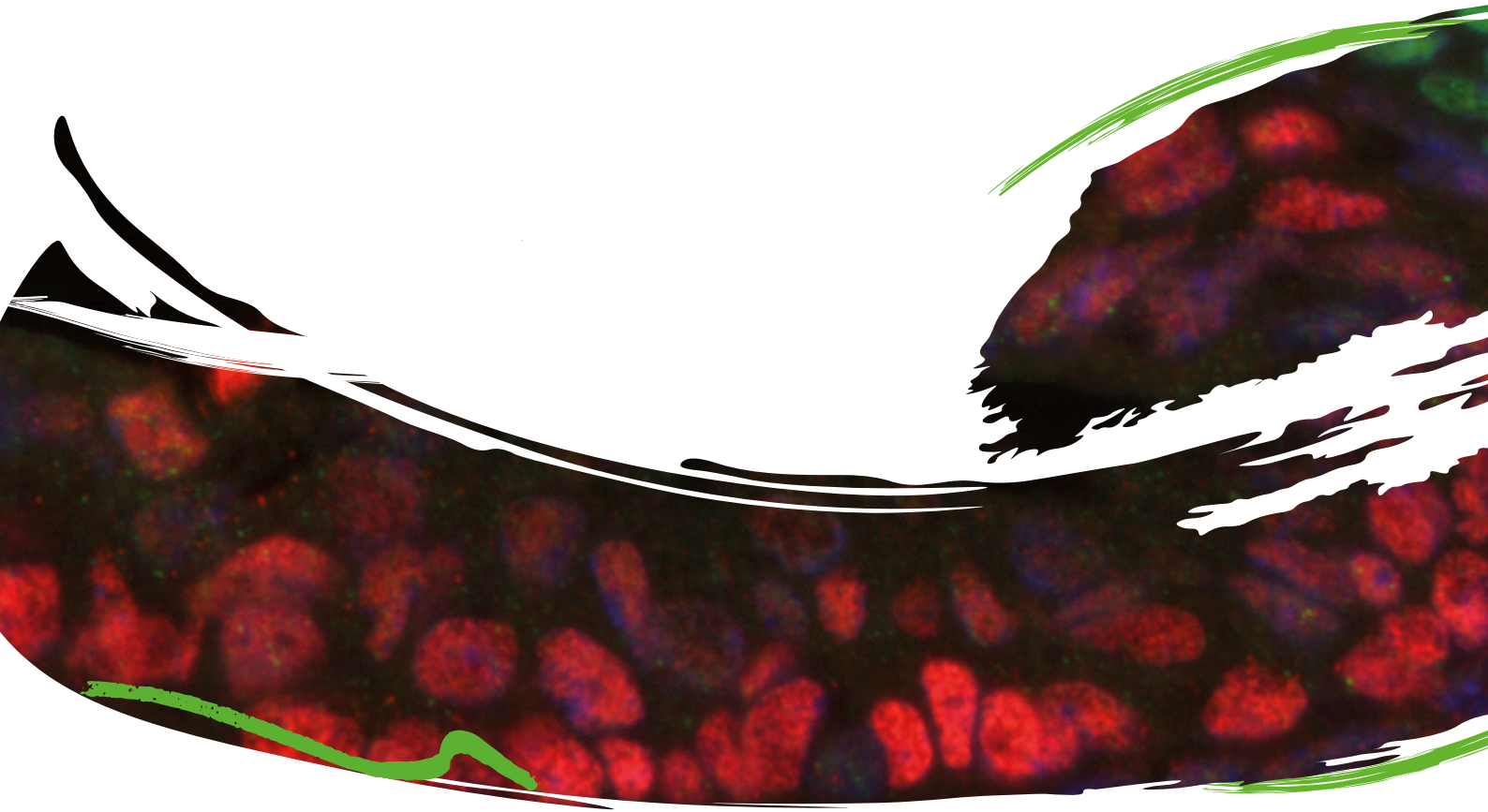

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