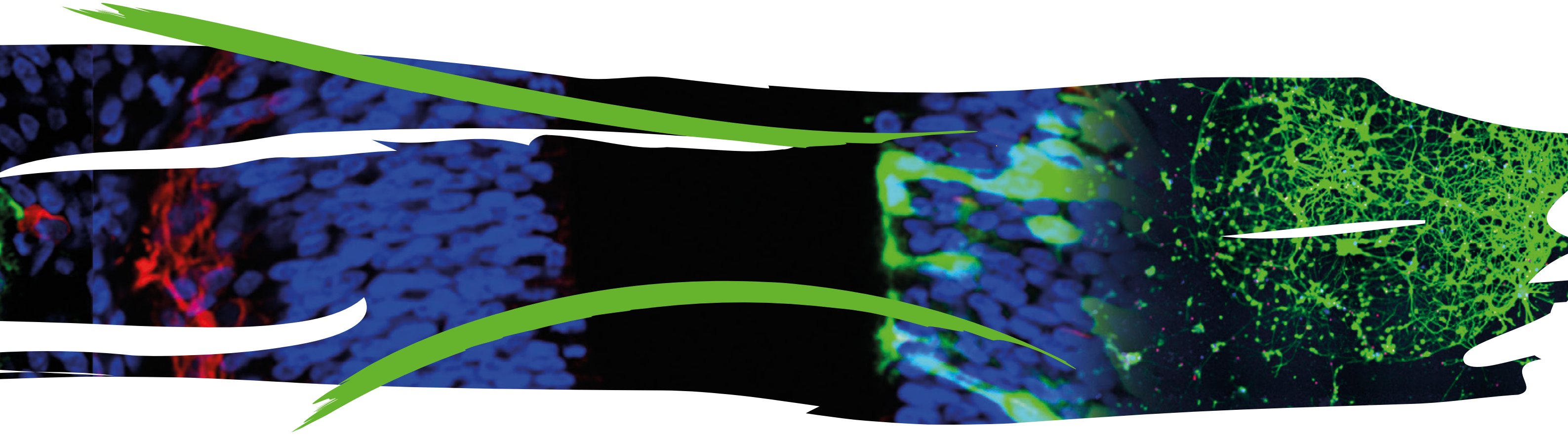
cabimer

Scientific **Report**

2021/2022



Junta de Andalucía

CSIC
Consejo Superior de Investigaciones Científicas

Universidad de Sevilla

Universidad Pablo de Olavide
Sevilla

cabimer

Scientific **Report**

2021/2022

Content

Director

Prof. Andrés Aguilera

Welcome

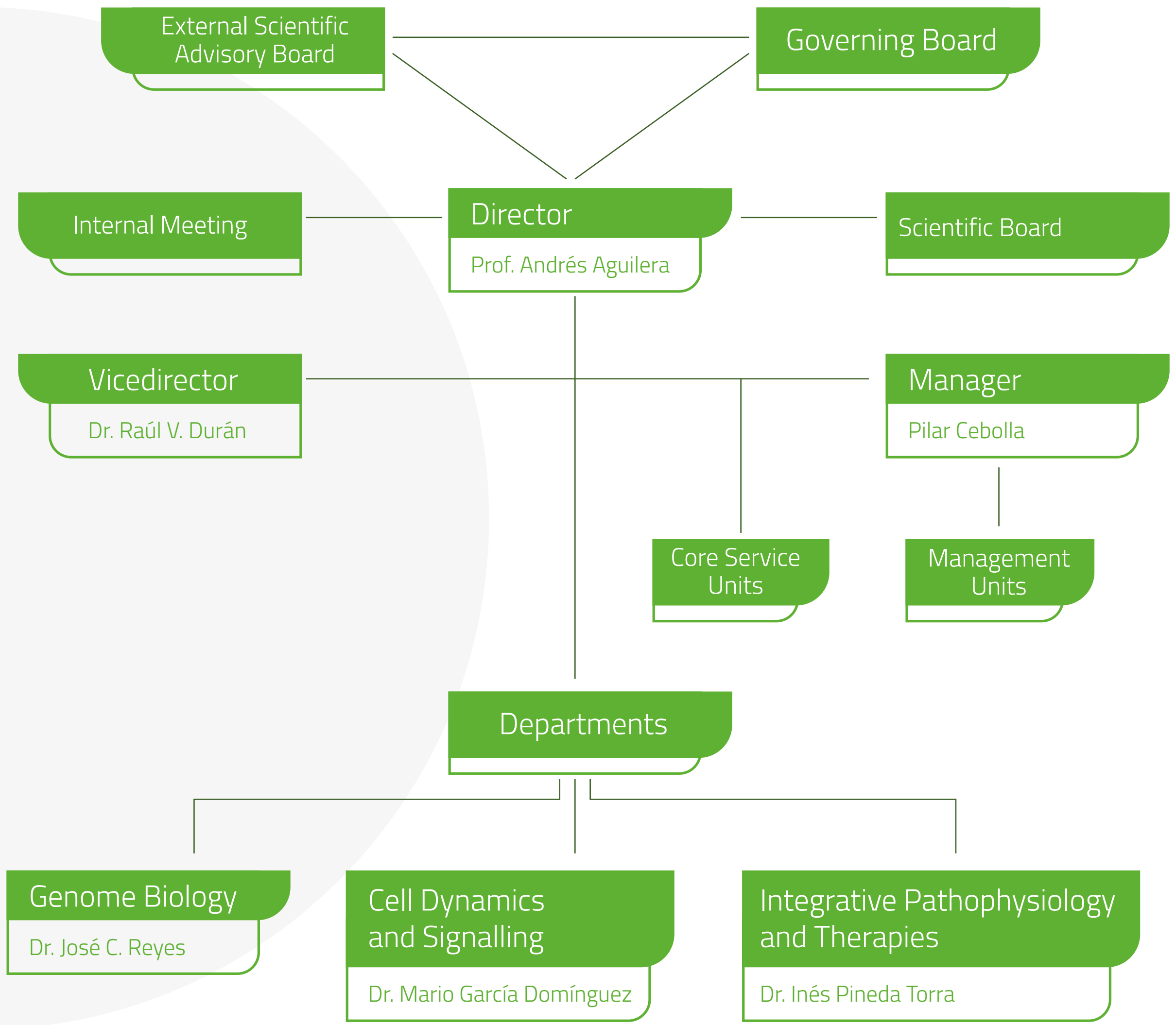
It is my pleasure to present the scientific report of CABIMER (Centro Andaluz de Biología Molecular y Medicina Regenerativa / Andalusian Centre of Molecular Biology and Regenerative Medicine) for the period 2021 to 2022. As a groundbreaking multidisciplinary biomedical research center in Andalusia, CABIMER 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 international scientists working at the Centre contribute to a stimulating and international atmosphere, and international seminars take 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 an important improvement of CABIMER activities and facilities to support the science undertaken by the 28 actual Principal Investigators (PI's), including 7 new emerging PIs, and their group members. In 2022, CABIMER has partially renovated the Scientific Advisory Board formed by prestigious international scientists and has stimulated the interaction and collaborations between the research groups of the Center with the celebration of internal scientific workshops and retreats for the young investigators, among other events. Some highlights of the past 2 years are the success of CABIMER researchers in obtaining funding from competitive calls from national and international agencies, such as the Caixa Research Foundation or the Juvenile Diabetes Research Foundation, as well as Marie Skłodowska Curie grants, a significant improvement of the quality of its publications and grant incomes, or the number and quality of PhD students and postdoctoral researchers, which has led to the defense of 12 PhD theses during this 2-year period and more than 119 publications, among other achievements. It is worth highlighting the celebration of the Second CABIMER International Workshop in February 2020 on "Trends in Cancer Biology and Advanced Therapies" with highly recognized invited speakers and attendants from all over the world that had a high international repercussion, and with which CABIMER initiates its series of annual international meetings. This represents an important achievement towards regularizing a scientific activity that not only stimulates collaborative science but helps place CABIMER in the international map of high standard research centers in Molecular Biology and Biomedicine. As a result of its scientific achievements in the last years, CABIMER has been distinguished as a Center of Excellence of Andalusia under the program QUALIFICA with a 689,000€ grant from the Andalusian Ministry of University, Research and Innovation.

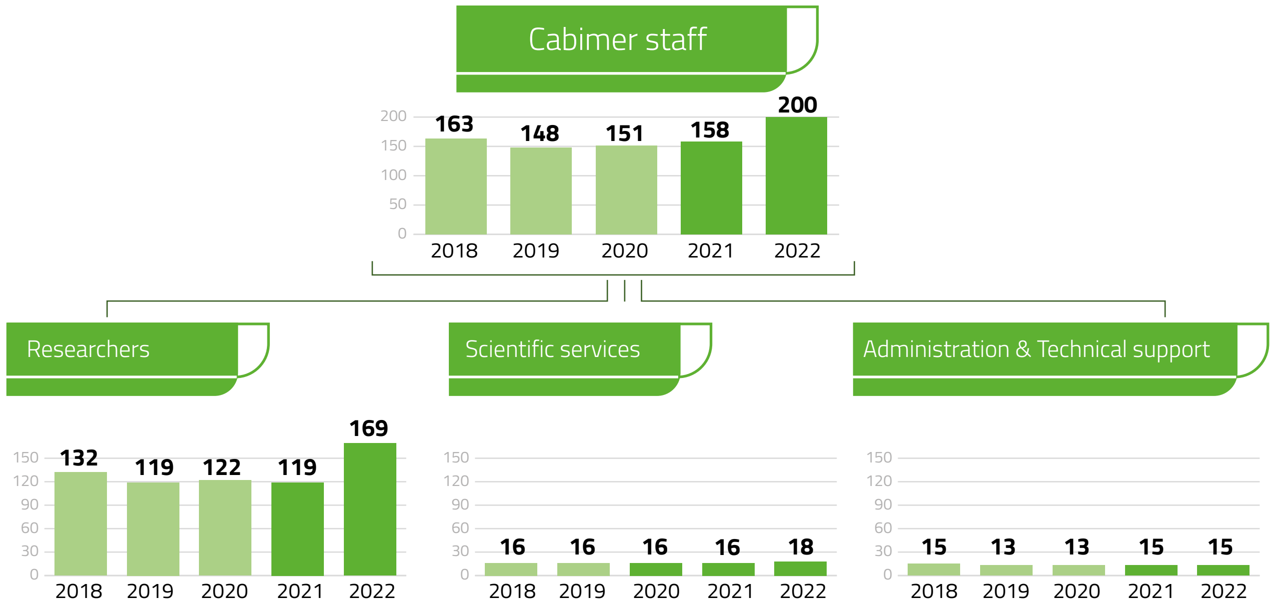
In the 2021-22 period CABIMER has updated the 9 fully-functional core services with technologies including the state of the art Biological Research Unit with a special service for the generation of genetically modified mice, the Genomic platform for the use of external and internal services, the advanced 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 in addition to genome-wide studies. A strong investment in image analysis and next-generation sequencing has strongly expanded our technical capabilities, but also that of many external laboratories all over Andalusia and beyond to which we provide high-standard NGS services.

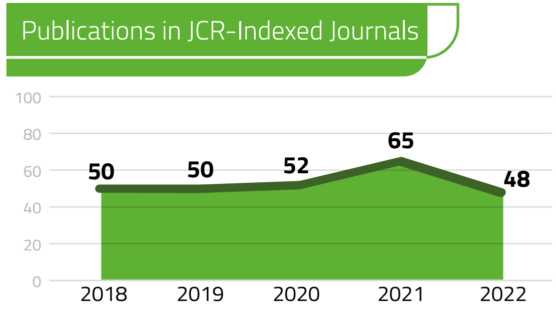
CABIMER is successfully increasing its reputation as an International Research Center and a major center of biomedical research in Spain. To accomplish these goals and improve its capabilities in the next future CABIMER has a new Strategic Plan for the next 4 years (2023-2026) that aim at expanding the 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, many of which pass through the ampliation of the lab space in the center to be able to incorporate new groups and technologies that allow us to work on the most actual problems in modern Biology and Biomedicine, and many new exciting discoveries lie ahead of us. I hope the information summarized in our Scientific Report conveys this ambition.

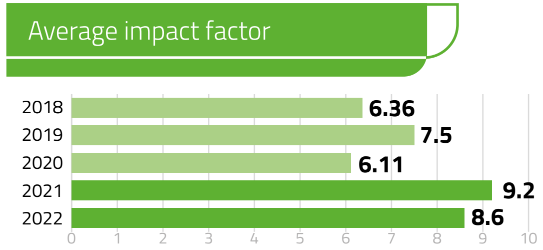
Organization and Research

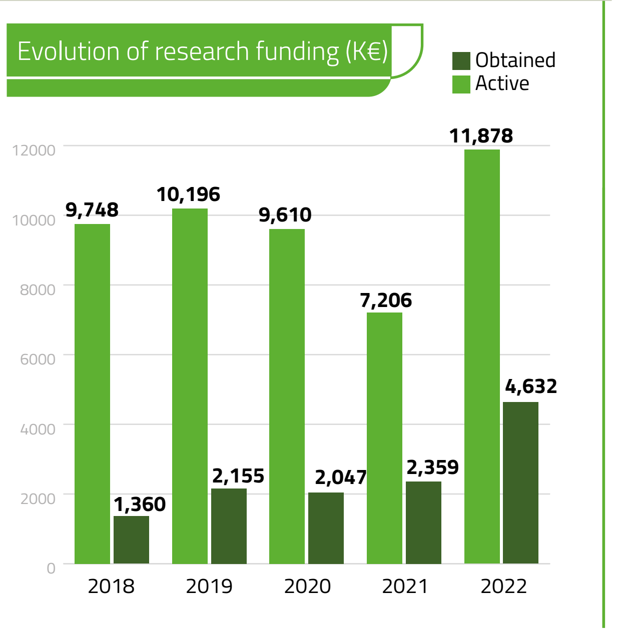


Organization and Research



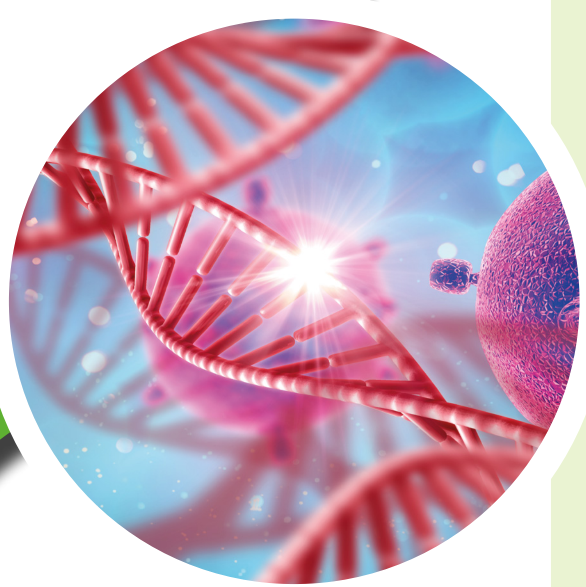






Genome Biology

The department of Genome Biology is dedicated to studying the processes involved in the expression and homeostasis of the genome, and how the alteration of these processes causes diseases. CABIMER's research covers various aspects of genome dynamics, including genome instability, DNA recombination, replication and repair, DNA damage response, chromatin integrity, epigenetics, and gene expression, with a special focus on genomics and epigenomics approaches analyzed using computational biology tools. Genomic instability is the cause of numerous congenital syndromes, rare diseases, as well as somatic diseases, especially cancer and aging. Therefore, an important part of our research interests is devoted to understanding how different aspects of genome metabolism are coordinated to avoid genomic instability, as well as the mechanisms and factors involved in genome protection. Other groups study the transcriptional and epigenetic regulation during cell differentiation, tissue plasticity, and cell signaling, and how alterations in these processes cause diseases. The department exhibits great thematic coherence, which enhances opportunities for collaboration among groups as they frequently utilize similar techniques, equipment, and molecular tools. Moreover, the extensive interrelationships between nuclear processes make it convenient to integrate various perspectives for a molecular understanding of the mechanisms of genome dynamics, which also increases cooperation between groups. Eleven groups are included in this area.



HEAD OF DEPARTMENT

Dr. José C. Reyes

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. G. Millán-Zambrano

Principal Investigator

Dr. José C. Reyes

Epigenetics and Gene Expression
Group Leader

Current position

- Since 2009: Scientific Researcher, National Council for Research, CSIC/CABIMER.
- Since July 2016: Chair of the Genome Biology Department of CABIMER, Seville, Spain.

Group Members

Postdocts
- María Ceballos-Chávez.
- Katiuska González Arzola.

PhD Students
- Laura Basurto.
- Elena Gómez-Marín.
- Elena Sánchez Escabias.
- Patricia Navarro Cansino.
- Lorena García Bernardo.

Technician
- María Escaño Maestre.

Former members
- **Postdocs:** José Antonio Guerrero-Martínez.

Research Activity

Overview
Development and cell differentiation are the result of a precise coordination of genes whose expression is controlled in a temporal and spatial manner. Alterations in gene expression can lead to various congenital malformations and diseases, including cancer. Chromatin, which is the complex formed by DNA and histone proteins, plays a fundamental role in gene expression. Our group's primary objective is to comprehend how chromatin changes in regulatory elements and gene bodies occur during transcription, how they are regulated and inherited, and which factors are responsible for them. We especially investigate how changes in these mechanisms are implicated in human disease, particularly cancer.

Epithelial-to-mesenchymal transition (EMT) is a biological process by which epithelial cells lose their characteristics and acquire mesenchymal properties. This process occurs during embryonic development, tissue remodeling, wound healing, and it is the origin of cancer metastasis. During EMT, the epithelial cells lose their cell-cell junctions and become more motile. They also undergo changes in their cytoskeleton and extracellular matrix, which allow them to move through tissues and invade other areas of the body. EMT is consequence of an intense transcriptomic and epigenomic reorganization that we have been studying during the last years.

Research Highlights

The main highlights for the period 2021-2022 are:

1.- RNA and protein factors involved in EMT.
In collaboration with different groups we have investigated the role of several protein and RNA factors in EMT. In collaboration with the group of Jose A. Pintor-Toro (CABIMER, Sevilla) we have shown that a long non coding RNA called lnc-Nr6a1 acts as a reservoir of miR-181 (a miRNA involved in EMT) and mediates assembly of a glycolytic complex (Polo-Generelo et al., Noncoding RNA 2022). In collaboration with Mario García-Domínguez (CABIMER, Sevilla) we showed that SENP7 overexpression protects cancer cells from oxygen and glucose deprivation and associates with poor prognosis in colon cancer (Gallardo-Chamizo et al., Genes & Diseases 2022). Finally, in collaboration with S. Chavez (IBIS, Sevilla) we have contributed to demonstrate that Prefoldin complex, another factor that has been also involved in EMT, modulates co-transcriptional pre-mRNA splicing (Payán-Bravo et al., Nucleic Acid Research 2021).

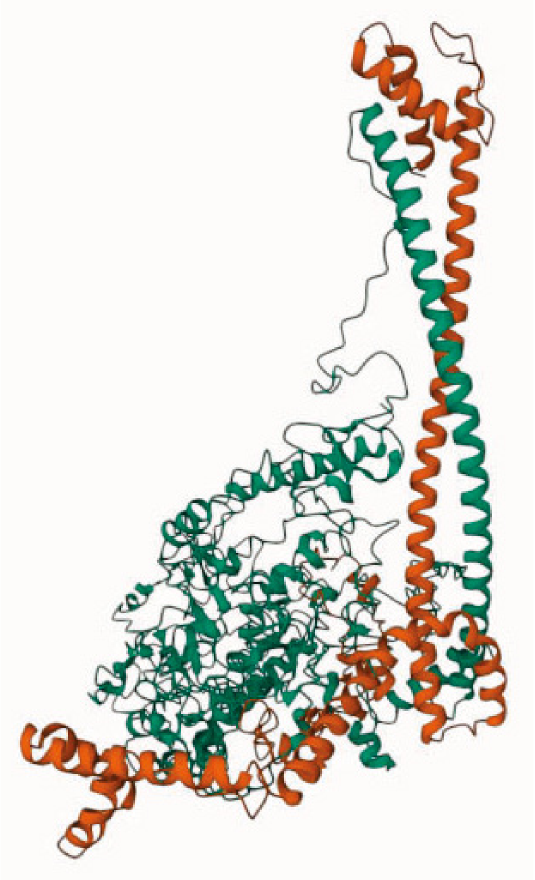


Figure 1. Artificial intelligence–based structure prediction of the HMG20A-PHF14 complex using Alphafold_multimers. HMG20A (orange), PHF14 (green).

2.- Co-transcriptional splicing efficiency is a gene-specific characteristic.
It is well-known that alternative splicing dramatically increases protein diversity. Now we have investigated whether splicing

efficiency of introns within the same gene is coordinated and eventually regulated as a mechanism to control mature mRNA levels. We have shown that co-transcriptional splicing (CTS) tends to be similar between the different introns of a gene. We have established that there are two strategies for CTS efficiency at the extremes of a gradient: short genes that produce high levels of pre-mRNA undergo inefficient splicing, while long genes with relatively low levels of pre-mRNA have an efficient splicing. We found that genes with efficient CTS displayed a relatively higher level of mature mRNA. TGFβ is the best known inductor of EMT. We showed that TGFβ regulates the general CTS efficiency, causing changes in mature mRNA levels (Sánchez-Escabias et al., Communications Biology 2022).

3.- Roles of HMG20A and PHF14 in EMT and disease.
HMG20A is a high mobility group protein that binds structured DNA without sequence specificity. Previously, we demonstrated that HMG20A regulates the LSD1/CoREST histone demethylase complex and is crucial for neuronal differentiation (Ceballos-Chavez et al., PNAS 2012). In collaboration with Dr. Gauthier (CABIMER, Seville), we have now discovered that HMG20A is also expressed in astrocytes and plays a crucial role in their function. Silencing HMG20A resulted in the repression of inflammatory, cholesterol biogenesis, and epithelial-to-mesenchymal transition pathways, which are hallmarks of reactive astrogliosis (Lorenzo et al., Theranostics 2021). Furthermore, we have found that HMG20A interacts with the histone reader PHF14 through the formation of a two-stranded alpha-helical coiled-coil structure (Figure 1). We have also shown that PHF14 and HMG20A collaborate in regulating several pathways involved in epithelial-mesenchymal plasticity, including the Hippo and TGFβ signal transduction pathways (Gómez-Marín et al., Nucleic Acid Research 2022).

Grants (2021-2022)

- 2020:2022: PY18-1962, Junta de Andalucía
- 2021:2024 Plan Nacional PID2020-118516GB-I00: Ministerio de Ciencia e Innovación/Agencia Estatal de Investigación
- 2013-present: VEC – 001/2014 FVEC-FPS. Fundación Vencer el Cáncer

Publication Highlights

Polo-Generelo S, Torres B, Guerrero-Martínez JA, Camafeita E, Vázquez J, Reyes JC, Pintor-Toro JA. **2022**. TGF-beta-upregulated lnc-Nr6a1 acts as a reservoir of miR-181 and mediates assembly of a glycolytic complex. **Noncoding RNA**. 8(5):62.

Gómez-Marín E, Posavec-Marjanović M, Zarzuela L, Basurto-Cayuela L, Guerrero-Martínez JA, Arribas G, Yerbes R, Ceballos-Chávez M, Rodríguez-Paredes M, Tomé M, Durán RV, Buschbeck M, Reyes JC. **2022**. The high mobility group protein HMG20A cooperates with the histone reader PHF14 to modulate TGFß and Hippo pathways. **Nucleic Acid Research**. 50(17):9838-9857.

Sánchez-Escabias E, Guerrero-Martínez JA, Reyes JC. **2022**. Co-transcriptional splicing efficiency is a gene-specific feature that can be regulated by TGFβ **Communications Biology**. 5(1):277.

Gallardo-Chamizo F, Lara-Ureña N, Correa-Vázquez JF, Reyes JC, Gauthier BR, García-Domínguez M. **2022**. SENP7 overexpression protects cancer cells from oxygen and glucose deprivation and associates with poor prognosis in colon cancer. **Genes & Diseases** 9(6): 1419-1422.

Lorenzo PI, Martin Vazquez E, López-Noriega L, Fuente-Martín E, Mellado-Gil JM, Franco JM, Cobo-Vuilleumier N, Guerrero Martínez JA, Romero-Zerbo SY, Perez-Cabello JA, Rivero Canalejo S, Campos-Caro A, Lachaud CC, Crespo Barreda A, Aguilar-Diosdado M, García Fuentes E, Martin-Montalvo A, Álvarez Dolado M, Martin F, Rojo-Martinez G, Pozo D, Bérmudez-Silva FJ, Comaills V, Reyes JC*, Gauthier BR* (*co-correponding authors). **2021**. The Metabesity Factor HMG20A Potentiates Astrocyte Survival and Reactivity Preserving Neuronal Integrity. **Theranostics**. 11(14):6983-7004.

Payán-Bravo L, Fontalva S, Peñate X, Cases I, Guerrero-Martínez JA, Pareja-Sánchez Y, Odriozola-Gil Y, Lara E, Jimeno-González S, Suñé C, Muñoz-Centeno MC, Reyes JC, Chávez S. **2021**. Human Prefoldin Modulates Co-transcriptional Pre-mRNA Splicing. **Nucleic Acid Research**. 49(11):6267-6280.

Principal Investigator

Prof. Andrés Aguilera

Genome Instability and Cancer

Group Leader

Current position

- Full Professor of Genetics, University of Seville (US).
- Director of CABIMER.
- Executive Responsible of the Genomics Unit of CABIMER.

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 (Assist. Prof., US).
- Sonia Barroso.
- Emilia Herrera-Moyano.
- M. Angeles Ortiz-Bazán.
- Sara Priego.
- Nibal Badra-Fajardo.

PhD Students

- M. Eugenia Soler-Oliva.
- Javier Marqueta-Gracia.
- Iván Núñez-Martín.
- Mar Bustamante-Sequeiros.
- Pablo Maraver-Cárdenas.
- Cristina Acedo-Rubio.

Technicians

- Pablo Cano Jiménez.

Former Members (2021-2022)

- **Postdocs:** Aleix Bayona-Feliu, Sonia P. Silva, José A. Mérida-Cerro.
- **PhD students:** Pedro Ortega, Cristina Guillén-Mendoza.
- **Master students:** Sandra Trujillo Sierra.
- **Visiting Scientists:** Prof. J. Lucas Argueso (Colorado State Univ, Forth Collins, CO, USA); Dr. Patrick Toolan-Kerr (Francis Crick Institute, London, UK); Dr. Giovana S. Leandro (Univ. Sao Paulo, Brazil); Renée Concetta-Durado (Erasmus+).
- **BSc students:** Marina Bejarano Franco.
- **Administration:** Zoë Cooper.

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 that caused by transcription-replication conflicts and R-loops. Our goals are:

1. to decipher the mechanisms by which cells prevent harmful R-loop accumulation and its associated genome instability;
2. to identify the main determinants of replication failures that lead to replication fork stalling and DNA breaks;
3. to understand how a replication-born DNA break is repaired to allow replication restart and prevent chromosome aberrations and genome instability;

Research Highlights

1. Prevention of R-loops and R-loop-mediated genome instability.

We have shown three different modes of resolving R-loops: i) by protecting the RNA by assembly; ii) by transiently closing chromatin by histone deacetylation, and iii) by removing occasional R loops co-transcriptionally. R-loops have different origins along the cell cycle with specific factors controlling their formation or resolution in nuclei and mitochondria. We have shown using *Saccharomyces* as a model system that whereas

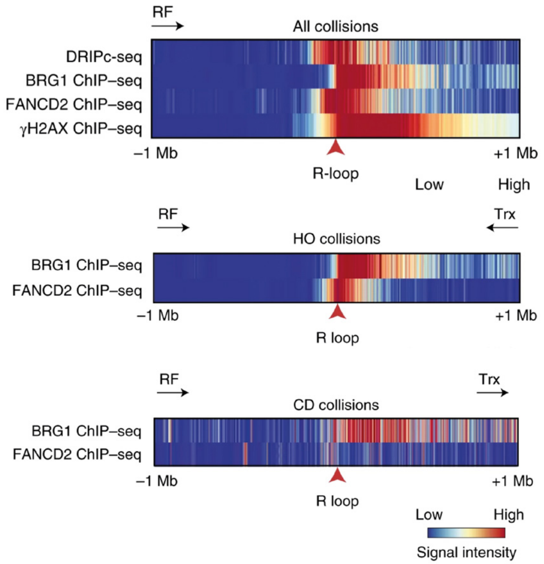


Figure 1. Modified from Bayona-Feliu et al, Nat Genet 2021

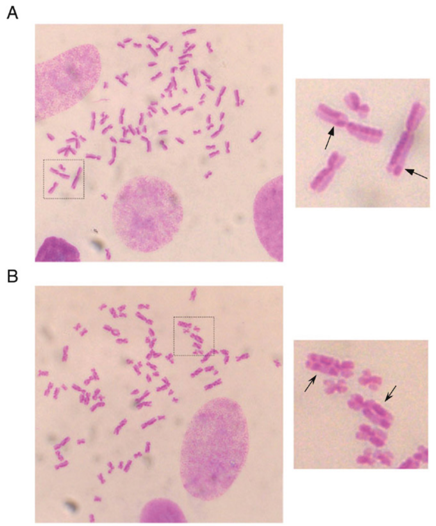


Figure 2. Tumini and Aguilera Methods Mol Biol, 2021

the THO complex protects cells from harmful R-loops in the G1 phase of the cell cycle, consistent with its transcriptional role, the Senataxin ortholog Sen1 only protects from R-loops during the S/G2 period, in line with a role related to the DNA damage response (San Martin-Alonso et al., Nat Commun 2021). The study has opened new perspectives on R-loops having different origins and different factors for its regulation. We have uncovered that histone acetylation by yeast Rtt109 counteracts R-loops (Cañas et al, Genetics 2022). In addition, we have shown that the human nucleoporin Tpr protects cells from RNA-mediated replication stress and R-loops in a collaborative effort that provides new clues about the link between the nuclear pore complex and genome integrity (Kossar et al, Nat Commun 2021). Finally, we have collaborated in the characterization and analysis of the involvement fo new factors involved in R-loop-mediated genome integrity, such as MutSß (Sakeliariou et al, Cell Rep 2022), ß-catenine (Dagg et al, Nat Commun 2021), ADAR (Jimeno et al, Nat Commun 2021) or Top1 (Sarni et al, Cell Rep 2022) in human cells. Also, we have shown that TREX2 protects from R-loops in *C. elegans* (Zheleva et al, J Cell Sci 2021) an collaborated on the study of the histone deacetylase inhibitor Romidepsin as a regulator of R-loop-mediated DNA breaks (Mol Cancer Res. 2021).

2. Transcription-replication conflicts and R-loop formation.

We have shown that DNA-RNA hybrids cause transcription-replication collisions rather than being a consequence. In human cells, we have shown that hybrids can be originated before or after DNA replication. In contrast to other reports, we have demonstrated that R-loops occur in cis and independently of Rad51 and are an obstacle to DSB repair. We demonstrated a role for chromatin remodeling and modification (SWI/SNF) in resolution of transcription-replication conflicts mediated by R-loops. SWI/SNF is indeed enriched at chromatin sites where R-loops are increased genome-wide in its absence, such R-loops causing transcription-replication collisions mainly at the head-on orientation (Figure 1) (Bayona-Feliu et al, Nat Genet 2021). Related to replication stress caused by other factors we have collaborated in the analysis of WASp (Han et al, Nat Commun 2022) and a chromatin segregase (Chacin et al, Nat Commun 2021) as new players in genome integrity and replication.

3. Repair of replication-borne DNA breaks.

Replication-borne DSBs are preferentially repaired with the sister chromatid, a reaction that we can infer form the number of sister chromatid exchanges in metaphase spreads of cultured human cells (Figure 2). With newly constructed tools we have shown that DNA-RNA hybrids accumulate at DSBs by live cell microscopy in human cells (Figure 3). We have observed in yeast that these hybrids have a negative role in DNA repair in addition to provide data indicating that Rad51 is not necessary for their formation, which adds a new view on the biological meaning of unscheduled hybrids formed at DSBs (Ortega et al, eLife 2021). Finally, in human cells in a collaborative effort, we have found that DDX5 is a DNA-RNA unwinding factor involved in the resolution of DNA-RNA hybrids formed at DSBs. DDX5 works with BRCA2 as a way to remove hybrids that interfere with the repair, providing thus additional evidence that DNA-

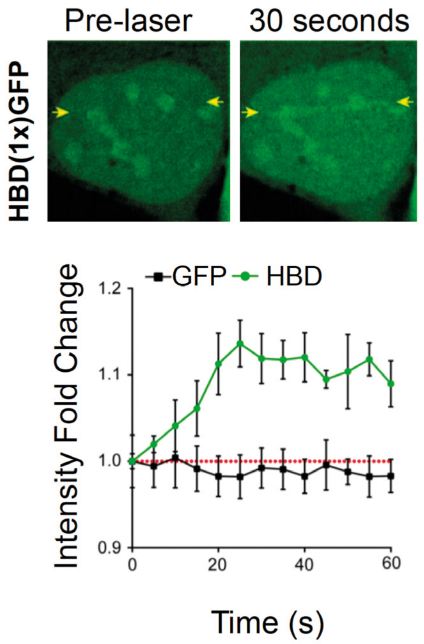


Figure 3. Silva et al Methods Mol Biol, 2022

RNA hybrids constitute an obstacle to DSB repair and we have found a BRCA2 cancer variant that impairs this interaction providing a possible cause for its role in tumorigenesis (Sessa et al, EMBO J 2021).

In addition to those highlights and others, we have edited 3 books of the *Methods Mol Biol* series (*R-loops and DNA recombination*), and contributed with specific chapters.

Grants
(The PI is the group leader unless otherwise specified).

- 2021-2023: US-1380058 Junta de Andalucía-US (PI: Gómez-González).
- 2021-2023: P18-FR-655 (PAIDI) Junta de Andalucía (co-PI: Rondón).
- 2020-2022: US-1258654 Junta de Andalucía-US (co-PI: Luna).
- 2020-2023: PID2019-104270G-100 Ministry of Science and Innovation.
- 2019-2023. Plan Propio Univ. Seville.
- 2022-2023. FIUS (Foundation Investigation Univ Seville-FIUS).
- 2022-2025. Caixa Research Foundation.
- Since 2013 : VEC001/2014 FVEC-FPS. "Vencer el Cáncer" Foundation.
- 2015-2021: ERC2014 AdG669898 TARLOOP. European Research Council.

Publication Highlights

(Corresponding author(s) indicated by *)

Cañas JC, García-Rubio ML, García A, Antequera F, Gómez-González B*, Aguilera A. 2022. A role for the Saccharomyces cerevisiae Rtt109 histone acetyltransferase in R-loop homeostasis and associated genome instability. Genetics 222(1):iyac108.

San Martin-Alonso M, Soler-Oliva ME, García-Rubio M, García-Muse T*, Aguilera A*. 2021. Harmful R-loops are prevented via different cell cycle-specific mechanisms. Nat Commun. 12(1):4451.

Ortega P, Mérida-Cerro JA, Rondón AG, Gómez-González B, Aguilera A*. 2021. DNA-RNA hybrids at DSBs interfere with repair by homologous recombination. Elife 10:e69881.

Bayona-Feliu A, Barroso S, Muñoz S, Aguilera A*. 2021. The SWI/SNF chromatin remodeling complex helps resolve R-loop-mediated transcription-replication conflicts. Nat Genet. 53(7):1050-1063

Sessa G, Gómez-González B, Silva S, Pérez-Calero C, Beaurepere R, Barroso S, Martineau S, Martin C, Ehlén Å, Martínez JS, Lombard B, Loew D, Vagner S, Aguilera A*, Carreira A*. BRCA2 promotes R-loop resolution by DDX5 helicase at DNA breaks to facilitate their repair by homologous recombination EMBO J. 2021 40(7):e106018.

Kosar M, Giannattasio M, Piccini D, Maya-Mendoza A, García-Benítez F, Bartkova J, Barroso SI, Gaillard H, Martini E, Restuccia U, Ramirez-Otero MA, Garre M, Verga E, Andújar-Sánchez M, Maynard S, Hodny Z, Costanzo V, Kumar A, Bachi A, Aguilera A*, Bartek J*, Foiani M*. 2021. The human nucleoporin Tpr protects cells from RNA-mediated replication stress. Nat Commun. 12(1):3937.

Principal Investigator

Dr. Félix Prado

Chromatin Integrity and Function

Group Leader

Current position

- Since 2006: Research Scientist- CSIC/ CABIMER.

Group Members

Postdocts
- Antonia María Romero Cuadrado.
- Marta Barrientos Moreno.

PhD Students
- Cristina González Garrido.
- Ana Amiama Roig.

Technicians
- Esther Cruz Zambrano.

Former Members (2021-2022)

- **Postdoc:** Marta Barrientos Moreno.
- **PhD students:** Aurora Yáñez Vílchez, Cristina González Garrido.
- **Master students:** Antonio Balconero Martín.
- **Technician:** Esther Cruz Zambrano.
- **JAE Intro:** Ana Utrilla Maestre.

Research Activity

Overview

Cells have to duplicate theirs genomes in a faithful and timely way to ensure the correct transmission of genetic information to the daughter cells. This implies replication of DNA and assembly into chromatin, two processes that can be challenged by multiple stress conditions that cause loss of DNA integrity and alterations in the pattern of nucleosome-associated epigenetic marks, which are linked to genetic disorders and cancer. Genome duplication is not an easy task, taking into account the number of physical, chemical and genetic agents that can pertub the advance of replication forks. The fate of stressed replication forks can be stalling or breakage, which will trigger common and specific responses. Our main goal is to get a deeper insight into the mechanisms that deal with stressed replication forks and the relevance that chromatin assembly plays in these processes.

Research Highlights

A major source of genetic instability is associated with the encounter of the replication fork with DNA adducts that hinder its advance. In this case, replication fork stability and genome integrity are maintained by a number of error-free and error-prone mechanisms that help the fork to pass through the lesions and to fill in the gaps of single-stranded DNA (ssDNA) generated during the process of fork blockage and lesion bypass. Consequently, this DNA damage tolerance (DDT) response is essential for cell cycle progression, genome integrity, and cancer avoidance. DDT relies on homologous recombination (HR) and translesion synthesis (TLS) mechanisms to fill in the ssDNA gaps generated during passing of the replication fork over DNA lesions in the template. Whereas TLS requires specialized polymerases able to incorporate a dNTP opposite the lesion and is error-prone, HR uses the sister chromatid and is mostly error-free. We have reported that the HR protein Rad52 acts in concert with the TLS machinery to repair MMS and UV light-induced ssDNA gaps through different non-recombinogenic mechanisms. Specifically, Rad52 facilitates the recruitment of the Rad6/Rad18 complex, required for PCNA ubiquitylation and subsequent recruitment of the TLS polymerases. Therefore, Rad52 facilitates the tolerance process not only by HR but also by TLS, providing a novel role for the recombination proteins in maintaining genome integrity.

The recombination proteins Rad51 and Rad52 physically interacts with the MCM helicase from yeast to human cells. We have shown in Saccharomyces cerevisiae that these interactions occur in a nuclease-insoluble scaffold enriched in replication/ repair factors. Rad51 accumulates in a MCM- and DNA binding-independent manner and interacts with MCM helicases located outside of replication origins and forks. MCM, Rad51 and Rad52 accumulate in this scaffold in

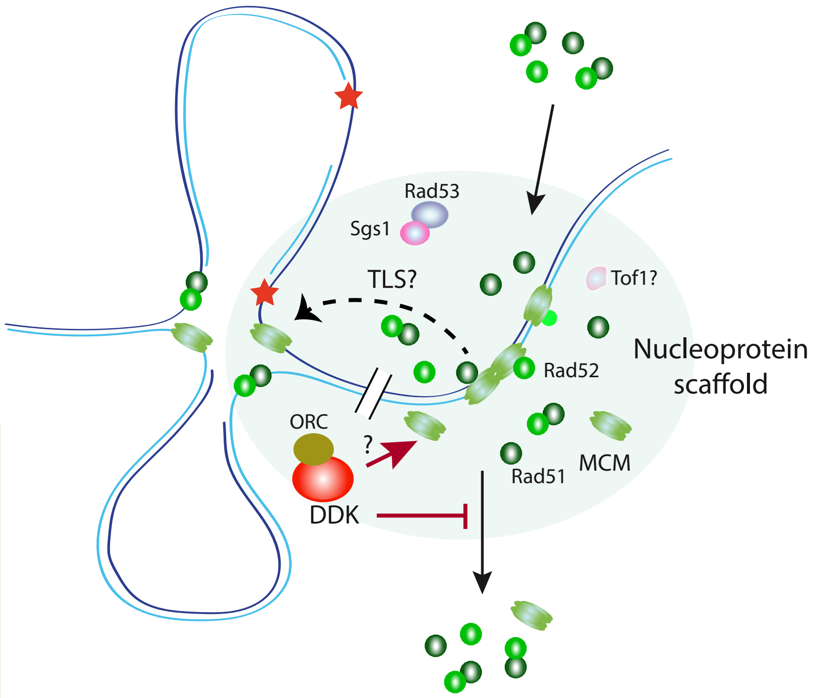


Figure 1. A hypothetical role for DDK controlling the integrity of a nucleoprotein scaffold for replication assistance. The MCM complex interacts in yeast with the HR factors Rad51 and Rad52 in a DNA damage and cell cycle-dependent manner. These interactions, which occur at a nuclease-insoluble nucleoprotein scaffold enriched in DNA replication and repair factors, facilitate replication fork progression and ssDNA filling during DDT. DDK maintains these interactions during S phase under conditions of replication stress by preventing the release of these HR factors from the scaffold. DDK might perform this function by controlling either the integrity of the nucleoprotein scaffold by acting upon MCM or the binding of the HR factors to this compartment.

G1 and are released during S phase. In the presence of replication-blocking lesions, Dbf4-dependent kinase (DDK) Cdc7 prevents their release from the scaffold, thus maintaining the interactions. We identified a rad51 mutant that is impaired in its ability to bind to MCM but not to the scaffold. This mutant is proficient in recombination but partially defective in ssDNA gap filling and replication fork progression through damaged DNA. Therefore, cells accumulate MCM/Rad51/Rad52 complexes at specific nuclear scaffolds in G1 to assist stressed forks through non-recombinogenic functions (Figure 1).

The advance and stability of replication forks rely on a tight co-regulation of DNA synthesis and nucleosome assembly. We have shown that mutants affected in parental histone recycling are impaired in the recombinational repair of the ssDNA gaps generated in response to DNA adducts that hamper replication, which are then fill in by TLS. 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 HR.

Grants (2021-2022)

- 2022-2025: PID2021-127486NB-I00. Ministerio de Ciencia e Innovación.
- 2021-2023: PY20_00750. Consejería de Conocimiento, investigación y Universidad.
- 2019-2021:PGC2018-099182-B-100. Ministerio de Ciencia, Innovación y Universidades.

Publication Highlights

Cristina González-Garrido and Félix Prado. **2022**. Novel insights into the roles of Cdc7 in response to replication stress. **FEBS J.** https://doi.org/10.1111/febs.16456

Félix Prado. 2021. Non-recombinogenic functions of Rad51, BRCA2, and Rad52 in DNA damage tolerance. **Genes** 12: 1550

María J. Cabello-Lobato, Cristina González-Garrido, María I. Cano-Linares, Ronald P. Wong, Aurora Yáñez-Vílchez, Macarena Morillo-Huesca, Juan M. Roldán-Romero, Marta Viciosos, Román González-Prieto, Helle D. Ulrich and Félix Prado. **2021**. Physical interactions between MCM and Rad51 facilitate replication fork lesion bypass and ssDNA gap filling through non-recombinogenic functions. **Cell Rep**. 36: 109440

María I. Cano-Linares, Aurora Yáñez-Vilches, Néstor García-Rodríguez, Marta Barrientos-Moreno, Román González-Prieto, Pedro San-Segundo, Helle D. Ulrich and Félix Prado. **2021**. Non-recombinogenic role for Rad52, Rad51 and Rad57 in translesion synthesis. **EMBO Rep.** 22: e50410

Román Gonzalez-Prieto, María J. Cabello-Lobato and **Félix Prado. 2021.** In vivo binding of recombination proteins to non-DSB DNA lesions and to replication forks. **Methods Mol. Biol** 2153: 447-458

Principal Investigator

Ralf Erik Wellinger

Mitochondrial Plasticity and Replication

Group Leader

Current position

- Full Professor at the Department of Genetics, University of Seville-CABIMER.

Group Members

Research Associates

- Helene Gaillard, Associate Professor of Genetics (University of Seville).

Postdocs

- Inés García de Oya.

Former Members (2021-2022)

- **PhD student:** Hayat Heluani-Gahete.
- **Erasmus + Master students:** Manuel Peral Vázquez (RW), Manuel Camacho Rivera (HG).

Research Activity

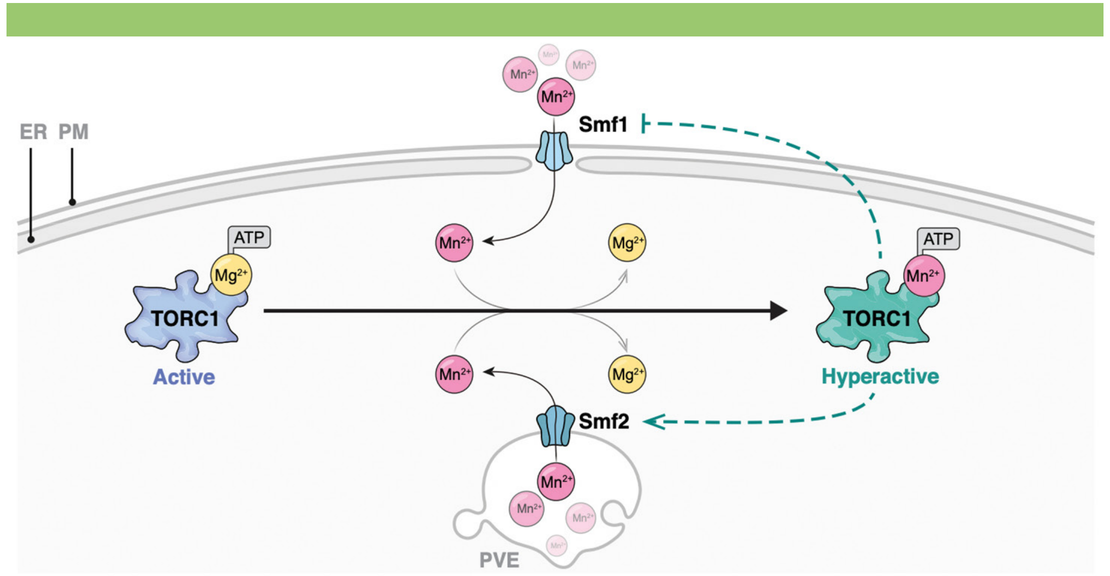
Overview

The correct metabolism of micronutrients, such as manganese (Mn) and iron (Fe), is essential for life. A main role of these transition metals consists in the activation of enzymatic activities. However, they also promote the formation of reactive oxygen species (ROS), thereby damaging lipids, proteins or nucleic acids. Moreover, Mn and Fe have a pivotal role in autophagy, stress signaling as wells as in DNA dependent processes such as transcription, DNA transposition, replication and repair. Our research aims at understanding why and how micronutrients are connected to human disease. A further objective is to take advantage of micronutrients as therapeutic tools to alleviate human disease and aging.

Research Highlights

Role of biometals in metabolic regulation

Biometals are essential micronutrients that are needed as cofactor for enzyme function. We initially became interested in biometals because we found that mitochondrial iron–sulphur cluster biosynthesis is important for the maintenance of nuclear genome stability,



Model for manganese-driven TORC1 activation.

and that cytosolic excess of manganese predisposes to genomic instability and bypasses the need for S-phase cell cycle checkpoints.

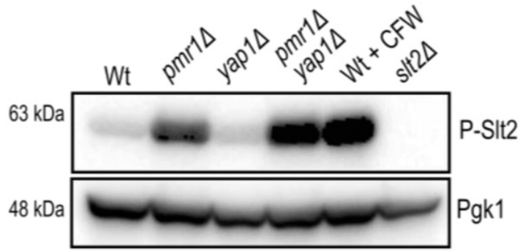
Follow up research based on our previous work on the impact of manganese on genome stability, during 2020 to 2022 the lab published original and collaborative work with groups from Spain and Switzerland. Interestingly, manganese excess was previously linked to rapamycin resistance suggesting a link between manganese and the TOR-pathway. To understand how manganese drives rapamycin resistance, we initiated a collaboration with the research group of Prof. Claudio de Virgilio at the University of Fribourg (CH). The results of this collaboration show that Mn serves as a metal-cofactor able to stimulate TORC1 kinase activity in vivo and in vitro.

We could show that Mn homeostasis is highly regulated and modulates key cellular processes such as autophagy, mitophagy, and mitochondrial retrograde response activation. Furthermore, by complementation assays, we also could show that NRAMP transporters are highly conserved from yeast to mice, and that Mn is a physiologically relevant TORC1 activator in yeast and human cells.

These findings could help to improve our understanding of disease phenotypes observed in Hailey-Hailey patients who suffer from impaired manganese/calcium homeostasis, skin ulceration, improper keratinocyte adhesion, and cancer predisposition. But even more importantly, our findings pinpoint to the molecular mechanisms underlying Mn-driven neuropathies in human.

Cellular Mn-stress response network

Each kind of stress requires an adequate response to optimize cell survival. How stress signalling networks manage to crosstalk with each other is not well understood, but mechanistic evidence has been provided on how oxidative stress inhibits pheromone signalling. Further characterization of stress signalling events in yeast revealed that Mn increases intracellular ROS levels. The corresponding signalling signature includes activation of stress activated MAP kinases (SAPKs) and the oxidative stress transcription factor Yap1.



Constitutive activation of the MAPKinase Slt2 in the absence of Pmr1.

A yet unanswered question is how stress signalling is channelled into the MAPK Slt2 in the absence of Yap1. In addition to the hitherto unknown role of Yap1 in manganese tolerance, we find that manganese induces a rapid reduction of Yap1 protein levels. However, the molecular bases of Mn-driven Yap1 decay remains to be explored in detail. Further exploration of these findings will open new perspectives for the understanding of neurodegenerative disorders and aging-related processes.

Grants (starting or ending 2021-2022)

- Un enfoque fosfoproteómico hacia la comprensión y el tratamiento de una enfermedad rara; P2020_01220_PAIDI I+D+i: Junta de Andalucía-European Union
- COST action CA21115; Iron-sulphur (FeS) clusters: from chemistry to immunology (FeSImmChemNet): European Union

Publication Highlights

Manganese is a Physiologically Relevant TORC1 Activator in Yeast and Mammals. Nicastro, R., Gaillard, H., Zarzuela, L., Péli-Gulli, MP., Fernández-García, E., Tomé, M., García-Rodríguez, N., Durán, R.V., De Virgilio, C., **Wellinger, R.E. eLife** (2022) https://DOI: 10.7554/eLife.80497 http://canalciencia.us.es/por-que-tienen-los-humos-dede-manganeso-un-efecto-tan-perjudicial-para-la-salud/

A new challenge for data analytics: transposons. Wellinger, R.E., Aguilar-Ruiz, J.S. **Biodata Mining** (2022) 15:9 http://DOI: 10.1186/s13040-022-00294-x

Manganese Stress Tolerance Depends on Yap1 and Stress-Activated MAP Kinases. de Oya I. G., Jiménez-Gutiérrez E., Gaillard H., Molina M., Martín H. and Wellinger R.E. **Int. J. Mol. Sci.** (2022), 23(24), 15706; https://doi.org/10.3390/ijms232415706

Adaptive Response of Saccharomyces Hosts to Totiviridae L-A dsRNA Viruses Is Achieved through Intrinsically Balanced Action of Targeted Transcription Factors. Ravoityte, B.; Luksa, J.; Wellinger, R.E.; Serva, S.; Serviene, **E. J. Fungi** (2022) 8:381 https://DOI: 10.3390/jof8040381

The p24 complex contributes to specify arf1 for copi coat selection. Sabido-Bozo S, Perez-Linero A.M., Manzano-Lopez J., Rodriguez-Gallardo S., Aguilera-Romero A., Cortes-Gomez A., Lopez S., Wellinger R.E., Muñiz M. **International Journal of Molecular Sciences** (2021) 22:423 https://doi.org/10.3390/ijms22010423

Principal Investigator

Dr. Pablo Huertas

DNA double strand breaks repair and human disease

Group Leader

Current position

- Research Scientist CABIMER.
- Associate Professor of the University of Seville.

Positions Held

- 2004-2010: The Gurdon Institute for Cancer Research and Developmental Biology, University of Cambridge, UK.
- 2010-2015: Ramón y Cajal, University of Seville/ CABIMER.
- 2015-2016: Profesor Contratado Doctor, University of Seville/CABIMER.

Group Members

Senior Researcher

- Sonia Jimeno.
- Fernando Romero Balestra.

Postdocs

- Néstor García Rodríguez.
- Rosario Prados Carvajal.

PhD Students

- Rosa Camarillo Daza.
- Andrés Domínguez Calvo.
- Andrea Moo Bajo.
- Amador Romero Franco.
- Guillermo Rodríguez Real.
- María del Carmen Domínguez Pérez.

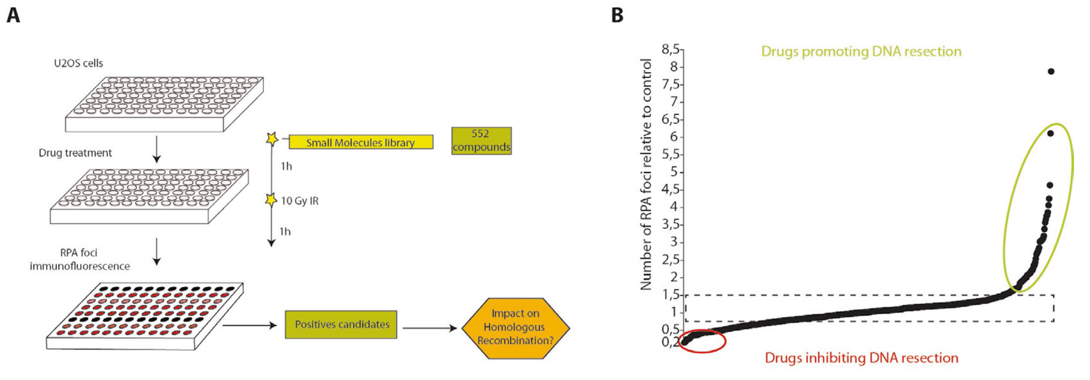
Research Activity

Overview

Double strand breaks (DSBs) repair is essential for cellular and organismal survival and fitness. While the complete inability to repair DSBs leads to embryonic lethality and cell death, mutations that hamper it causes the appearance of cancer or several genetically inherited syndromes. DSBs are repaired by two major mechanisms. The ends can be simple re-joined with little or no processing (non-homologous end-joining) or can be processed and engaged in a more complex repair pathway (homologous recombination). The balance between both pathways is exquisitely controlled and its alteration leads to the appearance of chromosomal abnormalities and contribute to the diseases aforementioned. Despite its importance, the network controlling the choice between both is poorly understood. In my laboratory, we pursue several research lines designed to investigate how such choice between is made, its relevance for survival and disease, and its potential as a therapeutic target for cancer or some genetically inherited disorders.

Research Highlights

The2021-2022 period has represented the consolidation of the lab in the international arena, with many publications and international collaboration in high tier journals. We have cemented our position as a referent in the field at national and international level. Regarding our research, keeping the focus on the regulation of the balance between non-homologous end-joining and homologous



Candidates from RPA foci screening >1,5 or <0,85 recombination, we have uncovered regulatory cues that expand from the most local issues, for example the presence of non-canonical structures at the DNA such as G4s quadruplexes or R-loops, to global signals such as the cell identity. We have made unexpected links with the metabolism of the RNA, uncover new factors involved in this regulation, and established connection with the genetically inherited Aicardi-Goutieres Syndrome that we propose can be explored for the search of therapeutics interventions.

Grants (starting or ending 2020-2022)

- 2019-2022: Fundación Ramón Areces.
- 2020-2022: US-1255532. Junta de Andalucía.
- 2020-2023: PID2019-104195G9. Ministerio de Ciencia e Innovación.
- 2020-2023: P18-RT-1204. Junta de Andalucía.

Publication Highlights

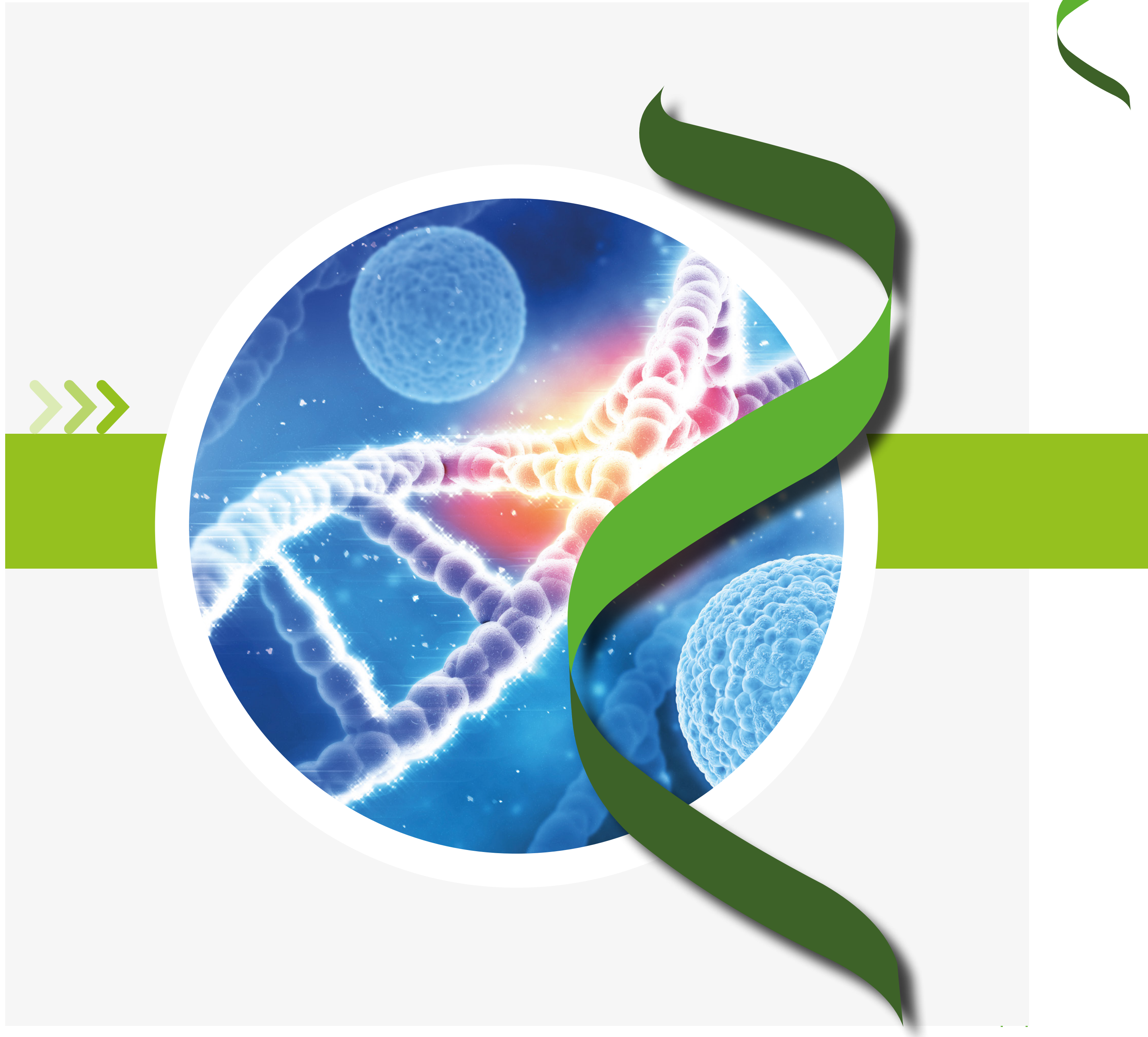
The Effect of Atypical Nucleic Acids Structures in DNA Double Strand Break Repair: A Tale of R-loops and G-Quadruplexes. Camarillo R, Jimeno S, **Huertas P.** Front Genet. 2021 Oct 8;12:742434. doi: 10.3389/fgene.2021.742434. eCollection 2021.

ADAR2-mediated RNA editing of DNA:RNA hybrids is required for DNA double strand break repair. Jimeno S, Prados-Carvajal R, Fernández-Ávila MJ, Silva S, Silvestris DA, Endara-Coll M, Rodríguez-Real G, Domingo-Prim J, Mejías-Navarro F, Romero-Franco A, Jimeno-González S, Barroso S, Cesarini V, Aguilera A, Gallo A, Visa N, **Huertas P.** Nature Communication, Volume 12, Issue 1December 2021 Article number 5512

The Emerging Role of RNA Modifications in DNA Double-Strand Break Repair. Jimeno S, Balestra FR, **Huertas P.** Front Mol Biosci. 2021 Apr 29;8:664872.

MRGBP, a member of the NuA4 complex, inhibits DNA double strand break repair. Rivero S, Rodríguez-Real G, Marín I, **Huertas P.** FEBS Open Bio. 2020 Dec 22. doi: 10.1002/2211-5463.13071.

CtIP-mediated alternative mRNA splicing finetunes the DNA damage response. Prados-Carvajal R, Rodriguez-Real G, Gutierrez-Pozo G, **Huertas P.** RNA. 2020 Dec 9:rna.078519.120. doi: 10.1261/rna.078519.120



Principal Investigator

Dr. Andrés J. López-Contreras

Molecular Oncology and Targeted Therapies

Group Leader

Current position

- Group Leader at CABIMER ("Científico Titular del CSIC").

Academic Background of PI

- 2004: Degree. University of Murcia, B.Sc. in Biochemistry.
- 2008: Degree. University of Murcia, B.Sc. in Medicine.
- 2008: PhD. University of Murcia, Ph.D. Thesis in Molecular and Cellular Biology.

Positions Held

- 2014-2020: Group Leader and Associate Professor at the "Center for Chromosome Stability" (CCS), Faculty of Health Sciences, ICMM Department, University of Copenhagen, Denmark.
- 2009 – 2014: Postdoctoral Researcher, Spanish National Cancer Research Centre (CNIO), Madrid, Spain.
- 2004 – 2008: PhD Student, Department of Biochemistry and Molecular Biology, University of Murcia, Spain.

Group Members

Postdocs

- María Castejón Griñán.
- Paula Aguilera Aguilera.
- Lucía Simón Carrasco.

Research assistant

- María José Morillo Chincoa (Contrato de Garantía Juvenil Junta de Andalucía).

PhD students

- Alba Guillén Benítez.
- Elena Pietrini.

Master Student

- Manuel Luque Pérez.

Research Activity

Overview

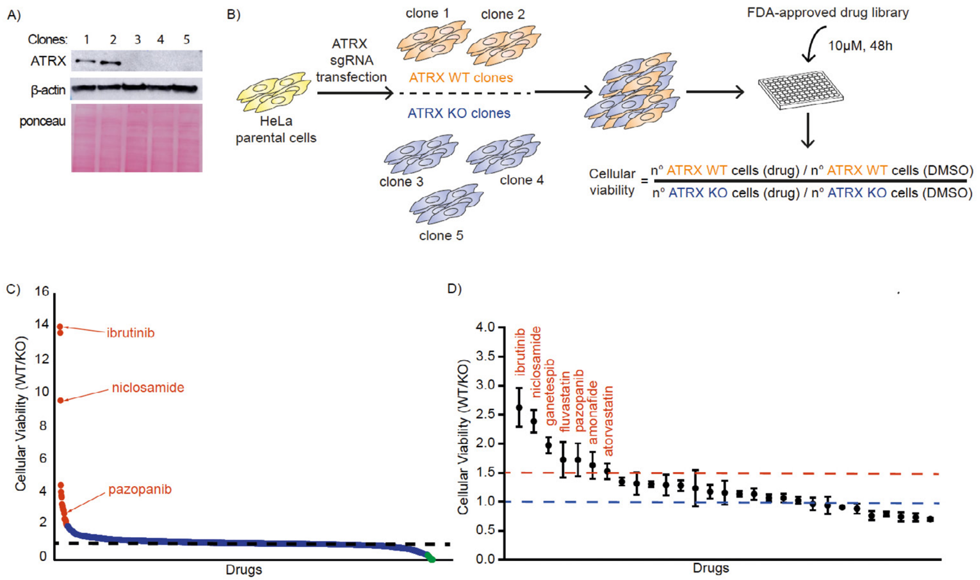
The focus of our group is the study of genomic instability and the DNA damage Response (DDR) in the context of cancer. The DDR is intimately linked to cancer development and cancer therapy. Indeed, many conventional chemotherapy agents and radiation therapy boost the levels of DNA damage to kill cancer cells. Our final aim is to identify novel therapeutic opportunities to treat cancer. For this, we perform cellular studies including proteomics, CRISPR genetic and drug screens to identify novel factors involved in the DDR. In addition, we use genetically modified mouse models and cellular systems to characterize the relevance of novel factors for cancer development and to develop novel anti-cancer therapies. My group is particularly interested in the study of the biology of Common Fragile Sites, which are conserved chromosomal regions with a high propensity to break in conditions of replication stress, and which are therefore frequently altered in cancer.

Research Highlights

Our group was established at the end of 2014 at the University of Copenhagen, Denmark, and moved to CABIMER/CSIC in July 2020. In the past years, we have contributed to the understanding of the effects of replication stress on aging, embryonic stem cell fate potential, and ovarian aging (Albers E et al., Aging 2020, Atashpaz S et al., eLife 2020; Ruth et al., Nature, 2021). In addition, we use genetically modified mouse models to characterize the relevance of novel factors for cancer and to develop novel anti-cancer therapies. We have generated a Pich conditional KO mouse model, identifying the DNA translocase PICH as an essential factor for embryonic development (Albers E et al., Cell Reports 2018) and a potential therapeutic target for cancer (Castejón-Griñán et al., in preparation). We are particularly interested in understanding the biology of the unstable genomic regions CFS and their contribution to cancer. We have generated the first CFS proteome, identifying among other factors the tumor suppressor ATRX to be essential to maintain CFS stability (Pladevall-Morera et al., Nucleic Acid Research 2018). We have recently developed a drug screen searching for compounds synthetic lethal with ATRX mutations (see figure below), frequently found in glioblastoma and other cancers. In this screen, we have identified RTK and specific PDGFR inhibitors (Pladevall-Morera et al., Cancers, 2021) and other molecules that we are currently characterizing. We have also identified SLX4IP as a regulator of CFS stability (Ingham et al., in preparation). Finally, we have generated the first mouse model with a deletion of a whole CFS that will be used to investigate the impact of FRA3B/FHIT loss in cancer and investigate whether FHIT deficiency can be exploited as a therapeutic opportunity.

Grants

- 2016-2022: ERC-2015-STG-679068, ERC Starting Grant
- 2021-2023: PY20-00755, Junta de Andalucía
- 2021-2024: PID2020-119329RB-I00, Ministerio de Ciencia e Innovación
- 2022-2026: Horizon-MSCA-2021-101072903, MSCA ITN network



FDA-approved drug screen identifies compounds synthetically lethal with ATRX deficiency. A) Immunoblotting of HeLa clones generated by CRISPR. B) Drug screen flowchart. C) Primary drug screen. Cellular viability (WT/KO) after 48h of drug treatment. Each point represents one drug. Red dots indicate drugs with a 2-fold higher lethality effect in the ATRX KO clones compared to WT clones. Green dots indicate compounds that induce higher toxicity in ATRX WT clones compared to ATRX KO clones. D) Secondary drug screen. Cellular viability (WT/KO) after 48h of drug treatment of the 29 top hits derived from the primary screen.

Publication Highlights

Pladevall-Morera D, Castejón-Griñán M, Aguilera P, Gaardahl K, Ingham A, Brosnan-Cashman JA, Meeker AK, **Lopez-Contreras AJ**. (2022) ATRX-Deficient High-Grade Glioma Cells Exhibit Increased Sensitivity to RTK and PDGFR Inhibitors. **Cancers (Basel)**, 14(7):1790.

Ruth KS, Day FR, Hussain J, Martínez-Marchal A, Aiken CE, Azad A, Thompson DJ, Knoblochova L,..., **Lopez-Contreras AJ**, Daniel JA, Stefansson K, Chang-Claude J, van der Schouw YT, Lunetta KL, Chasman DI, Easton DF, Visser JA, Ozanne SE, Namekawa SH, Solc P, Murabito JM, Ong KK, Hoffmann ER, Murray A, Roig I, Perry JRB. (2021) Genetic insights into biological mechanisms governing human ovarian ageing. **Nature**, 596(7872):393-397

Principal Investigator

Dr. Tatiana García-Muse

DNA Damage Response During Meiosis

Emerging PI

Current position

- Since 2021: Associated Professor, University of Seville-Andalusian Center for Molecular Biology and Regenerative Medicine, Seville, Spain.

Group Members

Postdocs

- Mariola Chacón Rodríguez.

Students

- Nuria Fernández Fernández.

Research Activity

Overview

Genomic DNA is exposed to both endogenous and exogenous DNA damaging agents. Without proper repair the resulting DNA damages would lead to 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.

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 phosphorylated 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).

We uncovered the in vivo relevance of one of this DNA damage-dependent phosphorylation identified by the peptide array, specifically the posttranslational modification of the C. elegans synaptonemal complex (SC) protein, SYP-1 (Figure 1). The SC is the structure that holds together the homolog chromosomes during meiosis, and it is crucial for proper meiotic recombination and chromosome segregation. The analysis of phosphomutants revealed how the phosphorylation of the synaptonemal complex bias the repair of persistent DSBs towards inter-sister recombination (Figure 2). Importantly this work validated our peptide array screening (Garcia-Muse et al., 2019).

We are characterizing the role of ATM/ATR-dependent phosphorylation of several proteins candidates from the peptide array. The analysis of some non-phosphorylable alleles has shown defects in DNA repair after IR, and we want to uncover the specific repair pathway involved. To further understand direct checkpoint regulation after DNA damage we are generating ATR/ATM versions that can be removed from the worm, especially at the germline, in a temporally controlled manner by introducing the TEV and/or AID epitopes.

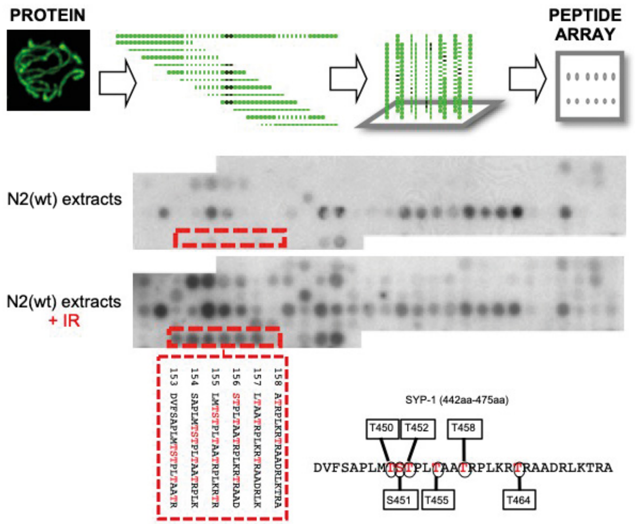


Figure 1: (Top) Scheme of the peptide array. The protein of interest can be scanned across its length by making 18 mer peptides that shifting by three residues. Each peptide is then spotted in a cellulose membrane. (Bottom) In vitro phosphorylation of the SYP-1 peptide array by N2(WT) extracts with/out 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.

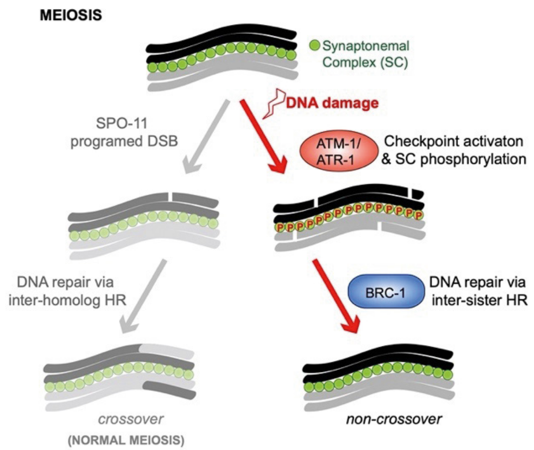


Figure 2: Model. During meiosis, SPO-11 DSBs are repaired by homologous recombination (HR) using the homolog chromatid as template (left). In a context where excessive DSBs are produced, the DNA damage checkpoint is activated and triggers phosphorylation of the SC component SYP-1 to bias repair through the sister chromatid as template that requires BRC-1 activity (right).

The long-term goal is to address at the molecular level the biological relevance of the DNA damage-dependent phosphorylations at meiotic proteins to ensure genome stability. Understanding this is of vital importance in order to have a major comprehensive view of the sources of errors that result in dramatically deleterious outcomes including infertility, miscarriages and birth defects such as Down syndrome. This knowledge of DDR regulation during meiosis should, therefore, provide important insights into fertility defects diagnosis and may present opportunities for therapeutic intervention.

Grants

- 2019-2022: PGC2018-101099B. Ministerio de Ciencia, Innovación y Universidades
- 2019-2021: 2019/00000463. Plan Propio US
- 2020-2022: 2020/00000690. VI Plan Propio Gestión I US
- 2022-2025: PID2021-123850N. Ministerio de Ciencia e Innovación
- 2022-2024: 2022/00000525. VII Plan Propio SuplemA US

Publication Highlights

Zheleva A, Camino LP, Fernández-Fernández N, García-Rubio M, Askjaer P, García-Muse T, Aguilera A. 2021. THSC/TREX-2 deficiency causes replication stress and genome instability in *Caenorhabditis elegans*. J Cell Sci. 134(20):jcs258435.

San Martin-Alonso M, Soler-Oliva ME, García-Rubio M, García-Muse T, Aguilera A. 2021. Harmful R-loops are prevented via different cell cycle-specific mechanisms. Nat Commun. 12(1):4451.

García-Muse T. 2021. Detection of DSB in *C. elegans* meiosis. Methods Mol. Biol. 2153:287-293

Principal Investigator

Dr. Silvia Jimeno-González

Transcription and mRNA processing

Emerging PI

Current position

- Since 2021, Assistant Professor, University of Seville, Andalusian Centre for Molecular Biology and Regenerative Medicine, Seville, Spain.
- 2016-2021: Ramón y Cajal Researcher, University of Seville, Andalusian Centre for Molecular Biology and Regenerative Medicine, Seville, Spain.

Academic Background of the Emerging PI

- 2001: Degree. University of Seville Biology.
- 2007: PhD. University of Seville, Doctor in Biology.

Positions Held

- 2007-2011: Postdoctoral Fellow, Molecular Biology Department, Aarhus University, Aarhus, Denmark.
- 2011-2014: Postdoctoral Fellow (Juan de la Cierva Researcher), CSIC, Andalusian Centre for Molecular Biology and Regenerative Medicine, Seville, Spain.
- 2015-2016: Postdoctoral Fellow, University of Seville, Andalusian Centre for Molecular Biology and Regenerative Medicine, Seville, Spain.

Group Members

PhD Students

- Clara Megías-Fernández.

Postdoctorals

- Sabrina Rivero.

Technicians

- Irene Delgado-Sainz.

JAEINTRO fellow

- Alberto León.

Former members (2021-2022)

- **Technicians:** Valentina Buglioni.
- **Master students:** Ainhoa Pérez; Cristina Peral.
- **Erasmus+ Master students:** Angelo Meoli.

Research Activity

Overview

The maintenance of cell homeostasis requires a dynamic regulation of gene expression. Transcription output is modulated at two main levels: transcription initiation and promoter-proximal pausing that takes place shortly after starting RNA synthesis. We have recently proposed that transcription repression through promoter-proximal pausing is coordinated with topoisomerase II (TOP2) activity. Considering that TOP2 produces transient breaks in the DNA to alleviate supercoiling, and that this can lead to DNA damage, genes regulated at pause level, could have more risk of genome instability. The use of TOP2 poisons, which stimulate the induction of TOP2 breaks, as chemotherapeutic agents, can increase that threat, and eventually trigger secondary malignancies. We aim to understand the mechanisms by which gene expression changes because of the generation of TOP2 breaks and the impact of such changes in DNA repair. Our work constitutes an attempt to exploit the scientific and biomedical potential of these avenues of research.

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 this 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. In this context, our group has discovered that TOP2 has also a negative function in transcription elongation through the removal of negative supercoiling at promoter regions. When TOP2 is inhibited, promoter-proximal pausing is disfavored and transcription of a subset of genes is upregulated (Figure 1). Early-response genes (ERG) expression is highly affected by changes in topoisomerase function because its regulation depends on the maintenance of promoter-proximal pausing.

Because of our previous work in which we established a connection between promoter-proximal pausing and TOP2 activity at promoters, we have decided to study 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 (⍰-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. Using this approach and ChIP-seq experiments, we have discovered that elongation factors are specifically important for the signaling of TOP2 breaks for the recruitment of repair factors.

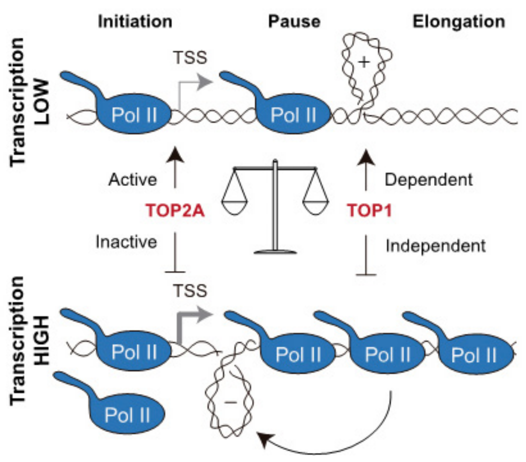


Figure 1. TOP2 function in regulation of transcription. TOP2A removes negative supercoiling at promoters while TOP1 removes positive supercoiling in the gene body. Under TOP2A inhibition, negative supercoiling accumulates, promoter-proximal pausing is destabilized, and transcription becomes TOP1 independent.

Overall, our results show that topoisomerase activity at promoter regions is closely related with the regulation of transcription elongation, more specifically, at promoter-proximal pausing level. Our future goal is to analyze the consequences of the generation of TOP2-breaks at promoters in gene expression at different levels, such as transcription elongation, termination, chromatin remodeling and RNA processing, and the interactions between TOP2 and regulatory factors. We will also study the relevance of changes in gene expression in response to TOP2-breaks in DNA repair. We also aim to determine whether these mechanisms are general or specific of certain subset of highly regulated genes.

Grants (2021-2022) (starting or ending 2021-2022)

- 2022-2025: ProyExcel_00835, Junta de Andalucía.
- 2020-2023: PID2019-104484G, Ministerio de Ciencia e Innovación
- 2020-2022: AYUDA SUPLEMENTARIA VI PP, Universidad de Sevilla

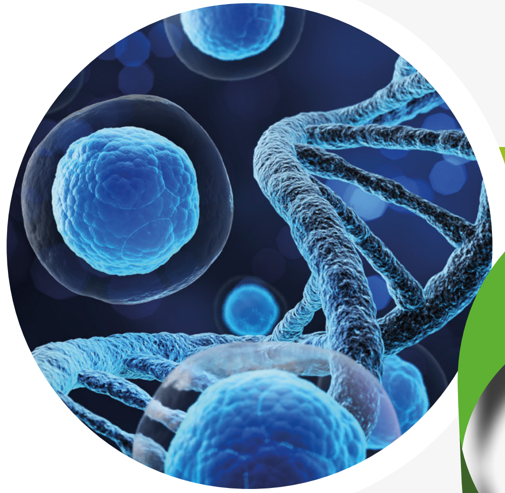
Publication Highlights

Delgado-Chaves, F. M., Martínez-García, P.M., Herrero-Ruiz, A., Gómez-Vela, F., Divina, F., Jimeno-González, S., Cortés-Ledesma, F. (2022) Data of transcriptional effects of the merbarone-mediated inhibition of TOP2. ***Data in Brief.*** Aug 1;44:108499.

Jimeno, S., Prados-Carvajal, R., Fernández-Ávila, M.J., Silva, S., Silvestris, D.A., Endara-Coll, M., Domingo-Prim, J., Mejías-Navarro, F., Rodríguez-Real, G., Romero-Franco, A., Jimeno-González, S., Barroso, S., Cesarini, V., Aguilera, A., Gallo, A., Visa N. And Huertas, P. (2021) ADAR2-mediated RNA editing of DNA:RNA hybrids is required for DNA double strand break repair. ***Nature communications.*** Sep 17;12(1):5512.

Payán-Bravo L., Fontalva S., Peñate X., Cases I., Guerrero-Martínez J.A., Pareja-Sánchez Y., Odriozola-Gil Y., Lara E., Jimeno-González S., Suñé C., Muñoz-Centeno M.C., Reyes J.C., Chávez S. (2021) Human prefoldin modulates co-transcriptional pre-mRNA splicing. ***Nucleic Acids Research***, Jun 7:gkab446.

Herrero-Ruiz, A., Martínez-García, P., Terrón-Bautista, J., Millán-Zambrano, G.; Lieberman, J.A., Jimeno-González, S.* and Cortés-Ledesma F.* (2021) Topoisomerase II⍰ represses transcription by enforcing promoter-proximal pausing. ***Cell Reports***, Apr 13;35(2):108977. *Corresponding authors

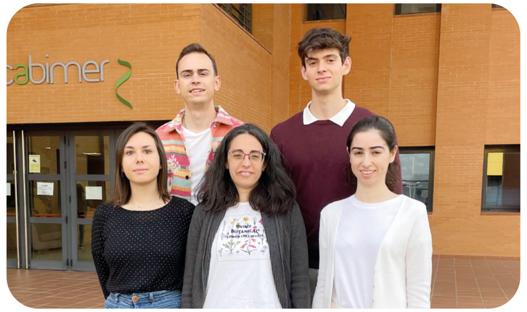


Principal Investigator

Dr. Cristina González-Aguilera

Replication and Nuclear Dynamics

Emerging PI



Current position

- Since 2020: Ramón y Cajal Researcher, University of Seville, Andalusian Center for Molecular Biology and Regenerative Medicine, Seville, Spain.
- Since 2020: Scientific responsible for the Genomic Unit at Cabimer.

Academic Background of the Emerging PI

- 2004: Degree in Biology. University of Seville.
- 2009: PhD in Biology. University of Seville.

Positions Held

- 2004-2005: Predoctoral Fellow "Formación de doctores" from Andalusian Government at Department of Genetics, University of Seville, Spain.
- 2005-2009: Predoctoral Fellow "FPU" from the Spanish Government at Andalusian Center for Molecular Biology and Regenerative Medicine associated with the Department of Genetics, University of Seville, Spain.
- 2009-2012: Postdoctoral contract at Andalusian Center for Developmental Biology, University Pablo de Olavide, Seville, Spain.
- 2012-2013: Postdoctoral contract at Andalusian Center for Developmental Biology, CSIC, Seville, Spain.
- 2013-2016: Postdoctoral Grant. Lundbeck Foundation. Biotech Research and Innovation Center, University of Copenhagen, Denmark.
- 2016-2018: Postdoctoral contract at Andalusian Center for Molecular Biology and Regenerative Medicine. CSIC.
- 2019-2019: Postdoctoral contract at Biomedicine Institute of Seville, FISEVI.

Group Members updated

PhD student

- Federica Bruno.

Technician

- Cristobal Coronel Guisado.

Master student

- María Marco Simancas.

Undergraduate student

- Roberto García Delgado.

Former Members (2021-2022)

- **Master student:** Adrián Núñez Sancho; María Navarro Riquelme.

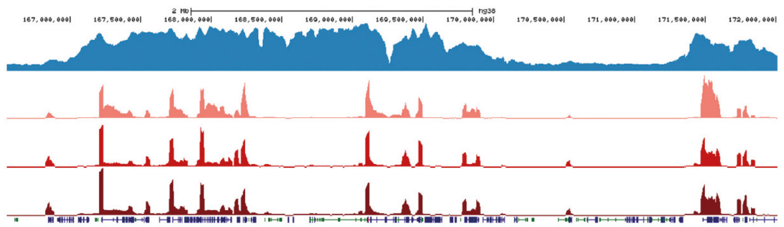
Research Activity

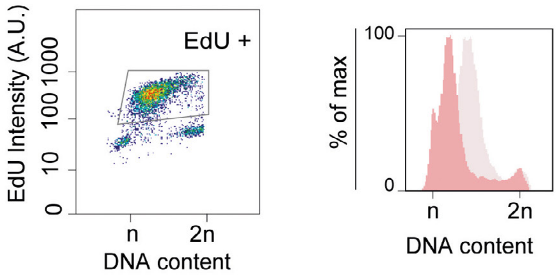
Overview

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 may take 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 alteration may impact gene expression regulation, nuclear dynamics and human diseases.

Research Highlights

During chromatin replication, parental histones have to be evicted from the DNA to allow the passage of the replication fork. Then, parental and newly synthetized histones are mixed together and relocate into the two daughter-strands to restore chromatin structure and nucleosome density. This parental histone recycling and its assembly with the new histones produce a genome-wide chromatin disorganization that includes reduction of chromatin accessibility and dilution of the epigenetic information.





Therefore, the cell has to assure that the epigenetic information is transmitted to the daughter's cells in a reliable way. Despite the great relevance that all these basic cellular activities could have in human diseases, our current knowledge of the regulation of chromatin maintenance after cell division is very limited.

In our lab we make use of ChOR-seq technology (Chromatin Occupancy after Replication), a cuting-edge technique that we developed and that it is able to purify proteins and histone PTMs associated to nascent chromatin in mammalian cells. The technique is based on the in vivo labelling of newly replicated DNA with 5-Ethynyl-2´-deoxyuridine (EdU), a thymidine analogue. After DNA labelling, chromatin bound proteins are sequentially purified first, by chromatin immunoprecipitation (ChIP) against the protein of interest and later by streptavidin-capture of biotinylated EdU-labelled DNA. Then, purified DNA is identified by next generation sequencing. With this technology, we have revealed that in human cells, parental histones carrying both active and silent histone PTMs are recycled precisely at their original pre-replicated positions, facilitating the maintenance of parental epigenetic patterns in the two newly replicated strands. However, the restoration of new histone PTM levels is mark and locus specific. Some marks are fast, as H3K4me3, which restoration is completed within 6 hours, before cell division. However, H3K27me3 restoration is slow and its methylation continues until the next round of chromatin replication in the daughter cell. All these findings confirm the existence of a complex epigenetic changes across the cell cycle that may play important roles on cellular function and human diseases.

Considering that the post-replicative chromatin rearrangements take hours to be restored, we are now also studying whether these changes may alter gene expression. In order to do that we are combining analysis of RNAPII activity and synthesis of nascent RNA.

Grants (2021-2022)

- 2021-2023 US-1381081. FEDER Universidad. Junta de Andalucía.
- 2020-2023 PID2019-105742GA-100. Ministerio de Ciencia e innovación.
- 2020-2024 RYC2018-025485-I. Ayuda Ramón y Cajal. Ministerio de Ciencia e Innovación.

Publication Highlights

Lopez-Alvarez, M., Gonzalez-Aguilera, C., Moura, D.S., Sanchez-Bustos, P., Mondaza-Hernandez, J.L., Martin-Ruiz, M., Renshaw, M., Ramos, R., Castilla, C., Blanco-Alcaina, E., et al. (2022). Efficacy of Eribulin Plus Gemcitabine Combination in L-Sarcomas. Int J Mol Sci 24. 10.3390/ijms24010680

López-Jiménez E, González-Aguilera C* (2022) Role of Chromatin Replication in Transcriptional Plasticity, Cell Differentiation and Disease. Genes. 13(6):1002. https://doi.org/10.3390/genes13061002 * Coresponding author.

Petryk N *, Reveron-Gomez N*, Gonzalez-Aguilera C*, Dalby M, Andersson R, Groth A. (2021) Genome-wide and sister chromatid-resolved profiling of protein occupancy in replicated chromatin with ChOR-seq and SCAR-seq. Nat Protocol. 16(9) 4446-4493 *EQUAL CONTRIBUTION.

Principal Investigator

Iván V. Rosado

Replication and endogenous DNA damage

Group Leader

Current position

- Research Scientist CABIMER.
- Associate Professor of the University of Seville.

Academic Background of the Emerging PI

- PhD in Molecular Biology.

Group Members

PhD student
- María José Peña-Gómez.

Technicians
- Jesús Cea García.
- Gonzalo Pinaglia Tobaruela.

Former Members (2020-2022)

- **PhD students:** Paula Moreno Gordillo.
- **Master student:** Marina Suarez Pizarro, JAEIntro.

Research Activity

Overview

Maintenance and faithful inheritance of genetic information is essential to avoid disease. Therefore, cells evolved a wide range of protection mechanisms responsible for the preservation of the genetic material. Special group of damaging agents are those produced within our cells during cellular metabolic reactions. Our group is interested in uncovering the nature of these endogenous metabolites inflicting DNA damage, to decipher the molecular mechanisms operating during replication, that help to avoid the catastrophic consequences of genetic instability caused by endogenous processes.

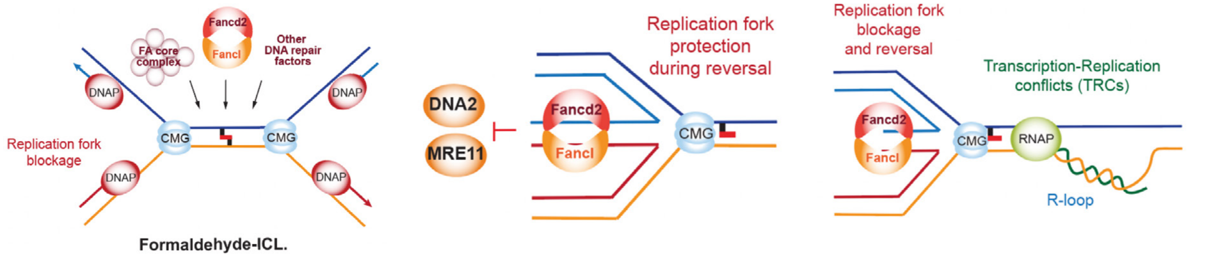


Figure 1. Cellular roles of the FA DNA repair pathway. Upon ICL formation, stalling of converging replication forks trigger a DNA damage response thus activating the FA pathway. The FA core complex monoubiquitinates the FANCD2-FANCI heterodimer to coordinate the incision steps during ICL repair. Upon replication fork stalling due to physical or chemical constrictions, the FA pathway maintains replication fork stability, avoids extensive DNA resection and promotes replication restart. The FA pathway prevents genomic instability at transcription-replication conflicts (TRCs)

Research Highlights

Accurate repair of damaged DNA is essential to ensure the faithful transmission of genetic information from a mother to its daughter cell. To avoid devastating consequences Therefore, cells have evolved several repair mechanisms that survey, detect and fix DNA lesions, in humans. Defective repair of DNA lesions underlies the Fanconi Anaemia (FA) syndrome, an ultrarare genetic instability syndrome featured by congenital abnormalities, stem cell loss and extreme cancer predisposition. The FA/BRCA repair pathway comprises 22 so far identified genes (FANCA to FANCW), essential for interstrand crosslink (ICL) repair at converging replication forks during S phase. The FA pathway received much attention since the FANCD1 gene was identified as BRCA2, the most frequently mutated gene in breast and ovarian cancer. The FA pathway play many crucial roles in genome maintenance like ICL repair, regulation of replication fork stability or avoidance of toxic DNA:RNA hybrids (R-loops) at transcription replication conflicts (TRCs) (Figure 1).

Many chemical and physical agents constantly challenge ongoing DNA replication. Our research effort focusses on the identification of metabolic agents endogenously produced that thread replication dynamics, leading to replication stress. Our research has identified 5-hydroxymethylated nucleosides (i.e. the cytosine demethylation base 5-hydroxymethyl-cytosine, 5hmdC), as potential hazardous nucleoside that threatens replication and genome integrity (Peña Gomez et al. IJMS, 2022; Peña-Gomez et al. Cell Death and Disease, 2022). We have revealed that misincorporation of 5-hydroxymethylated

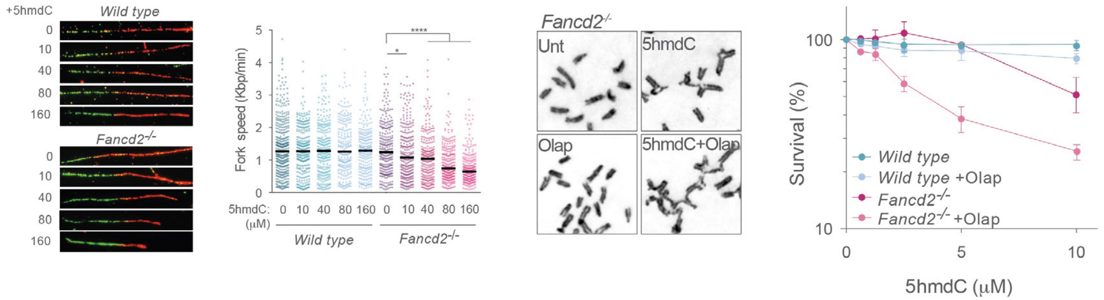


Figure 2. DNA fiber technique showing CldU (green) and IdU (red) tracks from wild type or *Fancd2*$^{-/-}$ MEFs exposed to increased concentration of 5hmdC (10, 20, 40 or 160 μM) for 30 minutes. Dot plot showing fork speed upon 5hmdC treatments. Chromosome fragility test of *Fancd2*$^{-/-}$ MEFs exposed to 5hmdC, the PARP1-trapping drug olaparib or combination of both compounds. Cell survival curve representing wild type or *Fancd2*$^{-/-}$ MEFs exposed to increasing dose of 5hmdC in the presence or absence of olaparib.

DNA bases disturb replication progression leading to genome instability in the absence of the Fanconi Anaemia DNA repair pathway (Figure 2).

We also uncovered that 5hmdC is deaminated to 5-hmdU, a toxic uridine analogue (Figure 3). 5hmdU is actively removed from the genome by the concerted actions of base excision repair factors. Thus, BER deficiency, either caused by pharmacological drugs targeting PARP1, or genetic inactivation by deletion of XRCC1 exacerbates 5hmdU genotoxicity. The mechanism by which 5hmdC and 5hmdU causes extensive genomic instability seems to be due to collisions of the replisome with ongoing BER intermediates undergoing fixation. BER intermediates, likely ssDNA gaps or AP sites are well-known potent replication stressors, therefore activating the FA pathway to promote fork stabilization.

Our main research lines are the following:

1-. Molecular mechanisms of Interstrand Crosslink (ICLs) repair: Our work uncovered endogenously reactive aldehydes as a novel source of ICLs, and how the Fanconi Anaemia DNA repair pathway orchestrates ICL-repair. We are currently deciphering the molecular mechanisms employed by cells to detect, signal and repair ICLs, by focusing on the identification of new players and the characterization of the molecular mechanisms of replication-associated DNA repair.

2-. Interplay of DNA repair mechanisms during replication fork impairment: During ICLs sensing and signaling by the ongoing replication fork, several distinct DNA repair pathways (Fanconi Anaemia, BER, HR, NHEJ, MMEJ...) converge to promote error-free repair. However, pathway choice determining the optimal consecution of repair event is poorly understood. We currently examine the interplay of different repair pathways in collaboration with the Fanconi Anaemia pathway to limit genome instability associated to replication fork defects during nucleoside misincorporation.

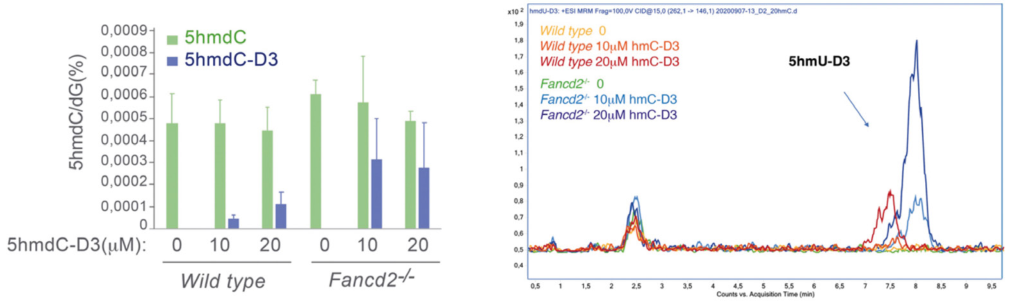


Figure 3. Levels of endogenous (5hmdC) and exogenous 5hmdC-D3 in genomic DNA samples from *wild type* or *Fancd2*$^{-/-}$ MEFs upon supplementation of culture medium with 5hmdC-D3 (10 or 20 μM) for 16 hours. HPLC chromatogram shows accumulation of the 5hmdC deamination product 5hmdU on genomic DNA samples from *wild type* or *Fancd2*$^{-/-}$ MEFs upon supplementation with 5hmdC-D3 (10 or 20 μM) for 16 hours.

3-. Molecular mechanisms of Interstrand Crosslink (ICLs) repair: Genetic mouse models of FA offers a great opportunity to investigate novel therapeutic approaches to improve bone marrow function and prevent cancer development in these patients.

Grants (starting or ending 2021-2022)

- 2022-2024: PID2021128988OB-100 Ministerio de Ciencia e Innovación.
- 2019-2022: P18-RT-1271 Junta de Andalucía.
- 2020-2022: US-1381081 Junta de Andalucía.

Publication Highlights

Peña-Gómez MJ, Moreno-Gordillo P, Narmonte M, García-Calderón CB, Rukšenaite A, Klimašauskas S, Rosado IV. 2022. FANCD2 maintains replication fork stability during misincorporation of the DNA demethylation products 5-hydroxymethyl-2'-deoxycytidine and 5-hydroxymethyl-2'-deoxyuridine. **Cell Death and Disease.** May 27;13(5): 503. doi: 10.1038/s41419-022-04952-0. PMID: 35624090

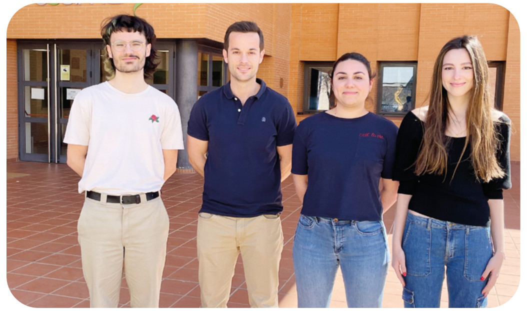
Peña-Gómez MJ, Suárez-Pizarro M, Rosado IV. 2022. XRCC1 prevents replication fork instability during misincorporation of the DNA demethylation bases 5-hydroxymethyl-2'-deoxycytidine and 5-hydroxymethyl-2'-deoxyuridine. **International Journal of Molecular Sciences.** Jan 14;23(2):893. doi: 10.3390/ijms23020893.

Principal Investigator

Gonzalo Millán Zambrano

Chromatin modifications

Emerging PI



Current position

- Since 2021: La Caixa Junior Leader, University of Seville, Andalusian Centre for Molecular Biology and Regenerative Medicine, Seville, Spain.

Group Members

Postdocs
- Patrick Toolan Kerr

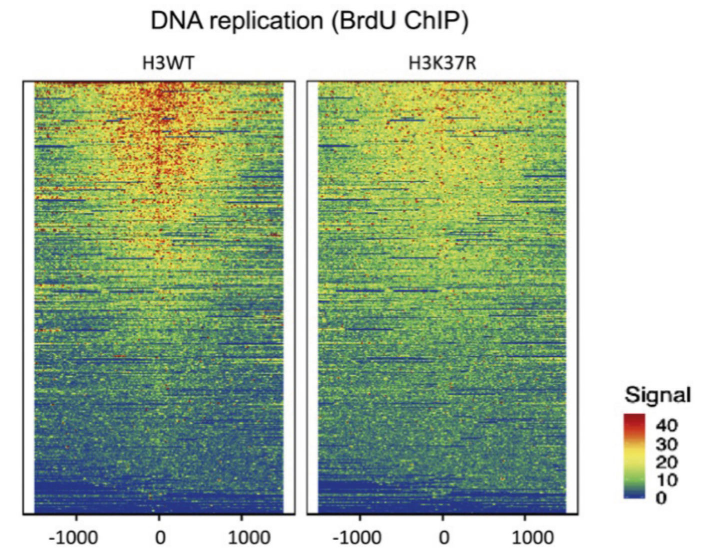
PhD students
- Laura López Hernandez

Master students
- Diego Polanco Alonso

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, 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.



H3K37me1 regulates DNA replication origin firing. Heatmap showing the distribution of BrdU incorporation at ARS in wild-type H3 and H3K37R mutant cells. Origins were aligned from highest to lowest BrdU signal in the wild-type strain and centered at ARS ACS (ARS consensus sequence).

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 still remains to be elucidated. During the 2021-2022 period we characterized the role of a novel histone PTM, mono-methylation of histone H3 at lysine 37 (H3K37me1). We demonstrated that H3K37me1 is catalyzed by Set1p and Set2p, and that it regulates DNA replication initiation (Santos-Rosa et al, Mol Cell 2021). In particular, 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 indicate that H3K37me1 safeguards the correct execution of the 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 tumor 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-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.

Grants (starting or ending 2021-2022)

- 2022-2024: PID2021–127432NA-100. Ministerio de Ciencia e Innovación.
- 2021-2024: LCF/BQ/PR21/11840007. Fundación La Caixa.

Publication Highlights

Millán-Zambrano G, Burton A, Bannister A, Schneider R. (2022) Histone post-translational modifications - cause and consequence of genome function. Nat. Rev. Genet. 23(9):563-580.

Santos-Rosa H*, Millán-Zambrano G*, Han N*, Leonardi T, Klimontova M, Nasiscionyte S, Pandolfini L, Tzelepis K, Bartke T, Kouzarides T. (2021) Methylation of histone H3 at lysine 37 by Set1 and Set2 prevents spurious DNA replication. Mol Cell. S1097-2765(21)00326-9. (*Joint first author).

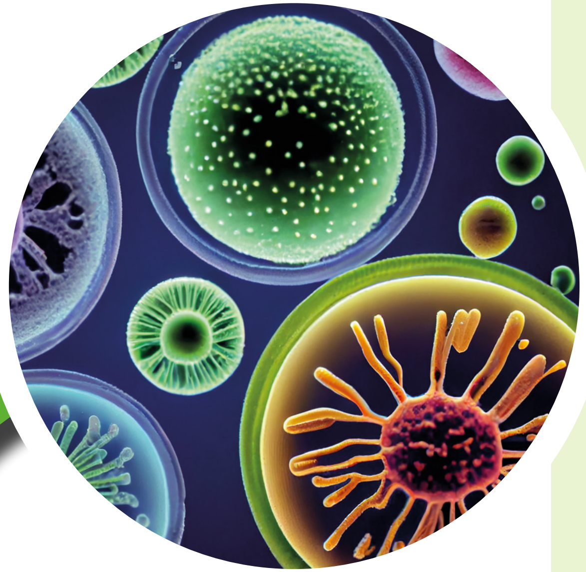
Herrero-Ruiz A, Martínez-García PM, Terrón-Bautista J, Millán-Zambrano G, Lieberman JA, Jimeno-González S, Cortés-Ledesma F. (2021) Topoisomerase IIα represses transcription by enforcing promoter-proximal pausing. Cell Rep. 13;35(2):108977.

Genome Biology

Cell Dynamics

Cell Dynamics and Signaling

The interest of the Department of Cell Dynamics and Signaling is focused on the knowledge of the mechanisms that ensure the normal functioning of the cell and that safeguard the homeostasis of the tissues. The correct development of cell division, migration, differentiation, morphogenesis and death processes is essential for the integrity of organisms. The alteration of these processes is normally associated with the development of different pathologies, among which cancer and degenerative diseases stand out. The alteration of cell division, as well as the processes of cell differentiation and death, is closely associated with the development of cancer, and together with the migratory and invasive capacity that tumor cells can acquire, are typical signs of cancer. The correct progression and coordination of these processes requires the existence of finely regulated signaling mechanisms, capable of integrating both internal and external signals to generate the appropriate responses and preserve the correct cellular physiology. The main objective of the Department is to advance in the knowledge of these signaling and response mechanisms, both in normal conditions and in altered or pathological conditions, thus contributing to define more precisely and effectively routes of therapeutic intervention against the aforementioned pathologies. Recently, two research groups initially assigned to the other departments (those of Dr. Anabel Rojas and Dr. Román González Prieto) have joined our six previously existing groups.



HEAD OF DEPARTMENT

Mario García Domínguez

RESEARCH GROUPS

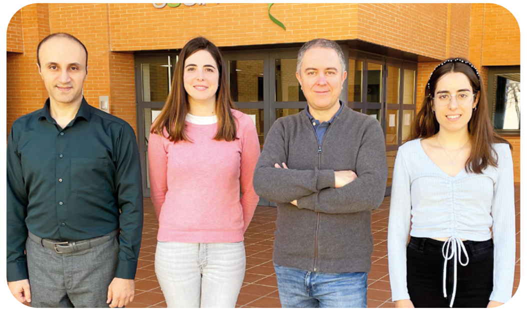
1. **Cell Differentiation**
   Dr. Mario García
2. **Cell Death Signalling**
   Prof. Abelardo López
3. **Cell Cycle and Oncogenesis**
   José Antonio Pintor
4. **Microtubule Dynamics in Health and Disease**
   Dr. Rosa M. Ríos
5. **Cell Division Control**
   Dr. Fernando Monje
6. **Metabolism and Cell 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

Principal Investigator

Mario García-Domínguez

Cell Differentiation Laboratory

Group Leader and Head
of the Department



Current position

- Since 2009: Research Scientist CSIC / Cabimer, Seville, Spain.

Group Members

PhD students

- Vahid Jafari.

Postdocs

- Nieves Lara Ureña.

Technicians

- Rosa Mª Troya Toledo.

Former Members (2020-2021)

- **Postdocs:** Pablo García Gutiérrez.
- **PhD students:** Juan Fco. Correa Vázquez.
- **Master students:** Alfonso Castañeda Segura; Olga Fernández Romero.

Research Activity

Overview

The main objective of our research is to decipher the molecular mechanisms that control the transition from proliferation to differentiation, especially in relation to the development of the nervous system. In particular, we study the post-translational modification of proteins by covalent binding of the SUMO polypeptide (sumoylation), focusing mainly on transcriptional control and the analysis of chromatin. We are also interested in the relationship of the molecular mechanisms underlying these processes with cancer and cell viability. SUMO is essential in eukaryotes and is involved in the regulation of many cellular processes, in particular through the control of gene expression. On the other hand, it has been described that protein sumoylation safeguards cell viability. Knowing in detail the components of the SUMO pathway involved in these processes and the associated regulatory mechanisms is of great therapeutic interest in relation to cancer and nervous system disorders.

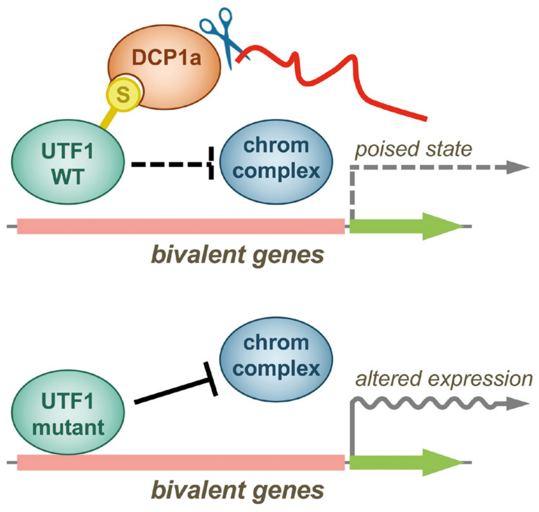


Figure 1. Sumoylation regulates transcriptional function of UTF1. Sumoylation of wild type (WT) UTF1 attenuates UTF1 binding to the chromatin to exert a milder exclusion effect on association of chromatin complexes to promoters of bivalent genes. SUMO also mediates recruitment of decapping enzyme DCP1a to degrade leakage mRNAs. Both mechanisms contribute to maintain transcription of bivalent genes in a poised state. A sumoylation mutant of UTF1 does not recruit DCP1a and stronger associates with the chromatin, which may alter normal association of chromatin complexes, resulting in altered expression of bivalent genes.

Research Highlights

The SUMO polypeptide is similar to the Ubiquitin and its covalent attachment to proteins has drastic consequences on protein properties and functions. A relevant role in the process of sumoylation is displayed by SUMO ligases and proteases, as they enhance and recycle SUMO from targets, respectively. To date, up to six different SUMO proteases belonging to the SENP family have been described (1-3, 5-7). Although SUMO is involved in the control of virtually all the cellular processes, very little is known about its role in initial steps of neurogenesis. To shed light on this, we have conducted a SILAC-based proteomic study to identify proteins sumoylated under proliferation and neuronal differentiation conditions, finding more than 300 proteins differentially sumoylated. This has allowed us to discover for the first time, the transcription factor UTF1, as a target of SUMO. Sumoylation modulates its chromatin affinity and mediates the recruitment of the decapping enzyme DCP1A to keep relevant development-associated bivalent genes in a transcriptional poised state for rapid activation in response to specific signaling.

We have also investigated changes in expression levels of genes coding for the different components of the sumoylation

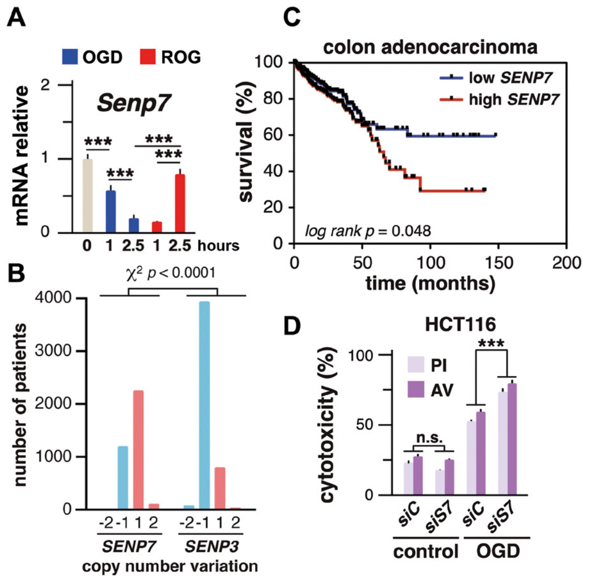


Figure 2. SENP7 promotes cell viability under limiting conditions of oxygen and glucose, and is a prognostic marker for colon cancer. **A)** *Senp7* expression is downregulated under oxygen and glucose deprivation (OGD) conditions and reactivates after restoration of oxygen and glucose (ROG). **B)** In most cancers, the SENP7 locus is associated with greater copy number variation, whereas the SENP3 locus (previously shown to promote cell death) is associated with less copy number variation. **C)** A worse prognosis is observed for patients with colon cancer with a higher expression of SENP7. **D)** In HCT116 colon cancer cells, siRNA (si)-mediated downregulation of SENP7 (S7) sensitizes cells to death under OGD conditions in comparison with control siRNA (siC), as determined by propidium iodide (PI) and Annexin V (AV) labeling.

pathway under ischemia simulated conditions. These conditions are achieved by oxygen and glucose deprivation (OGD), which also associates with the interior of solid tumors. We have interestingly observed that under OGD conditions, the SUMO protease SENP7 is dramatically downregulated, and that restoring of normal growth conditions leads to recovering of normal SENP7 levels. We have found that overexpression of SENP7 in tumor cells leads to enhanced viability in response to deleterious OGD conditions, in contrast to SENP3 overexpression that promotes cell death, as previously described. Specially for colon cancer, SENP7 is a prognosis marker, with poorer outcomes when overexpressed.

Grants

- 2022-2025: PID2021-125791NB-I00. Ministerio de Ciencia e Innovación.
- 2020-2022: PY18-1962. Consejería de Economía, Conocimiento, Empresas y Universidades, Junta de Andalucía.
- 2020-2021: CV20-93141. Consejería de Economía, Conocimiento, Empresas y Universidades, Junta de Andalucía.
- 2019-2022: PGC2018-094232-B-I00. Ministerio de Ciencia e Innovación.

Publication Highlights

Gallardo-Chamizo F, Lara-Ureña N, Correa-Vázquez JF, Reyes JC, Gauthier BR, García-Domínguez M. **2022.** SENP7 overexpression protects cancer cells from oxygen and glucose deprivation and associated with poor prognosis in colon cancer. **Genes Dis.** 9: 1419-1422

Lara-Ureña N, Jafari V, García-Domínguez M. **2022.** Cancer-Associated Dysregulation of Sumo Regulators: Proteases and Ligases. ***Int J Mol Sci.*** 23: 8012

García-Domínguez M. **2022.** Proper progression of neurogenesis relies on a defined pattern of SUMO-modified proteins. **Neural Regen Res.** 17: 1731-1732

Correa-Vázquez JF, Juárez-Vicente F, García-Gutiérrez P, Barysch SV, Melchior F, García-Domínguez M. **2021.** The Sumo proteome of proliferating and neuronal-differentiating cells reveals Utf1 among key Sumo targets involved in neurogenesis. **Cell Death Dis.** 12: 305

García-Gutiérrez P, García-Domínguez M. **2021.** BETting on a Transcriptional Deficit as the Main Cause for Cornelia de Lange Syndrome. **Front Mol Biosci.** 8: 709232

Lara-Ureña N, García-Domínguez M. **2021.** Relevance of BET Family Proteins in SARS-CoV-2 Infection. **Biomolecules.** 11: 1126

García-Jiménez MJ, Gil-Caballero S, Maza S, Corzana F, Juárez-Vicente F, Miles JR, Sakamoto K, Kadomatsu K, García-Domínguez M, de Paz JL, Nieto PM. **2021.** Midkine Interaction with Chondroitin Sulfate Model Synthetic Tetrasaccharides and Their Mimetics: The Role of Aromatic Interactions. **Chemistry.** 27: 12395-12409

García-Gutiérrez P, García-Domínguez M. **2021.** SUMO control of nervous system development. **Semin Cell Dev Biol.** 132: 203-212

Principal Investigator

Dr. Raúl V. Durán

Metabolism and cell signaling

Group Leader

Current position

- Since 2018: Senior Research Associate, Spanish National Research Council, CSIC/ CABIMER.
- Since December 2021: Deputy Director of CABIMER, Seville, Spain.

Group Members

Senior Researcher

- Dr. Socorro Murdoch (Associate Professor).

Postdoctorals

- María Jesús Fernández Ávila.
- Jonathan Martínez Fábregas.
- Dr. Macarena Morillo Huesca.
- Dr. Mercedes Tomé Montesinos.

PhD student

- Laura Zarzuela Moncada.
- Ignacio González López Cepero.

Technicians

- Ana Reina Bando.

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 normal functioning 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, a protein complex essential in the regulation of cell growth and metabolism. Our investigations have shed light on how glutamine and mTOR interact in an altered way in tumor cells through a mechanism that we have termed "glutamoptosis", and propose new therapeutic approaches to specifically target tumor cells in cellular and animal models.

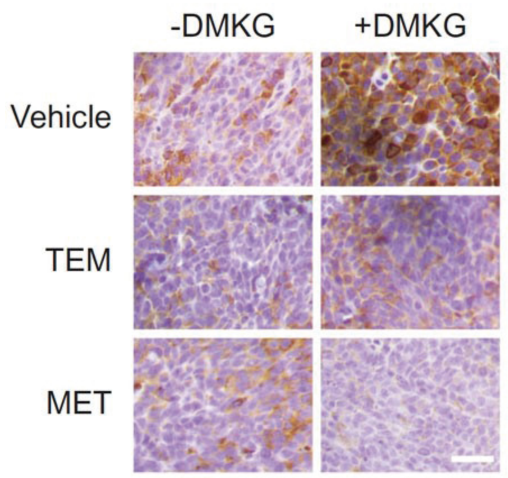


Figure 1. Glutamoptosis in vivo. Tumour cells increased mTORC1 signalling, as monitored by S6 phosphorylation, in mice harbouring colorectal tumours and treated with DMKG. Activation of mTORC1 was reversed by temsirolimus (TEM) or metformin (MET) treatment (Bodineau et al., Nat Comms 2021).

Research Highlights

Two parallel pathways connect glutamine metabolism and mTORC1 activity to regulate glutamoptosis

Glutamoptosis is the induction of apoptotic cell death as a consequence of the aberrant activation of glutaminolysis and mTORC1 signaling during nutritional imbalance in proliferating cells. The role of the bioenergetic sensor AMPK during glutamoptosis is not defined yet. Our recent results showed that AMPK reactivation blocks both the glutamine-dependent activation of mTORC1 and glutamoptosis in vitro and in vivo. We also show that glutamine is used for asparagine synthesis and the GABA shunt to produce ATP and to inhibit AMPK, independently of glutaminolysis. Overall, our results indicate that glutamine metabolism is connected with mTORC1 activation through two parallel pathways: an acute alpha-ketoglutarate-dependent pathway; and a secondary ATP/ AMPK-dependent pathway. This dual metabolic connection between glutamine and mTORC1 must be considered for the future design of therapeutic strategies to prevent cell growth in diseases such as cancer.

Glutamine, mTOR and autophagy: a multiconnection relationship

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/

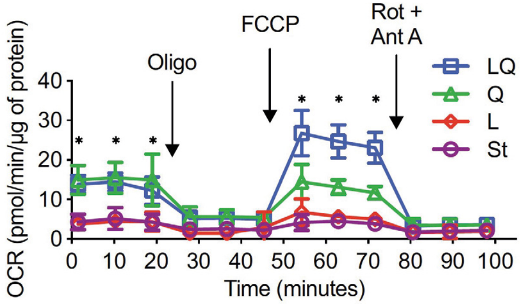


Figure 2. Glutamine is sufficient to induce bioenergetic increase in cancer cells. Seahorse analysis showed an increase in mitochondria-derived ATP production in cells treated with glutamine alone (Bodineau et al., Nat Comms 2021).

autophagy pathway through two independent branches involving glutaminolysis and ASNS-GABA shunt.

Glutamine Synthetase as a key factor for glutamine addiction in Notch-driven cancer

Previously, glutaminolysis inhibition has been proposed to synergise with anti-Notch therapies in T-cell acute lymphoblastic leukemia (T-ALL) models. In our investigations, we have recently demonstrated that Notch1 upregulation in T-ALL induces a change in the metabolism of the important amino acid glutamine, preventing glutamine synthesis through the downregulation of glutamine synthetase. Downregulation of glutamine synthetase is responsible for glutamine addiction in Notch1-driven T-ALL both in vitro and in vivo. Our results also confirm an increase in glutaminolysis mediated by Notch1. Increased glutaminolysis results in the activation of the mTORC1 pathway, a central controller of cell growth. However, glutaminolysis does not play any role in Notch1-induced glutamine addiction. From a clinical perspective, the combined treatment targeting mTORC1 and limiting glutamine availability has a synergistic effect to induce apoptosis and to prevent Notch1-driven leukemia progression. Our results place glutamine limitation and mTORC1 inhibition as a potential therapy against Notch1-driven leukemia.

Grants

- 2022 – 2025: PID2021-124251OB-I00), Ministry of Science and Innovation of Spain.
- 2021 - 2023: PY20_00757 Regional Ministry of Economy, Industry, Knowledge and Universities.
- 2021-2022: US-1381282, University of Seville and Regional Ministry of Economy, Industry, Knowledge and Universities.
- 2022: 2021AEP005, Spanish National Research Council - CSIC.
- 2019 - 2021: PGC2018-096244-B-I00, Ministry of Science, Innovation and Universities of Spain.

Publication Highlights

Gómez-Marín E, Posavec-Marjanović M, Zarzuela L, Basurto-Cayuela L, Guerrero-Martínez J, Arribas G, Yerbes R, Ceballos-Chávez M, Rodriguez-Paredes M, Tomé M, Durán RV, Buschbeck M, Reyes JC (2022) The high mobility group protein HMG20A cooperates with the histone reader PHF14 to modulate TGFβ and Hippo pathways, *Nucleic Acids Research* 50, 9838-9857.

Bodineau C, Tomé M, Murdoch PDS and Durán RV (2022) Glutamine, mTOR and autophagy: a multiconnection relationship, *Autophagy* 18, 2749–2750 (IF = 13.4).

Nicastro R, Gaillard H, Zarzuela L, Fernández-García E, Tomé M, García-Rodríguez N, Péli-Gulli MP, Durán RV, De Virgilio C and Wellinger RE (2022) Manganese is a physiologically relevant TORC1 activator in yeast and mammals, *eLIFE* 11, e80497.

Cabré N, Luciano-Mateo F, Chapski DJ, Baiges-Gaya G, Fernández-Arroyo S, Hernández-Aguilera A, Castañé H, Rodríguez-Tomàs E, París M, Sabench M, Del CastilloD, Del Bas JM, Tomé M, Bodineau C, Sola-García A, López-Miranda J, Martín-Montalvo A, Durán RV, Vondriska TM, Rosa-Garrido M, Camps J, Menéndez JA and Joven J (2022) Laparoscopic sleeve gastrectomy in patients with severe obesity restores adaptive responses leading to nonalcoholic steatohepatitis, *Int. J. Mol. Sciences* 23, 7830.

Sarmiento A, Durán-Díaz I, Fondón I, Tomé M, Bodineau C and Durán RV (2022) A method for unsupervised semi-quantification of immunohistochemical staining with beta-divergences, *Entropy* 24, 546.

Kaminski H, Marseres G, Yared N, Nokin MJ, Hooks K, Pitard V, Tarricone A, Zouine A, Garrigue I, Loizon S, Capone M, Gauthereau X, Mamani-Matsuda M, Durán RV, Pinson B, Pellegrin I, Thiébaut R, Couzi L, Merville P and Déchanet-Merville J (2022) mTOR inhibitors prevent CMV infection through restoration of functional αβ and γδ T cells in kidney transplantation, *J. Amer. Soc. Nephrol.* 33, 121-137.

Bodineau C, Tomé M, Courtois S, Costa ASH, Richard E, Sciacovelli M, Vacher P, Bessede E, Varon C, Rousseau B, Soubeyran P, Frezza C, Murdoch PS, Villar VH and Durán RV (2021) Two parallel pathways connect glutamine metabolism and mTORC1 activity to regulate glutamoptosis, *Nature Commun.* 12, 4814.

Nguyen TL, Nokin MJ, Terés S, Tomé M, Galmar O, Pasquet JM, Rousseau B, van Liempd S, Falcon JM, Richard E, Muzotte E, Rezvani HR, Priault M, Bouchecareilh M, Redonnet-Vernhet I, Fuentes P, Calvo J, Uzan B, Pflumio F, Fuentes P, Toribio ML, Khatib AM, Mudoch PS, Soubeyran P and Durán RV (2021). Downregulation of Glutamine Synthetase, not glutaminolysis, is responsible for glutamine addiction in Notch1-driven acute lymphoblastic leukemia, *Mol. Oncol.* 15, 1412-1431.

Klionsky et al. (2021). Guidelines for the use and interpretation of assays for monitoring autophagy (4th edition), *Autophagy* 17, 1-382.

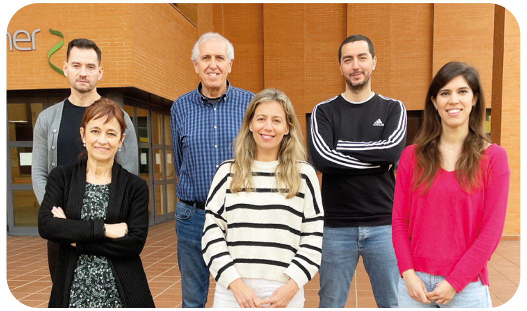
Courtois S, Haykal M, Bodineau C, Sifré E, Ménard A, Azzi-Martin L, Mégraud F, Lehours P, Durán RV, Varon C, and Bessède E (2021). Autophagy induced by *Helicobacter pylori* infection is necessary for gastric cancer stem cells emergence, *Gastric Cancer* 24, 133-144.

Principal Investigator

Prof. Abelardo López Rivas

Cell Death Signalling

Group Leader



Current position

- Since 2006, Research Professor CSIC, Andalusian Center for Molecular Biology and Regenerative Medicine, Seville, Spain.

Group Members

Research Associates

- Carmen Palacios Casanova.

Postdocs

- Rocío Mora Molina.

PhD Students

- Younes El Yousfi El Mourabit.

Technicians

- Francisco Javier Fernández Farrán.
- Belén Torres Agrela.

Former Members (2018-2020)

- **Postdocs:** Rosario Yerbes Cadenas.

Research Activity

Overview

The ability of tumor cells to adapt to various stress conditions generated in the tumor microenvironment, such as hypoxia, nutrient deprivation and oxidative stress, is decisive for the selection of aggressive tumor clones and to drive tumor progression. Despite recent advances in the field, there are still many questions to unravel concerning the mechanisms leading to an adaptive or to an apoptotic response after microenvironmental stress. The results of our group in recent years indicate that in response to microenvironmental stress, the pro-apoptotic receptor TRAIL-R2 promotes the formation in tumor cells of intracellular signalling platforms with other proteins of the extrinsic apoptosis pathway, the result of which may be cell death or a pro-tumoral response. Our project aims to decipher the molecular mechanisms regulating the activation of cell death by apoptosis upon metabolic stress and to determine the impact of the tumor microenvironment and the 3D architecture of tumor spheroids in tumor cell fate. This is essential to identify possible markers of tumor malignancy and potential therapeutic targets.

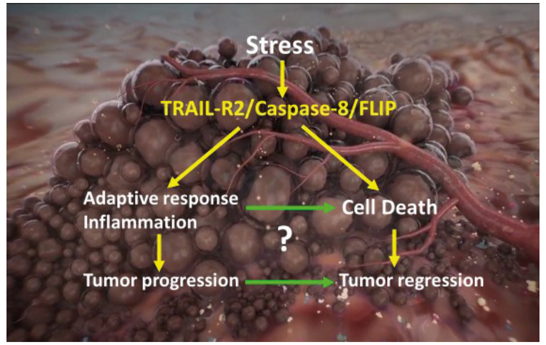


Figure 1. Dual role of Caspase-8 platfom in the control of tumor growth.

Research Highlights

Protein misfolding or unfolding and the resulting endoplasmic reticulum (ER) stress frequently occur in highly proliferative tumors. How tumor cells escape cell death by apoptosis after chronic ER stress remains poorly understood. We have investigated in both two-dimensional (2D) cultures and multicellular tumor spheroids (MCTSs) the role of caspase-8 inhibitor cFLIP as a regulator of the balance between apoptosis and survival in colon cancer cells undergoing ER stress. We have shown that downregulation of cFLIP proteins levels is an early event upon treatment of 2D cultures of colon cancer cells with ER stress inducers, preceding TNF-related apoptosis-inducing ligand receptor 2 (TRAIL-R2) upregulation, caspase-8 activation, and apoptosis. Maintaining high cFLIP levels during ER stress by ectopic expression of cFLIP markedly inhibits ER stress-induced caspase-8 activation and apoptosis. Conversely, cFLIP knockdown by RNA interference significantly accelerates caspase-8 activation and apoptosis upon ER stress. Despite activation of the proapoptotic PERK branch of the unfolded protein response (UPR) and upregulation of TRAIL-R2, MCTSs are markedly more resistant to ER stress than 2D cultures of tumor cells. Resistance of MCTSs to ER stress-induced apoptosis correlates with sustained cFLIPL expression. Interestingly, resistance to ER stress-induced apoptosis is abolished in MCTSs generated from cFLIPL knockdown tumor cells. Overall, our results suggest that controlling cFLIP levels in tumors is an adaptive strategy to prevent tumor cell's demise in the unfavorable conditions of the tumor microenvironment.

Tumor microenvironment is significantly different from normal tissue and increasing evidences suggest that apart from chemical input, the mechanical properties of the tumor microenvironment are important determinants for tumor cell behavior. Among the multiple physical parameters, extracellular matrix rigidity can especially affect intracellular signalling events,

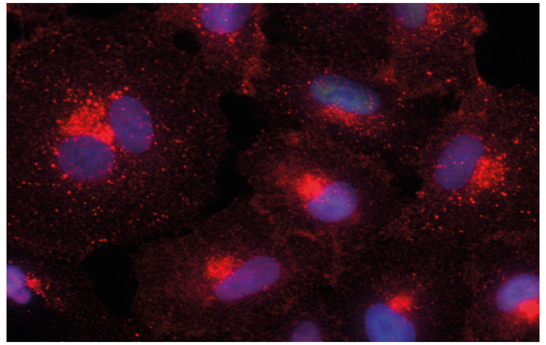


Figure 2. Inhibiting nuclear YAP/TAZ localization promotes intracellular TRAIL-R2 clustering and apoptosis in tumor cells.

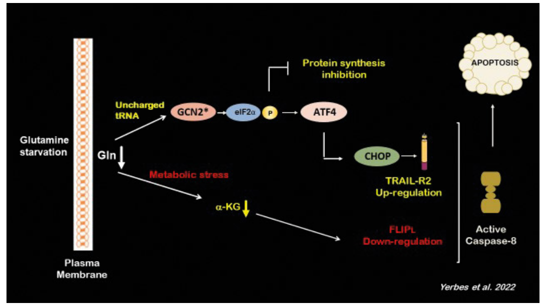


Figure 3. Limitation of glutamine in glutamine-addicted tumor cells will lead, on the one hand, to the elevation of TRAIL-R2 levels mediated by the activation of the GCN2 pathway and, on the other hand, to the decrease of FLIPL as a result of the metabolic defect caused by the loss of αKG. Both events will result in the activation of caspase-8 at the DISC, which will activate effector caspases and apoptosis.

influencing cancer progression and the tumor response to therapy. In this respect, our recent data revealed that matrix stiffness and nuclear localization of the transcriptional co-activators YAP/TAZ are key determinants of the apoptotic response to ER stress by controlling FLIPL levels and the activation of the extrinsic pathway of apoptosis in tumor cells.

Oncogenic transformation leads to changes in glutamine metabolism that make transformed cells highly dependent on glutamine for anabolic growth and survival. We have investigated the cell death mechanism activated in glutamine-addicted tumor cells in response to the limitation of glutamine metabolism. We have shown that glutamine starvation triggers a FADD and caspase-8-dependent and mitochondria-operated apoptotic program in tumor cells that involves the pro-apoptotic TNF-related apoptosis-inducing ligand receptor 2 (TRAIL-R2), but is independent of its cognate ligand TRAIL. In glutamine-depleted tumor cells, activation of the amino acid-sensing general control nonderepressible-2 kinase (GCN2) is responsible for TRAIL-R2 upregulation, caspase-8 activation, and apoptotic cell death. In addition, metabolic stress upon glutamine deprivation also results in GCN2-independent FLICE-inhibitory protein (FLIP) downregulation facilitating caspase-8 activation and apoptosis. Importantly, downregulation of the long FLIP splice form (FLIPL) and apoptosis upon glutamine deprivation are inhibited in the presence of a membrane-permeable α-ketoglutarate.

Altogether, our data support a model in which limiting glutamine utilization in glutamine-addicted tumor cells triggers a previously unknown cell death mechanism regulated by GCN2 that involves the TRAIL-R2-mediated activation of the extrinsic apoptotic pathway.

Collectively, our data suggest that cellular levels of FLIPL may play an important role in tumor cell fate decisions under the stressful conditions of the tumor microenvironment. Thus, in stressful situations, maintaining the levels of this protein that inhibits the extrinsic apoptosis pathway could enable the activation of an adaptive response in tumor cells and other tumor stromal cells, which would promote tumor growth and progression. More importantly, these results also reveal a dependence of tumor cells on maintaining FLIPL levels in the context of the tumor. Therefore, understanding the mechanisms that maintain high levels of FLIP in tumor cells should help in the design of therapeutic strategies that reduce the expression of this protein and, in this way, limit tumor growth.

Grants

- 2019-2022: PGC2018-093960-B-I00. Ministerio de Ciencia, Innovación y Universidades.
- 2017-2021: CB16/12/00421, Centro de Investigación Biomédica en Red (CIBERONC). Instituto de Salud Carlos III.
- 2021-2023: PY20_00754. Proyecto de Excelencia Junta de Andalucía.
- 2022-2024: PID2021-122226NB-I00. Ministerio de Ciencia e Innovación.

Publication Highlights

Mora-Molina R, Stöhr D, Rehm M and López-Rivas A. **2022**. cFLIP down-regulation is an early event required for endoplasmic reticulum stress-induced apoptosis in tumor cells. **Cell Death and Disease** doi: 10.1038/s41419-022-04574-6.

Mora-Molina R and López-Rivas A. **2022**. Restoring TRAILR2/DR5-mediated activation of apoptosis upon endoplasmic reticulum stress as a therapeutic strategy in cancer. **Int J Mol Sci**. 12;23(16):8987.doi: 10.3390/ijms23168987.

Yerbes R, Mora-Molina R, Fernández-Farrán F, Hiraldo L, López-Rivas A* and Palacios C* (*senior and corresponding author). **2022**. Limiting glutamine utilization activates a GCN2/TRAIL-R2/Caspase-8 apoptotic pathway in glutamine-addicted tumor cells. **Cell Death and Disease** doi: 10.1038/s41419-022-05346-y

Principal Investigator

Prof. José A. Pintor Toro

Cell Cycle and Oncogenesis

Group Leader

Current position

- Since 1986: Professor CSIC, Andalusian Center for Molecular Biology and Regenerative Medicine CABIMER, Seville, Spain.

Group Members

PhD Students

- Salvador Polo Generelo.

Technician

- Belén Torres Agrela.

Former Members (2018-2020)

- **PhD students:** Salvador Polo Generelo, Cristina Rodríguez-Mateo.
- **Postdoc:** Belén Torres Agrela.

Research Activity

Overview

The epithelial-mesenchymal transition (EMT) is a basic cellular process in which epithelial cells lose their epithelial characteristics and take on properties of mesenchymal cells. Our interest is focused on studying the immediate-early changes of this process. Two levels of regulation are the object of our study: transcriptional and post-transcriptional levels. Regarding the first level, our interest is identify non-coding RNA molecules (lnc-RNAs) that early regulate this process and act as “master genes”. Regarding the second level, our objective is to determine both coding and non-coding RNA molecules whose primary function is to sequester miRNA molecules that would be blocking the translation of transcripts whose proteins are essential for EMT. Since the appearance of these new proteins would not be correlated with changes in the expression of the corresponding mRNA, these ones would not be included in the transcriptomic profiles corresponding to the expression changes produced. Given the clinical relevance of EMT in tumorigenesis and metastasis, our ultimate interest is to explore the potential use as biomarkers and therapeutic targets of the new identified molecules.

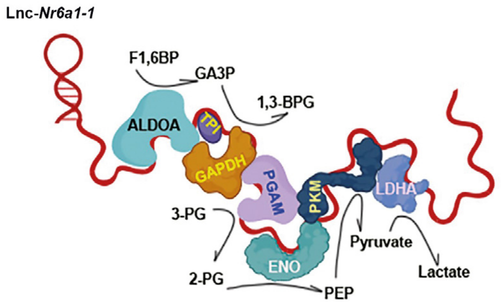


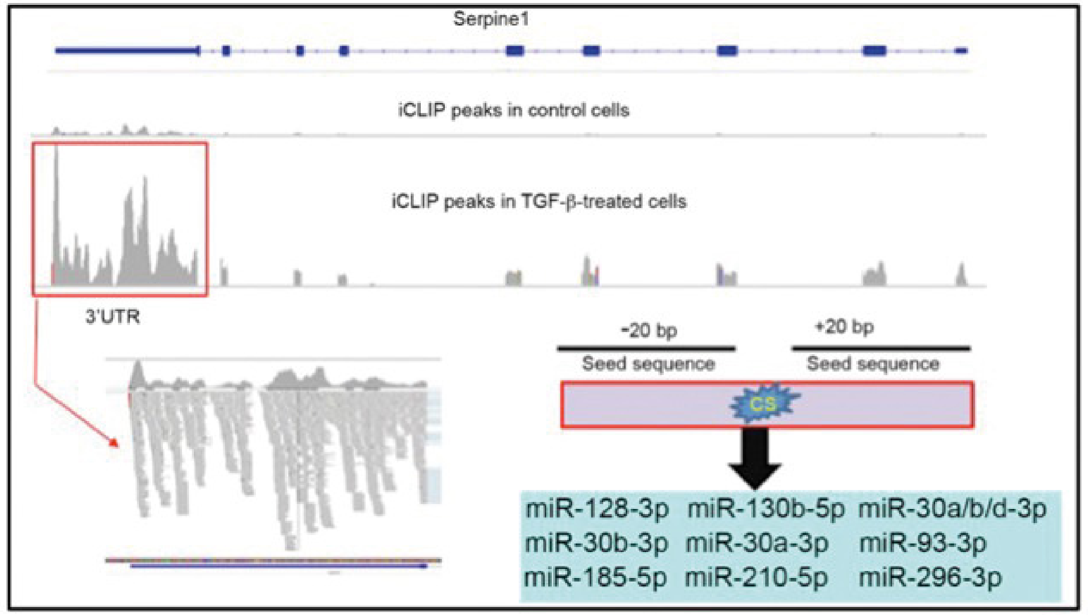
Figure 1. A proposed model for the role pf lnc-Nr6a1-1 as a scaffold molecule involved in the formation of a glycolytic complex. F1,6BP, fructose-1,6-bip`hosphate; GA3P, 3-phosphoglycerate; 1,BPG, 1,3-biphosphoglycerate; 3-PG, 3-phosphoglycerate; 2-PG, 2-phosphoglycerate; PEP, phosphoenolpyruvate

Lnc-Nr6a1 is an immediate-early regulator of EMT that is downregulated by TGF-b. *Lnc-Nr6a1* is processed giving rise to two abundant polyadenylated isoforms, *lnc-Nr6a1-1* and *lnc-Nr6a1-2*, and a longer non-polyadenylated microprocessor-driven *lnc-pri-miRNA* containing clustered *pre-miR-181a2* and *pre-miR-181b2* hairpins. Ectopic expression of polyadenylated isoforms enhance cell migration and invasive capacity of the cells, whereas the expression of these isoforms and the non-polyadenylated isoform conferred anoikis resistance. By identification of direct RNA interacting proteins (iDRIP) we defined a network of glycolytic proteins (ENO1, ALDOA, GAPDH, PKM, LDHA) interacting directly with the lnc-Nr6a1-1 isoform. This isoform acts as a scaffold molecule, supporting substrate channeling for efficient glycolysis.

Quantification of **RNAs-AGO2 interactions** at the beginning of EMT by iCLIP technique.

We have determined and quantified the RNA molecules present in the RISC complexes of TGF-β-treated NMuMG cells. The AGO2 protein interacts mainly with exons and 3'UTR regions. The percentage of RNAs that interact with AGO2 through exons is very similar to those that interact through the 3'UTR regions, underlining that the regulation mechanism carried out by RISC (RNA-induced silencing complex) complexes is also largely associated with exons. Remarkably the most enriched RNA in the RISC complexes after 4 hours of treatment with TGF-β was the mRNA Serpine1, it was also the mRNA that showed the greatest increase in crosslinking sites, going from 2 sites in untreated cells to 34 in TGF-β-treated cells.

The **Serpine1 gene** is strongly and rapidly induced by TGF-β treatment. It is paradoxical that in parallel the levels of *Serpine1* mRNAs



Scheme of the serpine1 gene showing the crosslinking sites of exons and 3'UTR region with AGO2 protein. 3'UTR sequence reads and miRNAs corresponding to crosslinking sites are shown.

increase strongly in the RISC complexes. This result indicates that *Serpine1* mRNA, in addition to act as a protein-coding gene, it may exert a non-coding biological function. In fact, *Serpine1* mRNA acts as a natural miRNA sponge to dampen the EMT inhibitor activity of miRNAs. By overexpressing ATGs-mutated *Serpine1* mRNA (*SerpineATG4*), we show that most of the effects so far associated with the SERPINE1 protein are also caused by the *Serpine1* mRNA. Proteomic analysis reveals that levels of the splicing factor TRA2b increase notably after *SerpineATG4* overexpression, while Tra2b mRNA levels are not affected. iCLIP and mutagenesis analysis indicate that miR-130b-5p regulates the levels of the TRA2b protein; as expected, *Serpine1* mRNA contains three binding sites for miR-130b-5p, one in exon 3 and two in the 3'UTR. An effect of the *SerpineATG4* overexpression, not described until now, is the downregulation of numerous genes, most of them related to the innate immunity. This same effect is also produced by Tra2b overexpression, sharing more than 60% of the expression changes (217 genes). Likewise, 25% of alternative splicing changes produced by *Tra2b* overexpression are also observed with *SerpineATG4*.

Ph.D. Theses Defended

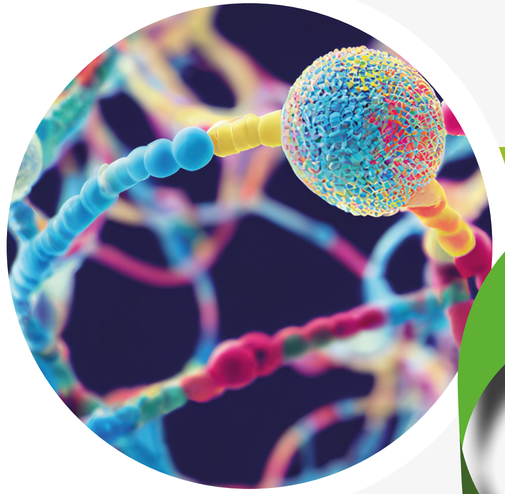
Salvador Polo Generelo. Identificación y análisis funcional del lnc-Nr6a1 y del mRNA *Serpine1* como reguladores tempranos de la transición epitelio-mesénquima. University of Seville. 2021

Grants

- 2018-2020 MICIIN. SAF 2017-86189-P: 157.300,00 €
- 2021-2023: MICIIN. PID2020-119732RB-100: 108.900,00 €

Publication Highlights

Polo-Generelo S., Torres B, Guerrero-Martinez J.A., Camafeita E, Vázquez J., Reyes J.C., and Pintor-Toro J.A. 2022. TGF-β-upregulated *Lnc-Nr6a1* acts as a reservoir of miR-181 and mediates assembly of a glycolytic complex. **Non-coding RNA** 8,(5), 62. doi.org/10.3390/ncrna8050070



Principal Investigator

Dr. Rosa M. Ríos

Microtubule dynamics
in health and disease

Group Leader

Current position

- 2011-Present: Senior Research Scientist - Spanish National Research Council CSIC-CABIMER.
- From 19/02/2019-06/11/2022: On temporary leave of absence as Secretary General of University, Research and Technology - Government of Andalucía.

Group Members

Research Associates

- Laura Martínez (Assit. Prof. US).

Postdocs

- María de la Paz Gavilán (Assit. Prof. US).
- Chiara Marcozzi.

PhD Students

- Carmen García.

Technicians

- Carmen Luque.
- María Montilla.

Former Members (2021-2022)

- **Postdocs:** Pablo Gandolfo.
- **PhD Students:** Jesús Roca.
- **Technicians:** Loida Pérez; Laura Díaz.

Research Activity

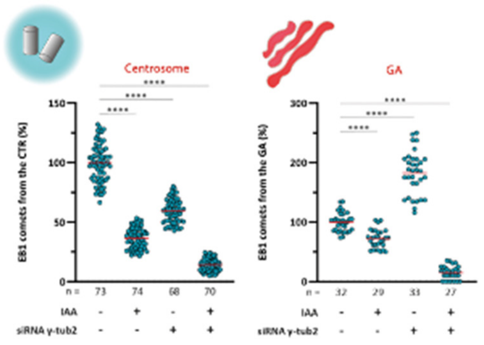
Overview

Microtubules (MTs) are cytoskeletal filaments that serve as tracks for intracellular transport, act as scaffolds to position organelles, contribute to cell shape and motility, and control cell division. During mitosis MTs form bipolar spindles that drive chromosome segregation into the daughter cells, whereas in interphase they organise in a cell type-specific fashion to support cell physiology. The event that initiates *de novo* formation of MTs, known as MT nucleation, occurs in specific subcellular structures globally called Microtubule Organising Centres (MTOCs). The centrosome and the Golgi Apparatus (GA) are the two major MT-organising centres (MTOCs) in actively dividing cells, and increasing evidence underpins the idea that there is a high degree of coordination and crosstalk between their activities along the cell cycle and during cell differentiation. The main focus of my lab is to unveil the molecular mechanisms underlying MT nucleation driven by the GA and the centrosome along the cell cycle or during cell differentiation. Our aim is to widen our current knowledge on the spatiotemporal control of MTOC activities and to gain a more mechanistic comprehension of the pathways involved. We are also investigating how chemokines regulate the organisation of chemokine receptors, and what are the associated cytoskeletal proteins that orchestrate T lymphocyte migration.

Research Highlights

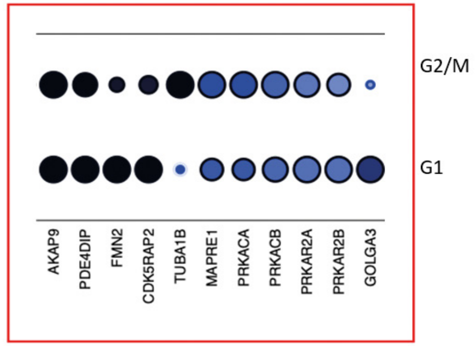
Microtubule (MT) nucleation, the event that initiates de novo formation of MTs, is a highly regulated process that enables cells to acquire specific architectures and to promptly respond to any cellular change. How cells assign MTOC locations and how the activities of different MTOCs are regulated and integrated in the same cell is far from being understood. During mitosis the MT nucleation activity of the centrosome greatly increases, while that of the GA is silenced. On the contrary, during cell differentiation centrosomes either disappear or become inactive, and the GA leads MT network organisation. Hence, it is of great importance to decipher the molecular mechanisms operated by these MTOCs.

The formation of new MTs is initiated by the activity of specific proteins, that act as nucleators and mediate the interaction between αβ-tubulin heterodimers. The only template-based MT nucleator identified so far is the gamma-tubulin ring complex (yTuRC), a conical structure consisting of 14 subunits of ytubulin held together by the ytubulin complex proteins (GCPs) and by additional factors. In mammalian cells, ytubulin is encoded by two genes, TUBG1 and TUBG2, that produce two isoforms differing in few amino acids at their C-terminus. In most cells, ytubulin 1 is much more abundant than ytubulin 2. Aware of the essentiality of ytubulin 1 for cell cycle progression, we generated a knockin cell line in which γtubulin 1 was tagged with the fluorescent protein mCherry2 and the mini-Auxin Induced Degradation motif (mAID) to achieve and monitor its rapid and controlled degradation. By combining γtubulin 1 induced-degradation with γtubulin 2 silencing, we have investigated the contribution of both γ-tubulin isoforms to MT nucleation from different MTOCs.



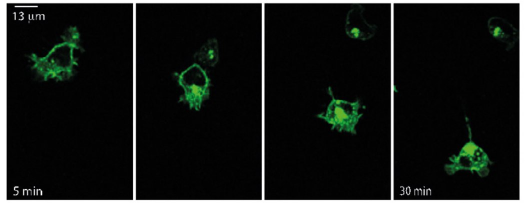
MT nucleation analysis by live-cell imaging under these conditions have revealed that both γtubulin isoforms contribute to MT nucleation and that γTuRC-mediated MT nucleation is the only mechanism operating in non-transformed epithelial cells, contrarily to what has been reported in cancer-derived cell lines. Interestingly, we found that whereas γtubulin 1 stimulates both centrosome and GA MT nucleation, γtubulin 2 plays an inhibitory role on MT nucleation at the GA.

Further investigation on the molecular mechanisms underlying this negative control revealed that γtubulin 2 exerts its inhibitory role by competing with γtubulin 1 for the binding sites on Golgi membranes. Interestingly, our studies unveiled a new regulatory mechanims of MT nucleation at the GA that involves the aggregation degree of the γTuRC receptors AKAP450 and CDK5Rap2 at Golgi membrane surfaces.



As mentioned before, at mitotic entry centrosome ability to nucleate MTs increases, while that of the GA is inhibited. Our previous work identified AKAP450 as responsible for GA-associated MT nucleation activity. For this reason, we focused on AKAP450 as the best candidate to mediate mitotic silencing of GA-MT nucleation. To identify cell-cycle regulated interactions potentially implicated in this mechanism, we have generated an AKAP450-mAID-m-Cherry2 knockin cell line and performed comparative proteomics of AKAP450-containing complexes purified from either G1 or G2/M synchronised cells.

This analysis revealed that most of previously reported interactions, i.e. AKAP450, CDK5Rap2, MMG, PKA, PDE4, EB1, Cep170, are cell-cycle independent. However, several new cell-cycle regulated interactions were also identified and been further confirmed perfoming co-IP experiments. We are now investigating the functional significance of such interations in mitotic Golgi silencing.



As an independent although related research line, we are also interested in the dynamic and organization of chemokine receptors during leukocyte migration. Leukocyte movement is mostly driven by chemokines and their receptors that organize at the cell membrane as monomers, dimers and small oligomers (nanoclusters). We have recently found that there is a correlation between the size of chemokine receptors nanoclusters and the cell migration capacity. The chemokine receptor CXCR4 and its ligand, the CXCL12 chemokine, form a key pair in lymphocyte trafficking. Using SPT-TIRF microscopy and a T cell line expressing endogenous CXCR4-AcGFP, we studied the CXCR4 dynamic organisation during T cell migration. We identified the cytoskeletal protein filamin A as a mechanical regulator of CXCL12-mediated CXCR4 nanoclustering, which orchestrates T cell motility.

Grants

- 2020-2023: PIE 202080E095. Intramural Project. CSIC.
- 2019-2021: PGC2018-095057-B-I00. National Plan for Scientific and Technical Research and Innovation. Ministry of Science and Innovation.
- 2019-2022: RTI2018-101789-J-I00, JIN/ Ministry of Science and Innovation (to L. Martinez).
- 2016-2022 AIO16163616GAVI. Spanish Association Against Cancer Scientific Foundation (AECC FC) (to M.P. Gavilán).
- 2021–2023 PY20_00615 Regional Government of Andalucía (to L. Martinez).

Cell Dynamics and Signaling

Publication Highlights

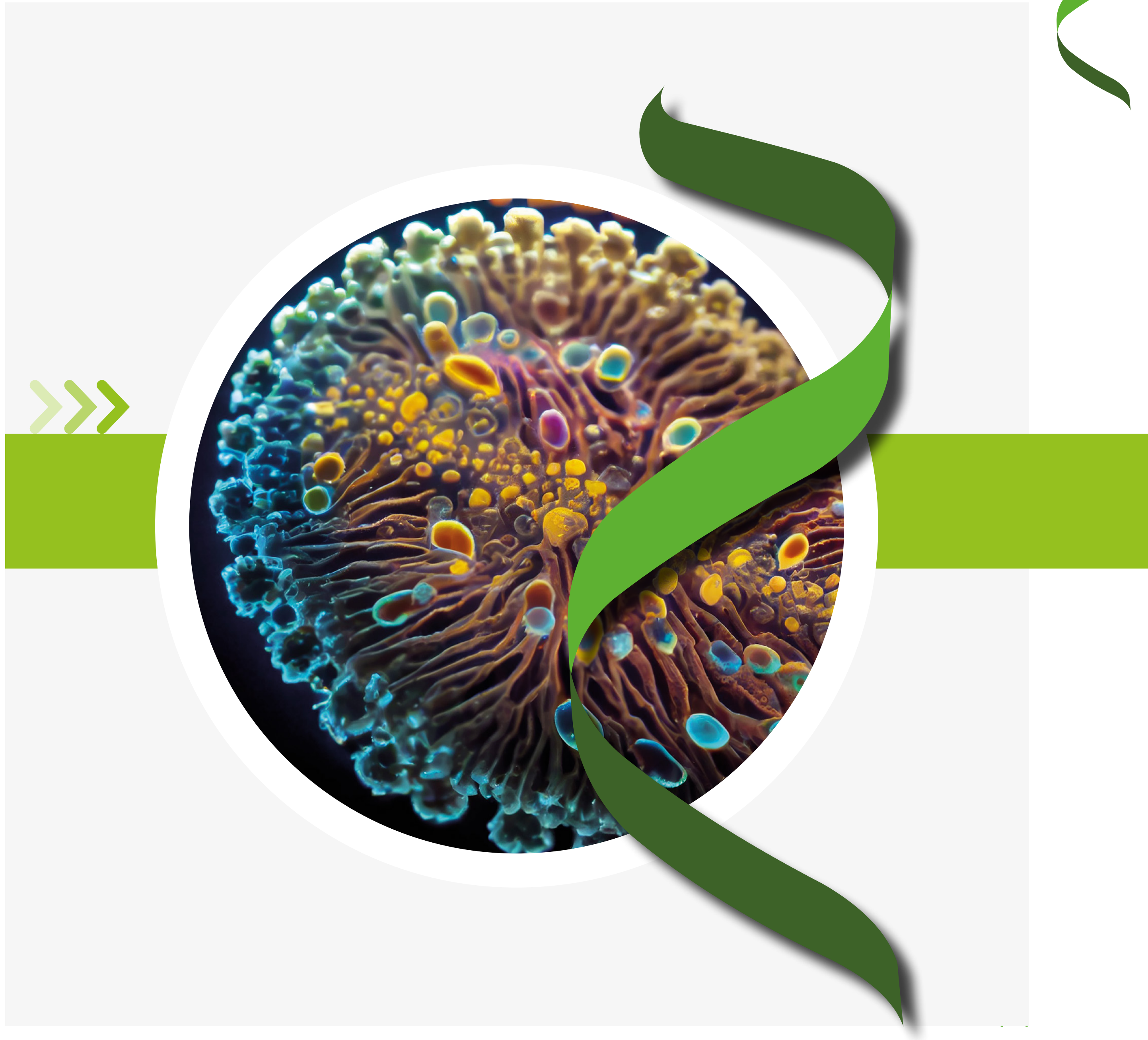
González-Arzola K, Díaz-Quintana A, Bernardo-García N, Martínez-Fábregas J, Rivero-Rodríguez F, Rivero-Rodríguez F, Casado-Combreras MA, Elena-real C, Velazquez-Cruz A, Gil-Caballero S, Velázquez-Campoy A, Szulc E, Gavilan MP, Ayala I, Arranz R, Rios RM, Salvatella X, Valpuesta J, Hermoso J, De la Rosa MA, Díaz-Moreno, I. Nucleus-translocated mitocondrial cytochrome-C liberates nucleophosmin-sequestered ARF tumor suppressor by changing nucleolar liquid-liquid pase separation. *Nature Structural and Molecular Biology* 2022; doi. org/10.1038/s41594-022-00842-3

García-Cuesta E.M., Rodríguez-Frade J.M., Gardeta S.R., D'Agostino G., Martínez P., Soler Palacios B., Cascio G., Wolf T., Martínez-Muñoz L and Mellado M. Altered CXCR4 dynamics at the cell membrane impairs directed cell migration in WHIM syndrome patients. *PNAS* 2022; 119 (21). doi: 10.1073/pnas.2119483119.

Balestra FR, Domínguez-Calvo A, Wolf B, Busso C, Buff A, Averink T, Lipsanen-Nyman M, Huertas P, Ríos RM, Gönczy P. TRIM37 prevents formation of centriolar protein assemblies by regulating Centrobin. *Elife* 2021. Jan 25;10:e62640. doi: 10.7554/eLife.62640.

Rivero-Rodríguez F, Díaz-Quintana A, Velázquez-Cruz A, González-Arzola K, Gavilan MP, Velázquez-Campoy A, Ríos RM, De la Rosa MA, Díaz-Moreno I. 2021. Inhibition of the PP2A activity by the histone chaperone ANP32B is long-range allosterically regulated by respiratory cytochrome c. *Redox Biology* 2021, 43:101967. doi: 10.1016/j.redox.2021.101967.

Olazábal-Morán M., Sánchez-Ortega M., Martínez-Muñoz L., Hernández C., Rodríguez M.S., Mellado M., Carrera A.C. Fluctuations in AKT and PTEN activity are linked by the E3 ubiquitin ligase c-CBL. *Cells* 2021; 10 (11). doi: 10.3390/cells10112803



Principal Investigator

Dr. Fernando Monje Casas

Cell division control

Group Leader

Current position

- Since 2016: Staff Scientist.
  Spanish National Research Council (CSIC).

Current Group Members

Postdocs

- María Galindo Moreno.
- Javier Manzano López.
- Ana María Rincón Romero.

PhD Students

- Alejandra Álvarez Llamas.

Technicians

- Macarena Gómez Carmona.
- Pablo Magán Osuna.

Master Students

- Darío López Muñoz.

Alumni (2021-2022)

- **PhD Students:** María de la Paz Vázquez Aroca.
- **Master Students:** María Pereira Hernández.
- **Technicians:** Cristina Maya Álvarez.

Research Activity

Overview

Our research aims to shed light on the mechanisms that control cell division and ensure a correct distribution of the genetic material during this process. Problems with genome partitioning can give rise to aneuploidy, an alteration of the normal number of chromosomes in the cells that is a hallmark of cancer and other genetic diseases. Accordingly, cells have developed checkpoints that verify DNA integrity and the fidelity of chromosome segregation during their division. Our goal is to better understand how checkpoint function is controlled and coordinated to regulate specific cell cycle transitions. Additionally, we are interested in evaluating how cells exploit the same machinery that allows genome distribution in order to generate polarity during cell division. Errors in the establishment of asymmetry during stem cell division can lead to neurodegenerative disorders and premature aging. Hence, advances in our knowledge about asymmetric cell divisions are of pivotal social and economic importance.

Research Highlights

The mitotic spindle constitutes the molecular machinery that facilitates the segregation of the chromosomes during the division of the cells. The spindle is formed by a bipolar array of microtubules that emanate from microtubule-organizing centers (MTOCs), located at both spindle poles. In higher eukaryotes, the spindle MTOCs are known as centrosomes, while in the budding yeast *Saccharomyces cerevisiae* these structures are named spindle pole bodies (SPBs). The MTOCs duplicate early in the cell cycle and nucleate both kinetochore microtubules that anchor the chromosomes to facilitate their distribution and astral microtubules that position the spindle, being therefore essential for proper spindle biogenesis, orientation and elongation. After their duplication, the pre-existent ("old") and the newly generated ("new") MTOCs differ in composition, size and age. Intriguingly, the old and new MTOCs can be differentially distributed during certain asymmetric divisions. This fascinating phenomenon was originally described in *S. cerevisiae*. During budding yeast division, the old SPB is inherited by the daughter cell, while the new is retained by the mother. Asymmetric centrosome distribution patterns have been later described in stem cells from different organisms, including humans. Remarkably, despite this evolutionary conservation, whether the asymmetric inheritance of spindle MTOCs played any biological role had been a topic of discussion during the last years. To answer this question, we recently generated a *S. cerevisiae* strain in which the old SPB is specifically retained by the mother cell during each division, thus displaying a constitutively reversed pattern of SPB inheritance. Excitingly, our analyses demonstrated that maintenance of the pre-established SPB fate plays a pivotal role in preserving budding yeast replicative lifespan. Specifically, asymmetric SPB inheritance is required to ensure normal levels

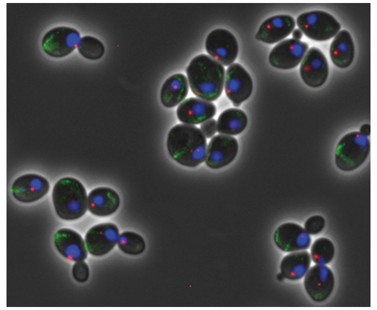


Fig.1. During mitosis, the pre-existent (old) and the newly generated (new) spindle pole bodies (SPBs) are asymmetrically distributed in S. cerevisiae cells. Based on the conservative nature of SPB duplication, this phenomenon can be observed by expressing a component of the SPB tagged with a slow-folding version of the red fluorescent protein RFP (in red), so that the old SPB displays a brighter signal than the new one. Also shown in the images are a mitochondrial protein (in green) and the nucleus (in blue).

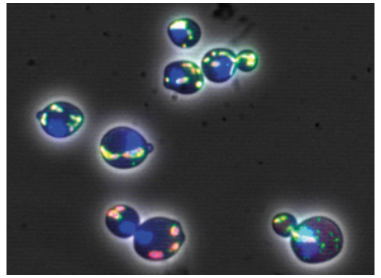


Fig. 2. The most functional and less oxidized mitochondria are preferentially inherited by daughter cells during yeast cell division, while mother cells retain the damaged mitochondria. To evaluate how the asymmetric inheritance of the spindle pole bodies affects mitochondrial distribution, we have developed a molecular redox probe (yo-mito-rxRFP, in red), which is targeted to mitochondria and displays maximum fluorescent intensity in its most oxidized form. The image also shows a marker for total mitochondria (in green) and the nucleus (in blue).

of the Sir2 sirtuin, a widely conserved lifespan modulator, and to properly distribute functional mitochondria and protein aggregates, which are selectively retained in the mother cell to reset replicative lifespan in the daughter cell, during cell division (Manzano-López et al. (2019), *Nat. Cell Biol.*). The research in our group has also allowed us to identify novel key regulators of non-random spindle inheritance of the MTOCs. In this way, we have recently unveiled a novel role of Polo-like kinases, a conserved family of key cell cycle regulatory proteins, in the regulation of non-random SPB inheritance (Matellán et al. (2020), *eLife*).

During the 2021-2022 period, we have been actively working to better understand the phenomenon of the differential distribution of the spindle MTOCs during asymmetric mitoses, both by more precisely describing the molecular mechanisms by which patterns of non-random MTOC distribution are established and also by further elucidating the consequences that interfering with these processes have on cellular aging. However, we have not only focused on this process, and we have additionally studied other mechanisms by which the cell can generate polarity during cell division that are dependent on the mitotic spindle but that do not rely on the intrinsic nature ("old" vs. "new") of the MTOCs that organize this structure. In this way, we have recently demonstrated that the Bfa1/Bub2 complex, a mitotic exit inhibitor that asymmetrically localizes to the SPB that enters the daughter cell during the division of *S. cerevisiae*, specifically interacts with a component of the nuclear pore complexes (NPCs). Our studies have also revealed that this association between SPB and NPC components is important during the autophagic degradation of damaged nuclear pores during cell division. Furthermore, the asymmetric localization of the Bfa1/Bub2 complex to the SPB that enters the daughter cell during anaphase suggests that its interaction with a nucleoporin could contribute to promote the specific autophagic degradation of damaged or non-functional NPCs that, despite the existence of different mechanisms for their retention in the mother cell during mitosis, could be nonetheless inherited by the daughter cell.

Finally, we have recently initiated a novel research line in the group to extent our analyses regarding the process of non-random inheritance of spindle MTOCs to human cells. Specifically, we are evaluating the mechanisms that orchestrate the asymmetric distribution of the centrosomes after their duplication during the division of human neuroblastoma cells. The use of human cell lines will contribute not only to widen our scientific horizon, but it will also potentiate the biomedical relevance of our findings. Defects during asymmetric cell divisions have been associated with tumorigenesis, neurodegeneration and developmental problems. Therefore, unveiling the basic mechanisms that regulate these divisions is of upmost relevance to better understand the causes for these diseases.

Grants

- 2020-2023: PID2019-105609GB-I00. Ministerio de Ciencia e Innovación.
- 2020-2022: PY18-3183. Junta de Andalucía.

Principal Investigator
Dr. Anabel Rojas

Pancreas and Liver Development and Disease
Group Leader

Current position

- Assistant Professor at University Pablo de Olavide.

Group Members

PhD Students
- Noelia Arroyo Del Alba.
- María Bermúdez Sauco.
- Ana Alicia Montero Cabrera.

Technicians
- Irene Díaz Contreras.
- Enrique Domínguez.

Research Activity

Overview

The severity and occurrence of diseases caused by liver malfunction demand a better understanding of the molecular bases that control the activity of this organ in normal conditions and in response to damage. In our laboratory, our lines of research are focused on the molecular basis underlying the ultimate consequence of hepatic chronic disease, liver fibrosis. We pursue: i) To unravel the transcription factors and signaling pathways controlling the phenotype of hepatic stellate cells in liver fibrosis and in the resolution of the fibrosis; ii) to search for pharmacological agents to modify the hepatic stellate cell phenotype; iii) To modulate the interplay between hepatic stellate cells and hepatocarcinoma cells. .

Research Highlights

Molecular and cellular basis of hepatic fibrosis

Hepatic stellate cells (HSCs) are specialized cells that are located in the liver and are involved in the storage and metabolism of vitamin A. However, in response to liver injury, HSCs can become activated and play a significant role in the development of liver fibrosis, a progressive scarring of the liver that can lead to liver dysfunction and failure. Activated HSCs are the main source of extracellular matrix components (ECM), such as collagen and laminin that form the fibrotic scars. The regression of liver fibrosis implies breakdown of ECM by metalloproteinases and the clearance of activated HSCs, by apoptosis or reversion to an inactive phenotype, thus allowing the hepatocyte to repopulate the damaged hepatic tissue. In recent years, one of the emerging therapies for liver fibrosis focuses in the study of the moleculas basis that control HSCs phenotype.

The GATA4 transcription factor is a protein that regulates gene expression and plays a critical role in the development and function of several organs, including the heart, gut, and liver. Our recent studies have uncovered a new role for GATA4 in the maintenance of HSC quiescence, a state of cell dormancy that helps prevent the activation of HSCs and the development of liver fibrosis. We have shown that overexpression of Gata4 in HSCs promotes the regression of liver fibrosis in mice with hepatic injury. Gene expression and Chip sequencing analysis have revealed that GATA4 alters the expression of fibrogenic and antifibrogenic genes and directly represses the Epas1 gene (which codes for the hypoxic inducible factor 2a, Hif2a), a gene that is

Figure 2

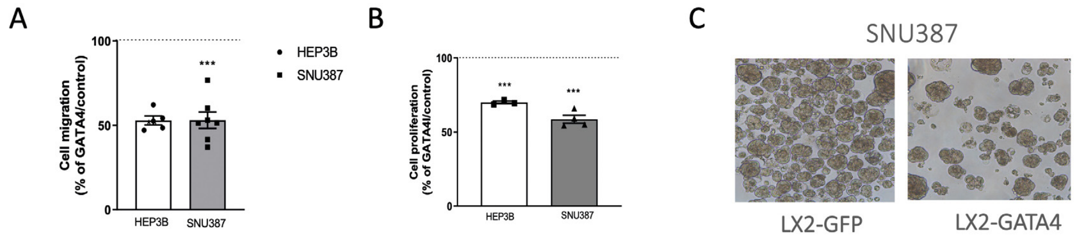


Figure 2. *(A)* Migration assay of Hep3B and SNU387 hepatocarcinoma cell lines coculture with Gata4-overexprerssing LX2 hepatic stellate cells (LX2-GATA4) or GFP-overexpressing LX2 cells (LX2-GFP) as control. **(B)** Cell proliferation assay of Hep3B and SNU387 hepatocarcinoma cell lines coculture with LX2-GATA4 or LX2-GFP. **(C)** Images of tumorospheres formation of SNU387 hepatocarcinoma cells coculture with LX2-GATA4 or LX2-GFP.

known to promote the activation of HSCs and the development of liver fibrosis. Our studies could ultimately help to develop novel therapeutic alternatives to treat hepatic fibrosis regardless the etiology.

Targeting HSCs has been proposed as a potential therapeutic strategy for the treatment of liver fibrosis and liver cancer, as activated hepatic stellate cells (HSCs) are also an important component of the tumor microenvironment in hepatocarcinoma. Several approaches have been investigated to target HSCs with relative success, including the use of small molecule inhibitors, gene therapy, immunotherapy, and small molecule inhibitors of HSC activation. Currently we are investigating the potential of GATA4 to inactivated HSCs in the context of hepatocarcinoma to inhibit or ameliorate the tumorigenic features. Our preliminary studies show that HSCs (LX2 cells) inactivation mediated by GATA4 reduces tumorogenic features of hepatocellular carcinoma cell lines (Hep3B and SNU387), such as migration, proliferation and tumorospheres formation (Figure 2).

Grants

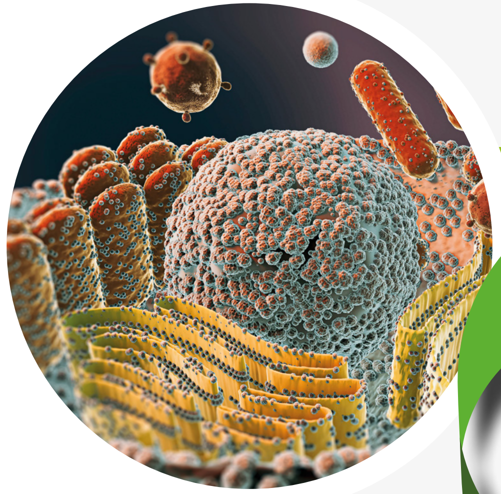
- GATA4: un modulador del fenotipo de las células estrelladas hepáticas. Nuevas estrategias terapéuticas para enfermedades hepáticas. **Principal Investigator:** Anabel Rojas. **Reference:** PID2020-114656RB-100. **Funding Agency:** Ministerio de Ciencia e Innovación. PID2020-114656RB-100. **Period:** 2021- 2023, 3 ayer. **Budget:** 143.000 euros.
- GATA4 como regulador de la activación de las células estrelladas hepáticas. Implicaciones para la fibrosis hepática. **Principal Investigator:** Anabel Rojas. **Funding Agency:** Consejería de Economía y Conocimiento. Junta de Andalucía. **Reference:** P20-00850. **Period:** Jan 2021- June2022, 1,5 years. **Budget:** 60.000 euros.
- GATA6: un nuevo regulador de la función de la célula beta pancreática. **Principal Investigator:** Anabel Rojas. **Funding Agency:** FEDER-Universidad. Consejería de Economía y Conocimiento, Empresas y Universidad. Junta de Andalucía. **Reference:** UPO-1380742. **Period:** Jan 2022- Jun 2023, 1,5 years. **Budget:** 28.248 euros.

Publication Highlights

Martin Vázquez E, Cobo-Vuilleumier N, Araujo Legido R, Marín-Cañas S, Nola E, Dorronsoro A, López Bermudo L, Crespo A, Romero-Zerbo SY, García-Fernández M, Martin Montalvo A, **Rojas A,** Comaills V, Bérmudez-Silva FJ, Gannon M, Martin F, Eizirik D, Lorenzo PI, Gauthier BR. NR5A2/LRH-1 regulates the PTGS2-PGE2-PTGER1 pathway contributing to pancreatic islet survival and function. iScience. 2022 May 2;25(5):104345.

Arroyo N, Villamayor L, Díaz I, Carmona R, Ramos-Rodríguez M, Muñoz-Chápuli R, Pasquali L, Toscano MG, Martín F, Cano DA, **Rojas A*.** GATA4 induces liver fibrosis regression by deactivating hepatic stellate cells. JCI Insight. 2021 Dec 8;6(23):e150059. doi: 10.1172/jci.insight.150059.

Extra virgin olive oil improved body weight and insulin sensitivity in high fat diet-induced obese LDLr-/-.Leiden mice without attenuation of steatohepatitis. Álvarez-Amor L, Sierra AL, Cárdenas A, López-Bermudo L, López-Beas J, Andújar E, Pérez-Alegre M, Gallego-Durán R, Varela LM, Martin-Montalvo A, Berná G, **Rojas A,** Robles-Frías MJ, Hmadcha A, Romero-Gómez M, Kleemann R, Martín F. Sci Rep. 2021 Apr 15;11(1):8250.



Principal Investigator

Román González-Prieto

Ubiquitin (-like) signalling & proteomics

Emerging PI

Current position

- Distinguished Researcher – EMERGIA20 program from Junta de Andalucía – Department of Cell Biology – University of Sevilla.

Academic Background of PI

- 2005 – Degree in Biology – University of Sevilla.
- 2012 – PhD. University of Sevilla – Microbial Technologies & Genetics.

Positions Held

- 2018-2022 : Senior Researcher and Principal Investigator – Leiden University Medical Center, Leiden, The Netherlands.
- 2013-2018 : PosDoc Researcher – Leiden University Medical Center, Leiden, The Netherlands.

Group Members

PhD Student

- Daniel Salas-Lloret (Leiden University Medical Center).
- Emily Esperanza Soto Hidalgo.

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.

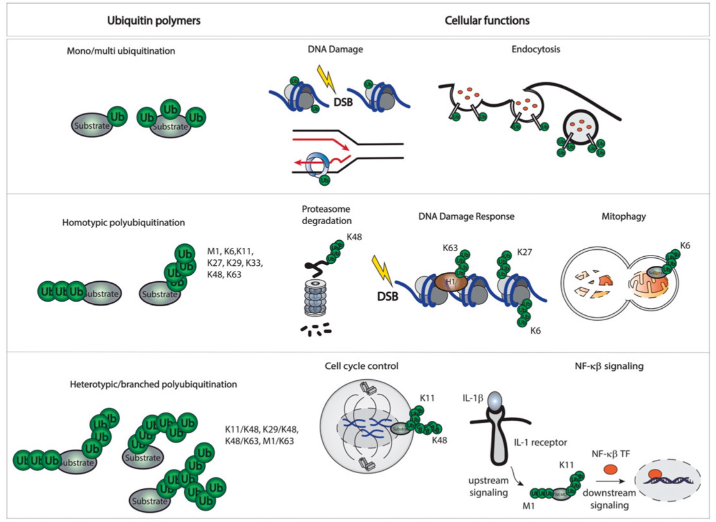


Figure 1. Ubiquitination polymers. Ub moieties can modify proteins at one (mono ubiquitination) or several (multiple mono ubiquitination) Lys residues. Ub can form eight distinctive homotypic linkages, either through M1 (linear Ub chain) or 7 internal Lys residues (K6, K11, K27, K29, K33, K48, and K63 Ub chains). Additional complexity is achieved through the formation of heterotypic Ub chains, which contain multiple Ub linkages and adopt mixed or branched topology. Cellular functions associated to these ubiquitin polymers are displayed. (From Salas-Lloret and González-Prieto (2022) Int. J. Mol. Sci. 23(6), 3281).

E3 enzymes confer substrate specificity for ubiquitin(-like) modifiers, and determining which E3 modifies which substrate is the next challenge in ubiquitin(-like) proteomics. I have optimized the TULIP(2) methodology, which enables to address such challenge. The human genome encodes for more than 600 different E3s.

Specifically, my research focuses on the function and relevance of E3s and their substrates involved in the biology of the genome, including DNA damage repair and tolerance mechanisms and genome organization.

Research Highlights

Developing a ubiquitin(-like) mass spectrometry-based proteomics toolbox.

To identify E3-specific ubiquitination substrates I developed the TULIP methodology which was later improved by two orders of maginitud in the second iteration, TULIP2. Using this methodology, to obtain novel insight in the cross talk between ubiquitin and the ubiquitin-like modifier SUMO, we discovered that the SUMO-targeted Ubiquitin Ligase RNF4 regulates SUMO signaling by targeting the SUMOylation enzymatic machinery for proteasomal degradation, rather than individual sumoylation substrates.

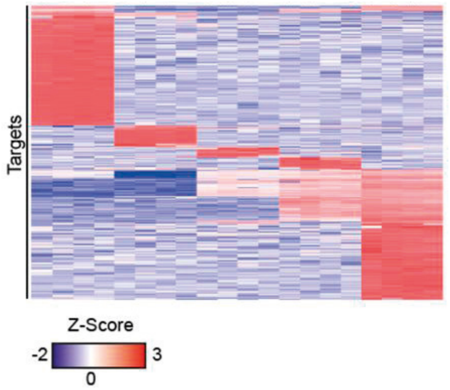


Figure 2. Heatmap depicting SUMO1 substrates for different SUMO E3 enzymes using TULIP2 methodology.

Identification of the non-covalent SUMO proteome.

In contrast to the covalent SUMO proteome, where more than 40k acceptor lysines for SUMO2/3 had been described, our knowledge about non-covalent SUMO interactome was more limited. We identified non-covalent binders that where specific for different SUMO isotypes and/or SUMO chains, additionally, we provided the most complete resource available of SUMO1 acceptor lysines.

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.

Grants (starting or ending 2021-2022)

- Title of the project: Consequences of the Ubiquitin-like modified genome. (SUMOTIN).
  Entity, Reference number: Agencia Estatal de Investigación, PID2021-122361NA-I00
  Dates Start/End: 01/09/2022 – 31/08/2025
  Total amount: €163,350
- Title of the project: Construcción y validación de herramientas para el estudio de huecos de DNA de cadena sencilla derivados de estrés replicativo.
  Entity, Reference number: Universidad de Sevilla, PPIIV.2
  Dates Start/End: 01/01/2022-31/12/2023
  Total amount: €30,000
- Title of the project: UbiGap: Understanding under-replicated DNA gaps signaling and processing with a focus on ubiquitin.
  Entity, Reference number: Junta de Andalucía Programa EMERGIA, EMERGIA20_00276
  Dates Start/End: 01/04/2022 – 31/03/2026
  Total amount: €135,000
- Title of the project: Identification of BRCA/BARD1 ubiquitin E3 ligase target proteins to obtain novel insight in breast- and ovarian cancer.
  Entity, Reference number: Dutch Cancer Society, YIG 11367/2017-2
  Dates Start/End: 01/06/2018 – 31/03/2022
  Total amount: €548,324

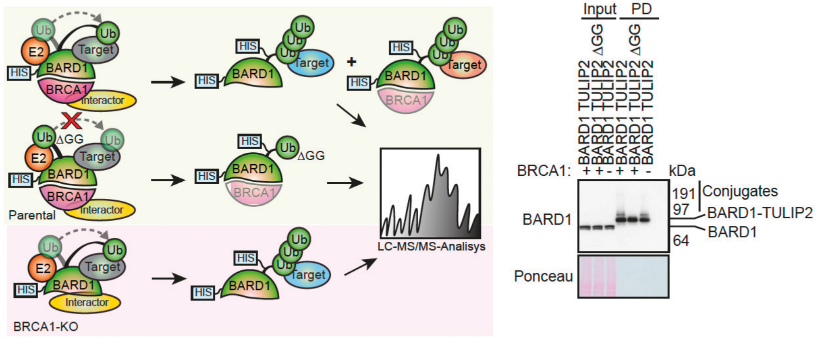


Figure 3. Cartoon depicting TULIP2 rationale for the BARD1 ubiquitin E3 enzyme and analysis by immunoblotting of BARD1-TULIP2 samples.

Publication Highlights

Tessier, S.*, Ferhi, O.*, Geoffroy, M.C.*, Gonzalez-Prieto, R., Canat, A., Quentin, S., Pla, M., Niwa-Kawakita, M., Bercier, P., Rerolle, D., et al. (2022). Exploration of nuclear body-enhanced sumoylation reveals that PML represses 2-cell features of embryonic stem cells. Nature communications 13, 5726. 10.1038/s41467-022-33147-6.

Salas-Lloret, D., and Gonzalez-Prieto, R.# (2022). Insights in Post-Translational Modifications: Ubiquitin and SUMO. Int J Mol Sci 23. 10.3390/ijms23063281.

Van der Weegen, Y., de Lint, K., van den Heuvel, D., Nakazawa, Y., Mevissen, T.E.T., van Schie, J.J.M., San Martin Alonso, M., Boer, D.E.C., Gonzalez-Prieto, R., Narayanan, I.V., et al. (2021). ELOF1 is a transcription-coupled DNA repair factor that directs RNA polymerase II ubiquitylation. Nature cell biology 23, 595-607. 10.1038/s41556-021-00688-9.

Van den Heuvel, D.*, Spruijt, C.G.*, Gonzalez-Prieto, R., Kragten, A., Paulsen, M.T., Zhou, D., Wu, H., Apelt, K., van der Weegen, Y., Yang, K., et al. (2021). A CSB-PAF1C axis restores processive transcription elongation after DNA damage repair. Nature communications 12, 1342. 10.1038/s41467-021-21520-w.

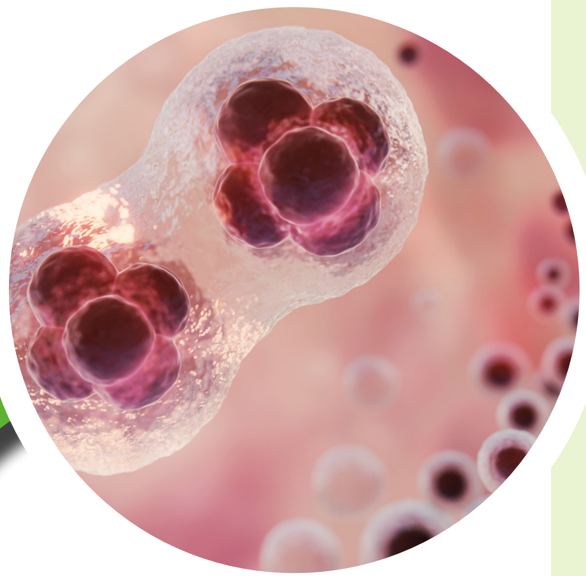
Gonzalez-Prieto, R.#, Eifler-Olivi, K., Claessens, L.A., Willemstein, E., Xiao, Z., Talavera Ormeno, C.M.P., Ovaa, H., Ulrich, H.D., and Vertegaal, A.C.O. (2021). Global non-covalent SUMO interaction networks reveal SUMO-dependent stabilization of the non-homologous end joining complex. Cell reports 34, 108691. 10.1016/j.celrep.2021.108691.

*: Equal contribution ; #: corresponding author

Integrative Pathophysiology and Therapies department

The name of this dept was changed to better reflect the scope and lines of research conducted by the groups integrating it. 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 finding drugs and therapeutic targets that promote healthy aging, cell survival, regeneration and the optimal function of organs to treat 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. Anabel Rojas till Dec 2022.
(Current Head: Dr. Inés Pineda-Torra)

RESEARCH GROUPS

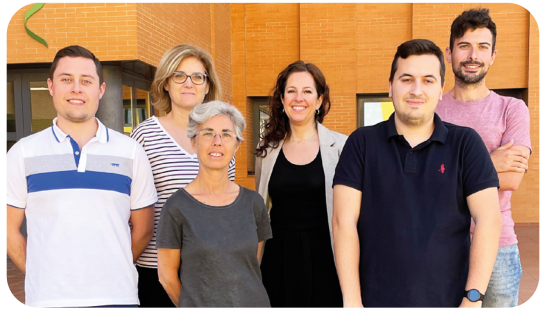
1. **Pancreatic Islets and Stem Cells**
   Dr. Franz Martin
2. **Pancreatic Islet Development & Regeneration**
   Dr. Benoit Gauthier
3. **Pancreas and Liver Development and Disease** (till Dec 2022)
   Dr. Anabel Rojas
4. **Cell Therapy for Neuropathologies**
   Dr. Manuel Álvarez Dolado
5. **Cellular and Molecular Neuroimmunology**
   Dr. David Pozo & **Misfolding Proteins and Molecular Chaperones in Immune Dysregulation**
   Dr. Cintia Roodveldt
6. **Metabolic Interventions for Successful Aging**
   Dr. Alejandro Martín-Montalvo
7. **Retinal Degeneration: from Genetics to Therapy**
   Dr. F. Díaz-Corrales
8. **Stem Cells and Translational Neurology**
   Dr. V. Capilla-González
9. **Metabolism, Immunology and Cardiovascular Risk**
   Dr. I. Pineda-Torra

Principal Investigator

Inés Pineda Torra

Metabolism, Immunology & Cardiovascular Risk

Group Leader
Head of the Department



Current position

- Since April 2022: Junta de Andalucía-Consejería de Salud y Familias Distinguish Scientist/Group Leader CABIMER, Seville, Spain.
- Since May 2022: Member of NURCAMEIN, Nuclear Receptors Spanish Excellence Network.

Current Group Members

PhD Students

- Laurel Woodbridge (UCL-London).

Technicians

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

Postdoctoral fellows

- Carlos Jimenez Cortejana.

Former Members (2021-2022)

- **PhD student:** Annalisa Maggio (UCL-London); Leda Coeleweij (UCL-London).
- **Career Development Fellows (sponsor in Charity funded Career Development Fellowship):** George Robinson (Versus Arthritis); Rens de Groot (British Heart Foundation).

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 affect systemic and intracellular metabolic and immune pathways and how that affects disease development. 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 increased cardiovascular risk in women with autoimmune disorders such as systemic lupus erythematosus (SLE). We used human and immune cells. During this period, I moved my research activities to CABIMER from the Division of Medicine at UCL.

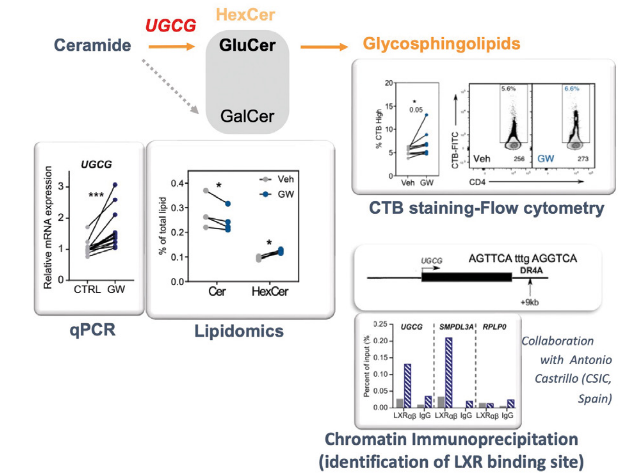


Fig. 1. LXR activation regulates the transcription of UGCG. Primary human CD3+ T cells (n = 4) were sorted by FACS and treated ± GW (2 µM) and total cellular lipid content analyzed by shotgun lipidomics. Schematic illustrating the role of UGCG in the conversion of ceramide to HexCer. Dot plots show overall change in Cer and HexCer levels.. Up-regulation of UGCG mRNA expression in CD4+ T cells by GW treatment (n = 13). LXR occupancy at the putative DR4 motif at UGCG compared with IgG control, positive control (SMPDL3A), and negative control (RPLP0) sequences. Cells were treated with DMSO (gray bars), LXR (GW, 1 µM), and RXR (LG100268; LG, 100 nM) ligands (hatched bars) . Representative of three independent experiments.) Two-tailed t tests: *P < 0.05, **P < 0.01, ***P < 0.001. Abbreviations: Cer, ceramide; HexCer, hexosylceramide.

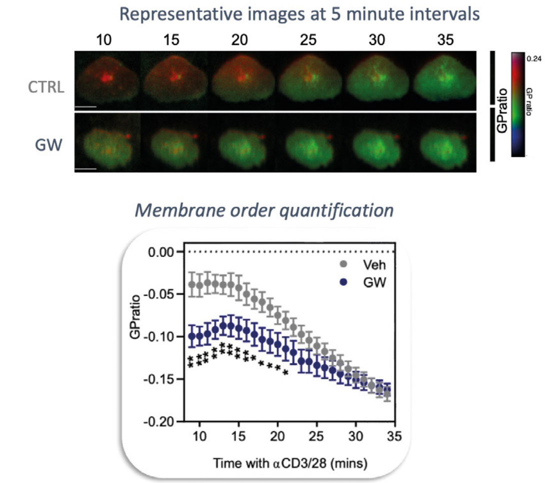


Fig.2: LXR activation regulates immune synapse formation. Magnetically purified CD4+ T cells were cultured ± GW before addition to chamber slides coated with anti-CD3/28 for immune synapse formation. (A and B) T cells were stained with di-4-ANEPPDHQ, and immune synapse formation was recorded for 30 min using TIRF microscopy. Shown are representative images at 5 min intervals. (Scale bar, 5 µM.) GP ratio was quantified at each minute (n= 10 to 12 cells/condition, mean ± SEM). Multiple unpaired t tests corrected for multiple comparisons: *P < 0.05, **P < 0.01, ***P < 0.001. *Shown in Waddington, PNAS 2021.*

Research Highlights

The main highlights for the period 2021-22 are:

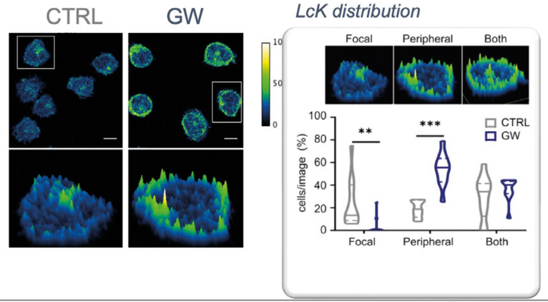


Fig.3: LXR activation modulates proximal TCR signaling. Magnetically purified CD4+ T cells were cultured ± GW before addition to chamber slides coated with anti-CD3/28 for immune synapse formation. Immune synapses (n = 2 donors) were fixed at 15 mins post activation and immunostained for Lck (CTRL = 68 cells, GW = 52 cells) and phosphotyrosine (pY) (CTRL = 59 cells, GW = 52 cells). Representative images and quantification of Lck distribution patterns . Violin plots show median and quartile values. Mann–Whitney U: *P < 0.05, **P < 0.01, ***P < 0.001. *Shown in Waddington, PNAS 2021.*

1. LXR and membrane lipid metabolism in human immune cells

We identified a novel mode of action of LXR involving regulation of membrane lipid rafts (specifically cholesterol and glycosphingolipids-GSLs) in lymphocytes (CD4+-T) and reported a GSL biosynthesis enzyme as a novel LXR target (Waddington et al. PNAS) and Fig.1. Changes in plasma membrane lipid composition in CD4+-T-cells are associated with altered immune synapse formation, T-cell receptor-mediated signalling, reduced T-cell proliferation and modified cytokine production. To examine the effect of LXR stimulation on the kinetics of lipid reorganization during the early stages of T cell activation, we used di-4-ANEPPDHQ staining and total internal reflection fluorescence (TIRF) microscopy to assess the interaction between CD4+-T cells and antibody-coated glass coverslips (thus mimicking the "immune synapse") (Fig. x2).

2. Cardiovascular risk and lipid metabolism: age & sex differences

We used blood metabolomic profiles and machine learning, combined with transcriptomic approaches to profile the increased cardiovascular risk in SLE. Our work identified metabolite and gene signatures, and interactions with clinical features associated with early atherosclerosis in adult and adolescent lupus (Robinson et al. 2022, Coeleweij et al. 2021, Woodridge et al. 2020, Robinson et al. 2021, Robinson et al 2020). Furthermore, in studies with cis and trans-gendered adolescents, we uncovered T-cell gender specific differences (gene expression and plasma membrane/serum lipid levels) (Robinson et al 2022, Robinson et al 2021) and evidenced that sex chromosomes and sex hormones drive different kind of changes (cell frequency vs function), respectively (Robinson et al 2021).

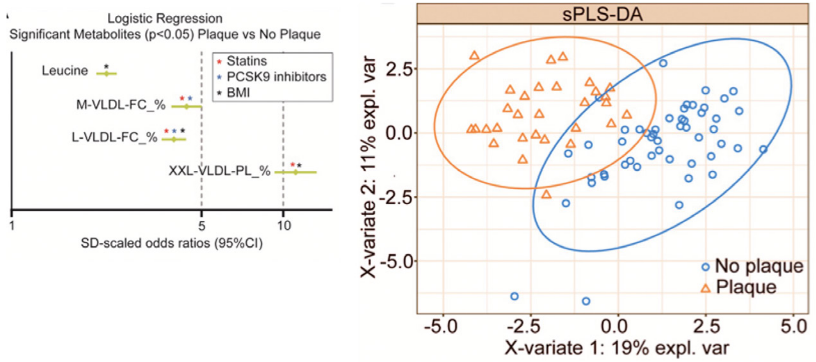


Fig.4. Identification of important metabolites separating patients with SLE with subclinical plaque (SLE-P) from patients with SLE with no subclinical plaque (SLE-NP). **Left**, Forest plot depicting statistically significant individual logistic regression results of metabolites in SLE-P (n=30) vs SLE-NP (n=50). Results given in odds ratio (95% CIs). Logistic regressions were adjusted for age, ethnicity, mean arterial blood pressure, global BILAG-2004, disease duration, and treatments at the time of scan. **Right,** Sparse partial least squares discriminant analysis (sPLS-DA) plot to validate top hits from the logistic regression with interactions.

Grants

- 2022-2025: Agencia Estatal de Investigación. Ministerio de Ciencia e Innovación (PI).
- 2022-2025, British Heart Foundation, Non-clinical PhD Studentship (Co-PI).
- 2020-2023: BBSRC-LIDO Studentship (PI).
- 2019-2022: UCL-Birbeck Medical Research Council Doctoral Training Program in Precision Medicine Studentship (PI).
- 2019-2022: UCB BioPharma-sponsored PhD Studentship (PI).
- 2020-2022: UCL NIHR BRC Inflammation, Immunity and Immunotherapeutics (co-PI).
- Oct 2020-March 2022: UCL NIHR BRC III theme (co-PI).
- Oct 2020-Sept 2021: UCL NIHR BRC Cardiovascular Diseases Theme-Omics (PI).
- 2019- 2021: Dunhill Medical Trust (Co-PI).
- 2018-2021: Diabetes UK Project Grant (co-PI).
- 2016-2021: British Heart Foundation Project Grant (co-PI).

Publication Highlights

Robinson, G.A., Peng, J., Peckham, H., Butler, G., **Pineda-Torra,I.** #, Jury, E.C. #, Ciurtin, C. # (#senior authors). **(2022)**, Investigating sex differences in T regulatory cells from cisgender and transgender healthy individuals and patients with autoimmune inflammatory disease: a cross-sectional study. **Lancet Rheumatology** 4 (10), e710-e724, https://doi.org/10.1016/S2665-9913(22)00198-9, commentary https://doi.org/10.1016/S2665-9913(22)00254-5

Robinson, G. A., Peng, J., Pineda-Torra, I., Ciurtin, C., & Jury, E. C. **(2022)**. Metabolomics defines complex patterns of dyslipidaemia in juvenile-sle patients associated with inflammation and potential cardiovascular disease risk. **Metabolites**, 12(1). doi:10.3390/metabo12010003

Robinson, G.A., Peng, J., Peckham, H., Radziszewska, A., Butler, G., **Pineda-Torra,I.** #, Jury, E.C. #, Ciurtin, C. # (#senior authors). **(2021)**. Sex hormones drive changes in lipoprotein metabolism, **iScience**, 24, 11,10325 https://doi.org/10.1016/j.isci.2021.103257

Waddington, K. E., Robinson, G. A., Rubio-Cuesta, B., Chrifi-Alaoui, E., Andreone, S., Poon, K. -S., ... Jury, E. C.#, **Pineda-Torra, I.** (#senior authors). **(2021)**. LXR directly regulates glycosphingolipid synthesis and affects human CD4+ T cell function. **Proceedings of the National Academy of Sciences of USA**, 118(21). doi:10.1073/pnas.2017394118

Schmidt, N.M., Wing, P.A.C., Diniz, M.O., Pallett, L.J., Swadling, L., Harris, J.M., ... **Pineda-Torra, I.**, Jolly. C., Jury, E.C., McKeating, J.A. & Maini, M.K. **(2021)**. Targeting human Acyl-CoA:cholesterol acyltransferase as a dual viral and T†cell metabolic checkpoint. **Nature Communications**, 12(1), 2814. doi:10.1038/s41467-021-22967-7

Coelewij L, Waddington KE, Robinson GA, ... Rahman A, Jury EC#, **Pineda-Torra I#** (#senior authors). **(2021)** Using serum metabolomics analysis to predict sub-clinical atherosclerosis in patients with SLE, **Arteriosclerosis, Thrombosis, and Vascular Biology**, Feb 1:ATVBAHA120315321. DOI: 10.1161/ATVBAHA.120.315321

Voisin M, Shrestha E, ... **Pineda-Torra I**, Fisher EA and Garabedian MJ **(2021)** Inhibiting LXRa Phosphorylation in Hematopoietic Cells Reduces Inflammation and Attenuates Atherosclerosis and Obesity. **Communications Biology** Mar 26;4(1):420, doi: 10.1038/s42003-021-01925-5

Editorials & Reviews

Fan, R., **Pineda-Torra, I.**, & Venteclef, N. **(2021)**. Editorial: Nuclear Receptors and Coregulators in Metabolism and Immunity. **Frontiers in Endocrinology**, 12. doi:10.3389/fendo.2021.828635

Robinson, G. A., **Pineda-Torra, I.**, Ciurtin, C., & Jury, E. C. **(2021)**. Predicting long-term cardiometabolic risk: Do childhood metabolomic signatures hold the key?. **EBioMedicine**, 74. doi:10.1016/j.ebiom.2021.103702

Robinson, G. A., **Pineda-Torra, I.**, Ciurtin, C., & Jury, E. **(2022)**. Lipid metabolism in autoimmune rheumatic disease: implications for modern and conventional therapies. **Journal of Clinical Investigation.** 10.1172/JCI148552

Thibaut T, Gage MC, **Pineda-Torra I**, Chabrier G, Venteclef N, Alzaid F **(2022)**, Liver Macrophages and Inflammation in Physiology and Physiopathology of Non-Alcoholic Liver Disease, **FEBS J**, 2022 Jun;289(11):3024-3057. doi: 10.1111/febs.15877

Pineda-Torra I, Siddique S, Waddington KE, Farrel R, & Jury EC **(2021)**, Disrupted Lipid Metabolism in Multiple Sclerosis: A Role for Liver X Receptors? **Front. Endocrinol.** 12, 345, | https://doi.org/10.3389/fendo.2021.639757

Voisin, M, Gage M, Becares N, Shrestha E, Fisher EA, **Pineda-Torra I** & Garabedian M, **(2021)** LXRα phosphorylation in cardiometabolic disease: insight from mouse models, **Endocrinology** https://doi.org/10.1210/endocr/bqaa089

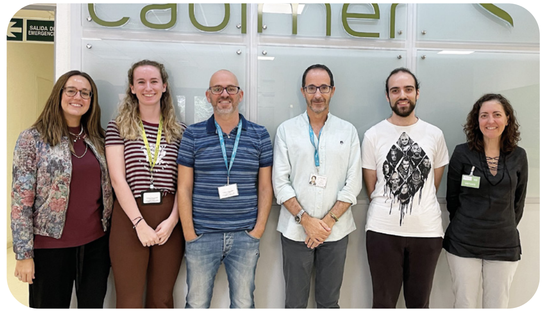
C Parikh, J Ponnampalam, G Seligmann, L Coelewij, **I Pineda-Torra**, Jury E, Ciurtin C. **(2021),** Impact of immunogenicity on clinical efficacy and toxicity profile of biologic agents used for treatment of inflammatory arthritis in children compared to adults. **Therapeutic Advances in Musculoskeletal Disease**, 3:1759720X211002685, DOI: 10.1177/1759720x211002685

Principal Investigator

Dr. Franz Martin

Nutrition and metabolic diseases

Group Leader



Current position

- Since 2006: Research Scientist CABIMER/ Seville, Spain.
- Since 2007: Principal Investigator of CIBER of Diabetes and Associated Metabolic Diseases (CIBERDEM).
- Since 2007: Full Professor at University Pablo de Olavide, Seville, Spain.
- Since 2016: Steering Committee Member of CIBERDEM.

Group Members

Senior Researchers

- Blanca Escudero.
- Mª Ángeles Ortega.

PhD Student

- Lucía López.

Technicians

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

Former Members (2020-2022)

- **PhD student:** Leticia Álvarez.
- **Technicians:** Amparo Luque.

Research Activity

Overview

Our main research line is to study the role of nutrients, foods and diets in the pathogenesis of diabetes, obesity, diabesity, metabolic syndrome and non-alcoholic fatty liver disease (NAFLD). Particularly we are involved in to understanding the mechanisms of actions by which hypercaloric high-fat and high-carbohydrate diets promote the onset of type 2 diabetes (T2DM) mellitus and NAFLD. We try to focus this issue from system physiology approach.

Research Highlights

Our main research highlights are:

1. We found, in mouse model of high fat diet (HFD)-induced obesity, T2DM and NAFLD, that extra virgin olive oil (EVOO) intake repaired HFD-induced hepatic damage via an anti-inflammatory effect in adipose tissue and modifications in the liver lipid composition and signaling pathways. Moreover, EVOO intake regulated glucose homeostasis, improving insulin sensitivity and pancreatic β-cell function. In addition, the intake of an EVOO much richer in phenolic compounds did not increase the beneficial effects of EVOO on liver injury, insulin sensitivity and β-cell function.

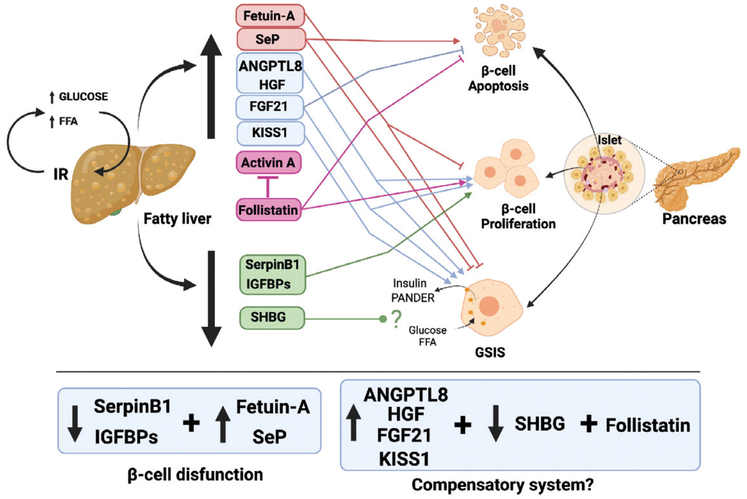


Figure 1. Liver factors involved in the talk of hepatic tissue with pancreatic β-cells in the presence of fatty liver.

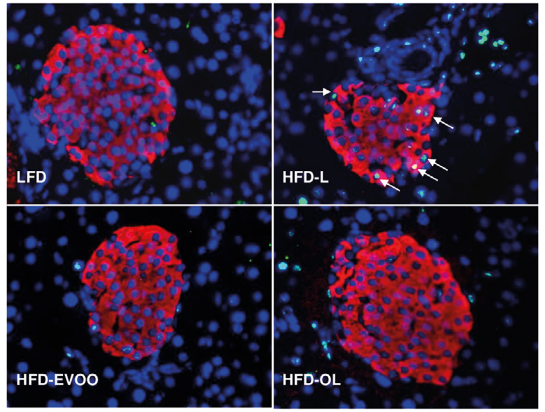


Figure 2. Effect of EVOO high fat diets on pancreatic β-cell number and apoptosis at the end of the 36-week interventional study. These are confocal images showing the nuclei stained with DAPI (blue), insulin (red) and TUNEL-positive (green) cells.

2. In a female *Ldlr-/-*.Leiden mice with HFD-induced non-alcoholic steatohepatitis (NASH), liver fibrosis, dyslipidemia and insulin resistance (IR), we found that EVOO intake improved body weight and IR but aggravated liver inflammation and fibrosis. The potential proposed mechanisms contributing to this EVOO effects are the upregulation of genes involved in liver inflammation, fibrosis and oxidative stress, as well as the downregulation of genes critical for liver lipid homeostasis.

3. In a female *Ldlr-/-*.Leiden mice with HFD-induced NASH/fibrosis, dyslipidemia and IR, EVOO intake attenuated adipose tissue hypertrophy and inflammation and exerted anti-atherosclerotic effects. The EVOO vasculoprotective effects are due to a modification in gene expression related with inflammation, a repression on monocyte/macrophage infiltration and a reduction of aortic peroxynitrites production A higher phenolic content of olive oil did not provide further benefits in the prevention of atherosclerosis.

4. When mothers are fed, during pregnancy and lactation with EVOO, there exist a protection in the offspring against the appearance of T2DM and NFLD, after the intake of hypercaloric diets (rich in sugars and saturated fats). This protection is different depending on the gender of the animal. EVOO intake cause transgenerational epigenetic modifications in miR expression that could contribute to offspring protection against the risk of T2DM and NAFLD development, depending on the gender.

Grants

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

Publication Highlights

Simón J, Goikoetxea-Usandizaga N, Serrano-Maciá M, Fernández-Ramos D, Sáenz de Urturi D, Gruskos JJ, Fernández-Tussy P, Lachiondo-Ortega S, González-Recio I, Rodríguez-Agudo R, Gutiérrez-de-Juan V, Rodríguez-Iruretagoyena B, Varela-Rey M, Gimenez-Mascarell P, Mercado-Gomez M, Gómez-Santos B, Fernandez-Rodriguez C, Lopitz-Otsoa F, Bizkarguenaga M, Dames S, Schaeper U, Martin F, Sabio G, Iruzubieta P, Crespo J, Aspichueta P, Chu KH, Buccella D, Martín C, Delgado TC, Martínez-Cruz LA, Martínez-Chantar ML. Magnesium accumulation upon cyclin M4 silencing activates microsomal triglyceride transfer protein improving NASH. J Hepatol, 2021, 75, 34-45.

Álvarez-Amor L, Luque-Sierra A, Cárdenas A, López-Bermudo L, López-Beas J, Andújar E, Pérez-Alegre M, Gallego-Durán R, Varela LM, Martin-Montalvo A, Berná G, Rojas A, Robles-Frías MJ, Hmadcha A, Romero-Gómez M, Kleemann R, Martín F. Extra virgin olive oil improved body weight and insulin sensitivity in high fat diet-induced obese LDLr-/-.Leiden mice without attenuation of steatohepatitis. Sci Rep, 2021, 11, 8250.

Gallego P, Luque-Sierra A, Falcón G, Carbonero P, Grande L, Bautista JD, Martin F*, Del Campo JA*. White button mushroom extracts modulate hepatic fibrosis progression, inflammation and oxidative stress in vitro in LDLR-/- mice. Foods, 2021, 10, 1788.

Lopez-Bermudo L, Luque-Sierra A, Maya-Miles D, Gallego-Duran R, Ampuero J, Romero-Gomez M, Berná G, Martin F. Contribution of liver and pancreatic islet crosstalk to beta-cell function/dysfunction in the presence of fatty liver. Front Endocrinol, 2022, 13, 892672.

Martín Vázquez E, Cobo-Vuilleumier N, Araujo Lehido R, Marín-Cañas S, Nola E, Dorronsoro A, López Bermudo L, Crespo A, Romero-Zerbo SY, García-Fernández M, Martín Montalvo A, Rojas A, Comaills V, Bermudez-Silva FJ, Gannon M, Martin F, Eizirik D, Lorenzo PI, Gauthier BR. NR5A2/LRH-1 regulates the PTG2-PGE2-PTGER1 pathway contributing to pancreatic islet survival and function. iScience, 2022, 25, 104345.

Principal Investigator

Dr. Benoit R. Gauthier

Pancreatic Islet Development and Regeneration Unit

Group Leader

Current position

- Since 2009: Junta de Andalucía-Consejería de Salud y Familias Staff Scientist/Group Leader CABIMER, Seville, Spain.
- Since 2017: Member of CIBERDEM, Madrid, Spain.

Group Members

Lab Manager

- Nadia Cobo Vuilleumier.

Senior Researcher

- Petra Isabel Lorenzo Ovejero.

Postdoctoral fellows

- Valentine Comaills.
- Christian Lachaud.
- Jaime Muños Franco.
- Akaitz Dorronsoro González.
- Livia López Noriega.

PhD student

- María Eugenia Martin Vázquez García.

Master student

- Sergio Manzano.

Technicians

- Cristina Cerrada Romero.
- Daniel De Lllano Teixeira.
- Pedro Antonio Soriano Gonzalez.

Former Members (2021-2022)

- **Postdoctoral Fellows:** Nestor Wenceslao Meza.
- **Master student:** Alejandro Andredas Cordero; Sebastian Bermeo (Erasmus, France).
- **Technicians:** Alejandra Crespo Barreda.

Research Activity

Overview

The hallmark of Diabetes Mellitus (DM) is hyperglycemia that develops as a consequence of either impaired insulin secretion from pancreatic islet beta-cells or resistance to its blood glucose lowering effect. Deficient insulin output stems from either beta-cell dysfunction (Type 2 DM; T2DM and gestational DM; GDM) or beta-cell obliteration by the immune system (Type 1 DM; T1DM). With this in mind, our research interests focus on the identification and characterization of DM associated genes implicated in beta-cell expansion, survival and function. We also seek to understand how the expression of these genes in other tissues such as brain and immune cells may influence islet function through an intricate cross talk between organs. We perform in depth genetic, molecular and cellular studies using human/mouse cell lines, mouse models and primary human tissues. The long-term goal is to develop innovative advanced and pharmacological therapies/cures for DM. Towards this goal, we have established and extensive International and National collaborative network with hospitals, research institutes and pharmaceutical partners as well as with private foundations.

Research Highlights

The main highlights for the period 2021-22 are:

1. LRH-1/NR5A21 mode of action in conveying alpha-to-beta cell transdifferentiation and anti-apoptotic capabilities to mouse islets

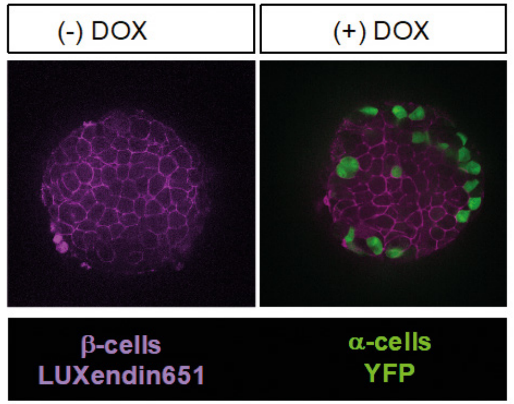


Figure 1: Live imaging of a pancreatic islet in which YFP expression was induced specifically in α-cells using doxycycline (DOX) in order to perform cell fate lineage tracing.

We previously shown that activation of the nuclear receptor LRH-1/NR5A2 using the small chemical agonist BL001, developed in house, reverts hyperglycaemia in several pre-clinical mouse models of T1DM via immuno-tolerization coupled to enhanced islet beta-cell survival and regeneration likely through alpha-to-beta cell trans-differentiation. In the past 2 years, we validated through lineage tracing studies in which alpha cells are irreversibly marked by YFP expression that BL001-mediated activation of LRH-1/NR5A2 in an hyperglycaemic environment prompts the genetic reprogramming of alpha cells towards a beta cell phenotype hallmarked by the expression of key markers such as INSULIN, PDX1 and GLP1R. This cellular rewiring was

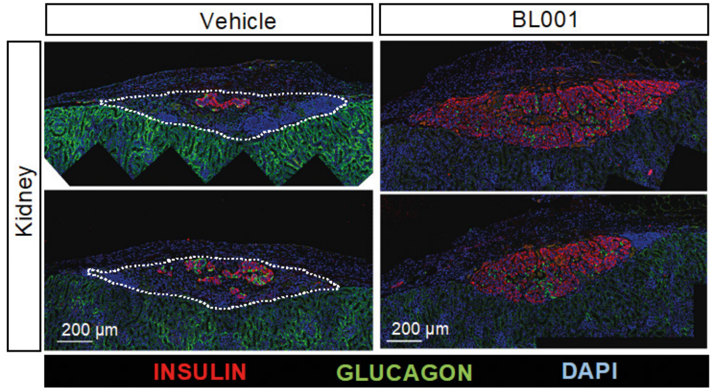


Figure 2: The LRH-1/NR5A2 agonist BL001 preserves the mass and functionality of human islets transplanted under the kidney capsule of immuno-competent mice.

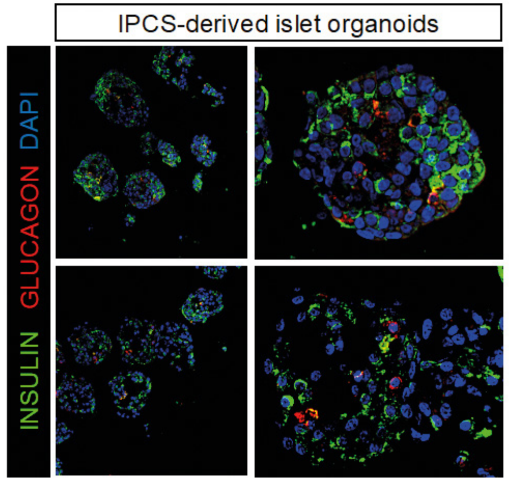


Figure 3: IPSCs were differentiated into pancreatic endocrine cells using a 7-step protocol. Representative immuno-fluorescent images of islet organoids.

enabled by BL001-mediated re-education of the immune attack, reminiscent of wound healing.

Furthermore we demonstrated that ablation of LRH-1/NR5A2 specifically in adult beta-cells abolished the BL001 anti-diabetic action in mice correlating with increased beta-cell destruction and blunted *Ptgs2* induction, one of the most up-regulated genes in BL001-treated islets. Islet PTGS2 inactivation led to reduced levels of its product PGE$_2$ and loss of BL001 protection against cytokines-induced apoptosis. We show that blocking the PGE$_2$ receptor PTGER1 negated BL001-mediated islet survival against cytokine induced apoptosis. Our results define the LRH-1/PTGS2/PGE$_2$/PTGER1 signalling axis as a key pathway mediating BL001 survival properties.

2. LRH-1/NR5A2 agonism rewires immunometabolism favouring an anti-inflammatory phenotype to immune cells of type 1 diabetes mellitus donors and fosters islet graft stealthiness

We also translated our murine data to human immune cells obtained from individuals with T1DM, demonstrating the capacity of BL001 to reduce the expression of pro-inflammatory markers and cytokines from macrophages as well as dendritic cells while increasing regulatory T-cells (Tregs) important for suppressing the autoimmune attack. More importantly, the expansion of cytotoxic T-cell implicated in the destruction of beta-cells was blunted by dendritic cells and Tregs exposed to BL001. At the molecular level, BL001 immuno-paralyzed pro-inflammatory macrophages through mitohormesis while increasing oxidative phosphorylation and fatty acid oxidation in dendritic cells promoting a tolerogenic phenotype. We also demonstrate that BL001 administration to diabetic immune-competent mice transplanted with human islets improved survival rate correlating with reduced hyperglycaemia and preserved beta-cell mass. Our results establish that BL001 can induce a pro-to-anti-inflammatory phenotypic switch to human immune cells, and improve human islet engraftment/function, supporting the therapeutic benefits of BL001 in human. To further understand the complex interaction between immune and islet cells and the impact of BL001 holistically, we are now modelling T1DM in vitro using IPC-derived islet organoids and immune cells obtained from diseased individuals.

3. HMG20A, a multicellular integrator to stress adaptation

We have shown that the 'Metabesity' factor HMG20A regulates islet beta-cell functional maturity as well as astrocyte polarization as a gluco-adaptive/neuro-protective response to physiological stress such as obesity and diabetes. We also found that HMG20A transcript levels were increased in adipose tissue of obese non-diabetic individuals as compared to obese diabetic patients indicating a key role of HMG20A in a coordinated adaptive organ response to pathophysiological conditions. Accordingly, treatment of obese mice with ORY1001, a pharmacological inhibitor of the LSD1/CoREST complex that mimic the effect of HMG20A, normalized glucose intolerance paving the way for a new therapeutic approach for Type 2 Diabetes Mellitus.

Grants

- 2022-2025: 3-SRA-2023-1307-S-B Juvenile Diabetes Research Foundation.
- 2022-2024: DiabetesCERO.
- 2021-2024: PID2021-123083NB-I00 Ministerio de Ciencia, Innovación y Universidades.
- 2020-2023: Vencer el Cáncer.
- 2020-2024: Amarna Therapeutics S.L.
- 2019-2021: JDRF, SRA-2019-837-S-B. Juvenile Diabetes Research Foundation.
- 2018-2022: PRE2018-084907. Ministerio de Ciencia, Innovación y Universidades.

Publication Highlights

Martin Vázquez E, Cobo-Vuilleumier N, Araujo Legido R, Marín-Cañas S, Nola E, Dorronsoro A, López Bermudo L, Crespo A, Romero-Zerbo SY, García-Fernández M, Martin Montalvo A, Rojas A, Comaills V, Bérmudez-Silva FJ, Gannon M, Martin F, Eizirik D, Lorenzo PI, Gauthier BR. **2022.** NR5A2/LRH-1 regulates the PTGS2-PGE2-PTGER1 pathway contributing to pancreatic islet survival and function. **iScience.** 25:104345.

Gauthier BR, Rubio-Contreras D, Gómez-Rosado JC, Capitán-Morales LC, Soria Escoms B, Lachaud CC. **2022.** Human Omental Mesothelial Cells Impart an Immunomodulatory Landscape Impeding B and T-cell activation **Int. J. Mol. Sci.** 22:4239.

González-Mariscal I, Pozo-Morales M, Romero-Zerbo SY, Espinosa-Jimenez V, Escamilla-Sánchez A, Sánchez-Salido L, Cobo-Vuilleumier N, Gauthier BR, Bermúdez-Silva FJ. **2022.** Abnormal cannabidiol ameliorates inflammation preserving pancreatic beta cells in mouse models of experimental type 1 diabetes and beta cell damage. **Biomed. Pharmacother.** 145:112361.

Lorenzo PI, Martin Vazquez E, López-Noriega L, Fuente-Martín E, Mellado-Gil JM, Franco JM, Cobo-Vuilleumier N, Guerrero Martínez JA, Romero-Zerbo SY, Perez-Cabello JA, Rivero Canalejo S, Campos-Caro A, Claude Lachaud C, Crespo Barreda A, Aguilar-Diosdado M, García Fuentes E, Martin-Montalvo A, Álvarez Dolado M, Martin F, Rojo-Martinez G, Pozo D, Bérmudez-Silva FJ, Comaills V, Reyes JC, Gauthier BR. **2021.** The Metabesity Factor HMG20A Potentiates Astrocyte Survival and Reactive astrogliosis Preserving Neuronal Integrity. **Theranostics.** 11:6983.

Lorenzo PI, Cobo-Vuilleumier N, Martín-Vázquez E, López-Noriega L, Gauthier BR. **2021.** Harnessing the Endogenous Plasticity of Pancreatic Islets: A Feasible Regenerative Medicine Therapy for Diabetes? **Int. J. Mol. Sci.** 22:4239.

Principal Investigator

Dr. Manuel Álvarez-Dolado

Cell-Based Therapies for Neuropathologies

Group Leader

Current position

- Since 2008: Tenure Research Scientist. CSIC/ CABIMER, Seville, Spain.

Group Members

Postdocs

- Maurizio Riga.

PhD Students

- Mª Mercedes Pérez Fernández.
- Benito Domínguez Velasco.

Technicians

- Sara Vázquez Ávila.

Former Members (2021-2022)

- **Postdocs:** Magdalena Martínez-Losa; Inmaculada Márquez Noriego.
- **Master Students:** Miriam Gonzalvo Ramón.
- **Erasmus+PhD students:** Adrianna Wittek.

Research Activity

Overview

We develop new cell-based therapeutic strategies for the treatment of diseases that affect the nervous system, with special interest in those related to GABAergic interneurons such as infantile epilepsy (Dravet and Stxbp1 syndromes), mental disorders (depression, schizophrenia), or neurodegenerative disease (ataxia, Alzheimer).

We perform pre-clinical assays to verify the therapeutic potential of GABAergic interneurons transplanted into the brain of infantile epilepsy models at early time points (P3-5), analysing the ability to stop their seizures and to revert cognitive and behavioural alterations.

We are also deciphering the role of the sodium voltage-gated channel Nav1.1 in the function of GABAergic interneuron of the prefrontal cortex. This is relevant to better understand the symptoms of Dravet syndrome and also the etiology of some mental disorders.

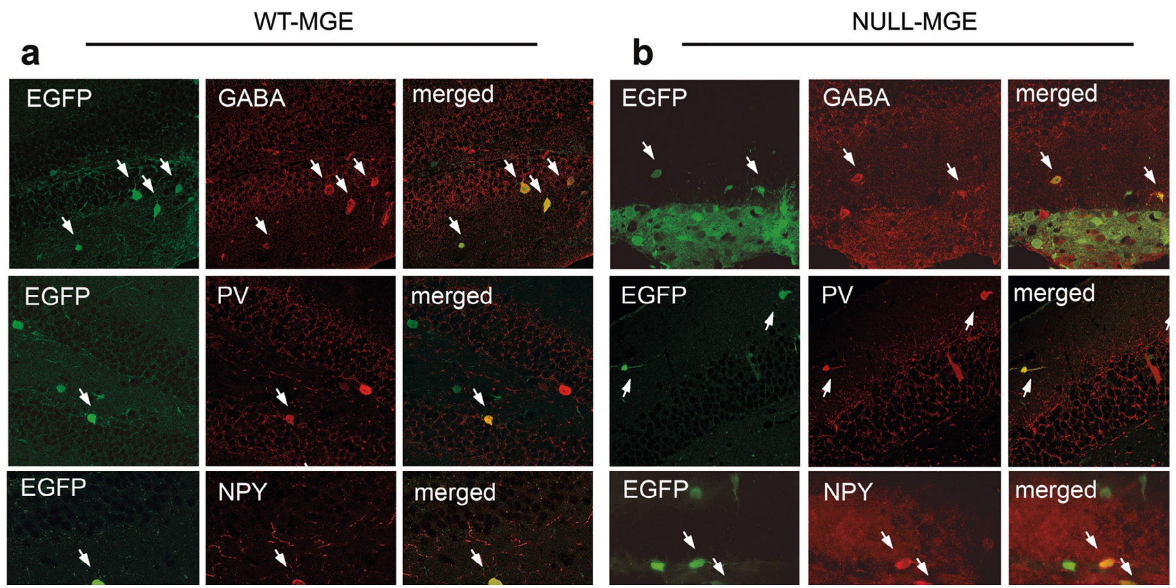


Figure 1. Subtype characterization of grafted EGFP+ GABAergic neuronal precursors in the dentate gyrus (DG) of WT-MGE (a) and LPA1-null mice (b). Representative sections show individual and merged images of immunocolocalization (arrows) of EGFP with GABA, PV, NPY, and SOM. Scale bars, GABA, PV, CB, and CR, 100 μm; NPY and SOM, 50 μm. Modify from Rosell-Valle C, et al. Brain Struct Funct. 2021.

Finally, we collaborate in the design of new miniaturized neuronal recording and stimulation devices in order to improve the detection and control of brain activity through new optogenetic technologies. These systems have a possible therapeutic application in the early detection of epileptic seizures and their prevention.

Research Highlights

During these years a major effort was devoted to show the ability of GABAergic neuronal precursors to rebalance the excitatory/ inhibitory equilibrium in certain pathological conditions. They increase the inhibitory tone in the transplanted area, what recover normal brain function by reducing hyperactivity. This leads to improvements in cognitive deficits and behavioral alterations. Especially relevant was our work (Neuron, 2018) showing that it is possible to rescue cognitive deficits and restore brain rhythms in an animal model of familial Alzheimer Disease characterized by reduced expression of the voltage-gated sodium channel Nav1.1. Similarly, the transplant of these neuronal precursors has the potential to reduce anxiety-like behaviors, and neutralize passive coping in a model of metal disorders (Brain Struct Funct, 2021). Currently, we are exploring a possible cell-based therapeutic application for Dravet syndrome, a refractory infantile epilepsy originated by inactivating mutations in Nav1.1. We are transplanting GABAergic precursors that overexpress Nav1.1 in a mouse model of DS and analyzing its antiepileptic effects. In addition, we are studying the role of Nav1.1 in the prefrontal cortex and its relation with the etiology of mental disorders. In parallel, we are also applying the GABAergic neuronal precursors in a model of Stxbp1 Syndrome, another infantile epileptic encephalopathy, with promising results.

Besides, we have participated in a study about the influence of the Reelin secreted by interneurons in the lamination process of the cerebral cortex during development (PNAS USA, 2022). We also collaborated with the CSIC Microelectronics Institute to generate a multiplexed neuronal recording interface that was experimentally verified in vitro with primary neuronal cultures (IEEE Trans Biomed Circuits Syst, 2021).

In resume, our results strongly suggest that naïve or genetically-modified GABAergic interneuron precursors are a promising source of cells for regenerative medicine to treat mental conditions.

Grants

- 2021-2023 Fundación Alicia Koplowitz.
- 2020-2023 Technological contract CSIC - Stxbp1 Syndrome Association.
- 2018-2021 Ministerio de Ciencia, Innovación y Universidades (RTI2018-099768-B-I00).
- 2020-2022 Apoyo Dravet Association.
- 2020-2022 Junta de Andalucia. Programa Operativo FEDER Andalucía (US-1264375).

Publication Highlights

GABAergic deficits in absence of LPA1 receptor, associated anxiety-like and coping behaviors, and amelioration by interneuron precursor transplants into the dorsal hippocampus. Rosell-Valle C, Martínez-Losa M, Matas-Rico E, Castilla-Ortega E, Zambrana-Infantes E, Gómez-Conde AI, Sánchez-Salido L, Ladrón de Guevara-Miranda D, Pedraza C, Serrano-Castro PJ, Chun J, Rodríguez de Fonseca F, Álvarez-Dolado M*, Santín LJ*, Estivill-Torrús G*. (* Co-corresponding authors). Brain Struct Funct. 2021 Jun;226(5):1479-1495. doi: 10.1007/s00429-021-02261-4. Epub 2021 Apr 1. PMID: 33792787

The metabesity factor HMG20A potentiates astrocyte survival and reactive astrogliosis preserving neuronal integrity. Lorenzo PI, Martin Vazquez E, López-Noriega L, Fuente-Martín E, Mellado-Gil JM, Franco JM, Cobo-Vuilleumier N, Guerrero Martínez JA, Romero-Zerbo SY, Perez-Cabello JA, Rivero Canalejo S, Campos-Caro A, Lachaud CC, Crespo Barreda A, Aguilar-Diosdado M, García Fuentes E, Martin-Montalvo A, Álvarez Dolado M, Martin F, Rojo-Martinez G, Pozo D, Bérmudez-Silva FJ, Comaills V, Reyes JC, Gauthier BR. Theranostics. 2021 May 12;11(14):6983-7004. doi: 10.7150/thno.57237. eCollection 2021. PMID: 34093866 Free PMC article.

A 32-Channel Time-Multiplexed Artifact-Aware Neural Recording System. Perez-Prieto N, Rodriguez-Vazquez A, Alvarez-Dolado M, Delgado-Restituto M. IEEE Trans Biomed Circuits Syst. 2021 Oct;15(5):960-977. doi: 10.1109/TBCAS.2021.3108725. Epub 2021 Dec 9. PMID: 34460384 Free article.

Specific contribution of Reelin expressed by Cajal-Retzius cells or GABAergic interneurons to cortical lamination. Vílchez-Acosta A, Manso Y, Cárdenas A, Elias-Tersa A, Martínez-Losa M, Pascual M, Álvarez-Dolado M, Nairn AC, Borrell V, Soriano E. Proc Natl Acad Sci U S A. 2022 Sep 13;119(37):e2120079119. doi: 10.1073/pnas.2120079119. Epub 2022 Sep 6. PMID: 36067316 Free PMC article.

Principal Investigator

Dr. David Pozo

Cellular and Molecular Neuroimmunology

Group Leader

Current position

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

Academic Background of the PI

- 1992: University of Seville, B.Sc. in Biological Sciences
- 1998: PhD. University of Seville, PhD in Biochemistry & Molecular Biology

Group Members

Postdoctoral

- Zaira González Sánchez.

PhD students

- Victoria Areal Quecuty.
- Jesús A Pérez Cabello.
- Raquel García García.
- Lucía Silvera Carrasco.

Technicians

- Daniel Tejada Moreno.

Former Members (2021-2022)

- **Postdoctoral Fellows:** Aurea Simón-Soro.
- **Master Students:** Enrico Tebaldi, Dimitris Giannacopoulos, Sabine Vernon.

Principal Investigator

Dr. Cintia Roodveldt

Immune Signalling in Neurodegenerative Proteinopathies

Emerging PI

Current position

- Associate Professor of Biochemistry and Molecular Biology, University of Seville Medical School

Academic Background of the Emerging PI

- 2005: Ph.D., The Weizmann Institute of Science, Rehovot, Israel
- 1999: Licenciatura en Biotecnología, Universidad Nacional del Litoral (UNL), Argentina

Research Activity

Overview

The Cellular and Molecular Neuroimmunology 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, with particular emphasis on the role of key cell populations as microglia, astrocytes, and different T regulatory cell subsets in the development of Amyotrophic Lateral Sclerosis (ALS) and other protein misfolding diseases. The activities at CMNL merge basic disease-oriented research on primary cell cultures and cell line cultures, preclinical studies in mouse models of human ALS and patient-driven research in clinical studies in ALS.

The active research lines are as follows:

a. Modulation of innate and adaptive immunity by endogenous neuropeptides in neurodegeneration.
b. Nanoparticles for controlled and targeted drug delivery: improving the drugability of neuropeptides and smart reprogramming

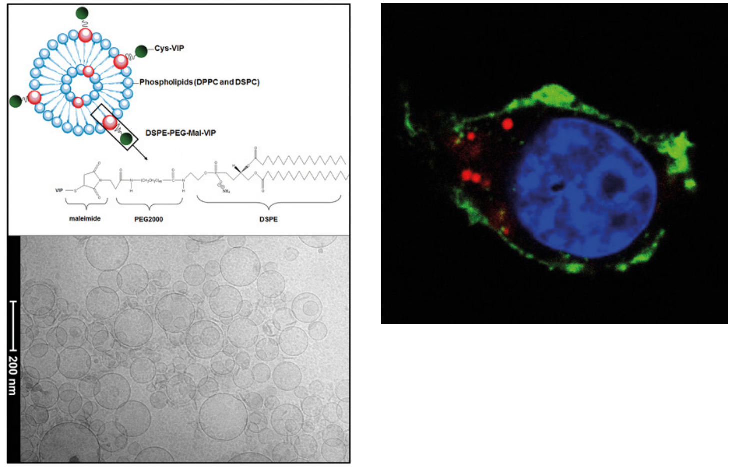


Figure 1. Left. Scheme of the neuropeptide VIP functionalization via DSPE-PEG-Mal and liposome composition and cryo-TEM of VIP-liposomes (Scale bar 200nm). The top figure shows VIP functionalization via its N-terminal cysteine to the maleimide-modified termini of PEG chains located on the external surface of liposomes, forming a stable carbon-sulphur bond. Moreover, the bottom figure shows a microscopy analysis which is in close agreement with the DLS results, displaying an average size of approximately 120 nm for VIP-liposomes. Right. Specific PSMA-micelles targeting microglia. Red (rewiring cargo), Blue (Nucleus), Green (Phalloidin/actin).

of glia in neurodegenerative diseases. Biomaterial-based modulation of the immune system: engineering immunomodulatory nanomaterials.

c. Energy metabolism as modifier of the immune response: tissue crosstalk in Amyotrophic Lateral Sclerosis (ALS) and neurodegeneration.

d. Molecular mechanisms of immune signalling and immune dysregulation in neurodegenerative proteinopathies, including ALS and Parkinson.

e. Role and mechanisms of immune kinases in microglial responses and neuroinflammation elicited in amyotrophic lateral sclerosis (ALS)

f. Molecular mechanisms and transcriptional dynamics in immune responses and neurodegenerative proteinopathies.

Research Highlights

Research line 1 (D. Pozo): A common feature among several neurodegenerative diseases including ALS is an impairment of neuroprotective mechanisms associated to immune imbalance. 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 (CNS) plasticity and homeostasis. We identified neuropeptide activity-dependent neuroprotective protein (ADNP) and NAP-derived peptide as new neuroimmunodulators by using different models (acute brain inflammation, septic shock, EAE, ALS and Adnp haploinsuffiency mice) disclosing an emerging role in brain immune homeostasis. Ongoing studies are focused on the unknown molecular mechanisms of ADNP on microglial phenotype based on CRISPR/Cas9 KO cell lines, chromatin remodeling studies and their impact on primary motor neuron function (Silvera-Carrasco & Pozo, under preparation). 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. Recently, we have disclosed a new phenotype in the transgenic mouse model of ALS linking energy homeostasis and ALS onset and severity (Franco & Pozo, under review). As a follow-up of those findings, we are leading the development of a clinical trial based on drug repurposing as an alternative to the high risk and lengthy procedure of traditional drug development. Clinical and translational studies involve collaborations with S. Martinez (Instituto Neurociencias Alicante, CSIC) and FJ. Quintana (Harvard University, USA).

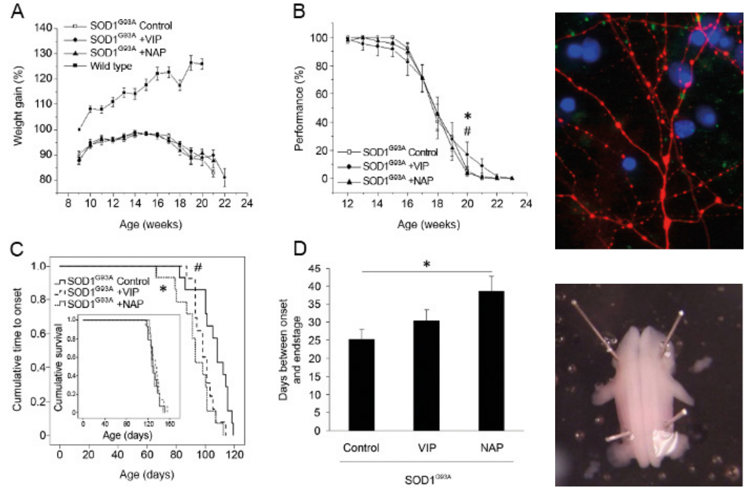


Figure 2. Left. Neuropeptide regulation of ALS onset/disease. Right. Primary motor neurons (up) from mice embryo spinal cord (down).

Research line 2 (C. Roodveldt): In addition to the progressive loss of motor neurons, a typical characteristic of ALS is the development of neuroinflammation. Several studies have shown that microglia, the main immunocompetent cells in the central nervous system, become activated and neurotoxic, thereby contributing to motor neuron loss and disease onset and

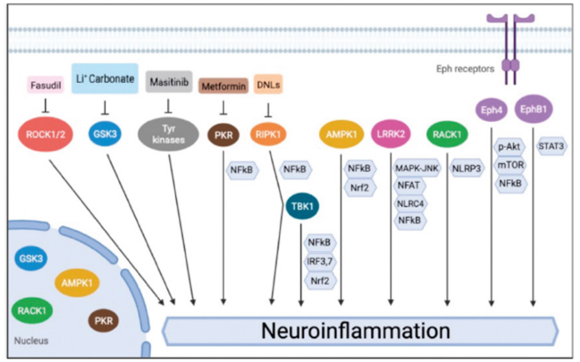


Figure 3. Immune-related kinases involved in ALS/FTD physiopathology. Schematic overview of immune-related kinases that have been implicated in in ALS or FTD, based on in vitro, ex vivo, in vivo or genetic studies. (Top): Signaling kinases known to display immune-related functions (ovals) and the identified signaling pathways in which they participate (hexagons) from various inflammation-based disease models. Specific kinase inhibitors (rectangles) that have been used to modulate some of these kinases are also shown. (Bottom): Different cell types located within the CNS or in the periphery, through which immune-related kinases may play a role in ALS/FTD pathophysiological processes.

progression. The mechanisms driving microglia activation and neurotoxicity in ALS remain incompletely understood.

Another key hallmark of ALS is the intracellular accumulation of TDP-43 protein aggregates, which are thought to play a central role in ALS pathophysiology. Previously, we had identified a poorly characterized signalling Ser/Thr kinase, MAPK/MAK/MRK overlapping kinase (MOK), as a protein that interacts with internalized TPD-43 aggregates and alters its activation state in primary microglia and organotypic spinal cord cultures (Leal-Lasarte et al., 2017). Given that signalling kinases are known be central mediators in immune responses, and whose functions may be dysregulated in neuroinflammation-associated neurodegenerative diseases (García-García et al., 2021) we sought to dissect the signalling pathways regulated byf MOK in ALS-linked microglial responses. By using ALS-based cellular models and organotypic culture, chemical inhibitors and MOK-KO cells, we could assess the participation of MOK in microglial immune responses. Apart from identifying an epigenetic reader as the first downstream-regulated molecule for MOK reported, we showed that MOK regulates both its phosphorylated levels and its functions in the cell nucleus (Pérez-Cabello & Roodveldt, under review). 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. Remarkably, we also found that MOK is altered in spinal cord tissue from ALS patients and mouse models, particularly in microglial cells (Pérez-Cabello & Roodveldt, under review). Overall, our results support a role of MOK in the pathogenic mechanisms of ALS and contributes to the search of novel and effective therapeutic targets against ALS and other neurodegenerative diseases.

Grants

- 2019-2023: RTI2018-098432-B-I00. Retos-Investigación Plan Nacional, Ministry of Science.
- 2019-2021: US-1265227. Programa Operativo FEDER-Junta de Andalucía.
- 2019-2023: CIVP19A5938. Fundación Ramón Areces.
- 2022-2024: PI-232. Consejería de Salud y Familias. Junta de Andalucía.

Publication Highlights

García-García R, Martín-Herrero L, Blanca-Pariente L, Pérez-Cabello J, Roodveldt C. **2021.** Immune signaling kinases in Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal Dementia (FTD). **Int. J. Mol. Sci.**, 22(24): 13280.

Lorenzo PI, Martin Vazquez E, López-Noriega L, Fuente-Martín E, Mellado-Gil JM, Franco JM, Cobo-Vuilleumier N, Guerrero Martínez JA, Romero-Zerbo SY, Perez-Cabello JA, Rivero Canalejo S, Campos-Caro A, Lachaud CC, Crespo Barreda A, Aguilar-Diosdado M, García Fuentes E, Martin-Montalvo A, Álvarez Dolado M, Martin F, Rojo-Martinez G, Pozo D, Bérmudez-Silva FJ, Comaills V, Reyes JC, Gauthier BR. **2021.** The metabesity factor HMG20A potentiates astrocyte survival and reactive astrogliosis preserving neuronal integrity. **Theranostics** 11(14):6983-7004.

Leal-Lasarte M, Mannini B, Chiti F, Vendruscolo M, Dobson CM, Roodveldt C, Pozo D. **2021.** Distinct responses of human peripheral blood cells to different misfolded protein oligomers. **Immunology.** 164(2):358-371.

D. Pozo, **2021.** Cell-based drug delivery harnesses inflammatory and autoimmune responses in neurodegeneration. **J. Mol. Med.** 99(5):673-674.

González-Sánchez Z, Areal-Quecuty V, Jimenez-Guerra A, Cabanillas-Balsera D, Gil FJ, Velasco-Ortega E, Pozo D. **2022.** Titanium Surface Characteristics Induce the Specific Reprogramming of Toll-like Receptor Signaling in Macrophages. **Int J Mol Sci.** 13;23(8):4285.

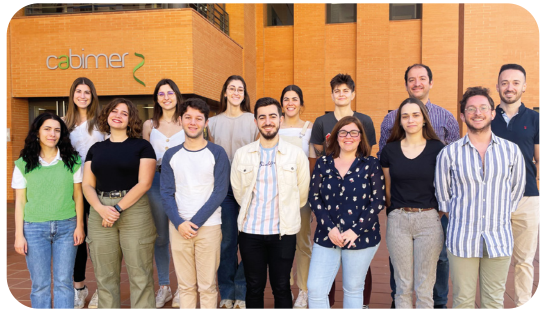
Quintana FJ, Pozo D. **2022.** Nanoparticle-Mediated Signaling Rewiring and Reprogramming of Immune Responses. **Front Immunol. 2022.**13:927733.

Principal Investigator

Dr. Alejandro Martín-Montalvo

Metabolic Interventions for Healthy Aging

Group Leader



Current position

- Científico titular del CSIC.

Group Members

Postdoctoral

- Isabel Espadas Villanueva.
- Raúl López-Fernández.

PhD Students

- Alejandro Sola García.
- María Ángeles Cáliz Molína.

Technicians

- Daniel González Morán.
- Naym El Kharoubi Zamudio.

Master Students

- Ryan Conesa Bakkali.
- Mercedes Ruiz Yuste.
- Ángela Vega Blanco.

Former Members (2021-2022)

- **Postdoctoral:** Guillermo Martínez Corrales.

Research Activity

Overview

During the last decades, life expectancy has increased considerably, but in many cases this increase in longevity is not accompanied by an optimal quality of life during old age and ~50% of people older than 80 years of age are dependent. Given the high incidence of pathologies associated with aging (e.g. sarcopenia, diabetes, neurodegenerative diseases and cancer, among others), there is an urgent need to develop therapies to prevent and treat these conditions and promote active-healthy aging. For this reason, the definition of new interventions aimed at maintaining optimal health during the old age is of great importance for our current society. Our purpose is to evaluate the potential of novel geroprotective strategies to promote healthy aging as a therapeutic weapon to prevent disorders that impedes having an optimal quality of life and independence in the elderly.

Research Highlights

The use of geroprotectors for healthy aging

Our purpose is to evaluate the potential of novel geroprotective strategies to promote healthy aging as a therapeutic weapon to prevent disorders that impedes having an optimal quality of life and independence in the elderly. At mechansitic level, we are focused to determine the relevance of two biological process which could have great potential in the modulation of aging and age-related diseases:

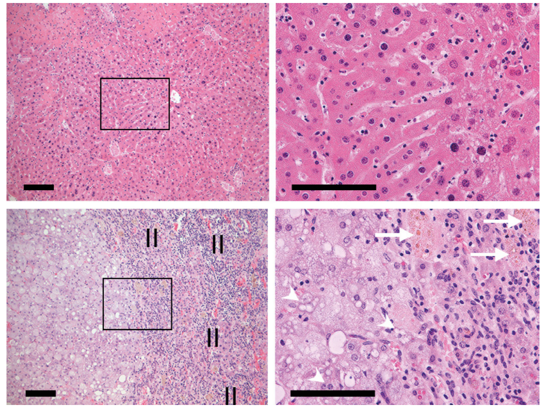


Figure 1. Representative images of liver sections of normal liver and liver tumors. Liver sections were stained with hematoxylin and eosin at necropsies from mice without cancer and mice with liver cancer II: inflammatory infiltrate; White head arrow: steatosis. Scale bar 100 µm. Left: normal liver. Right: primary liver epithelial neoplasm.

The potential of the modulation of Ac-CoA metabolism in healthspan and lifespan.

We will determine the physiological effects of the pharmacological inhibition of the main enzyme responsible for Acetyl-Coenzyme A generation, the ATP-citrate lyase, in mice. We will feed wild type mice with standard or high-fat diets supplemented with inhibitors of the ATP–citrate lyase the effects on physical health, memory, metabolic homeostasis and longevity at different ages. Mechanistic studies will decipher whether alterations in the transcriptional and proteomic profile exist and the main molecular processes leading to these changes.

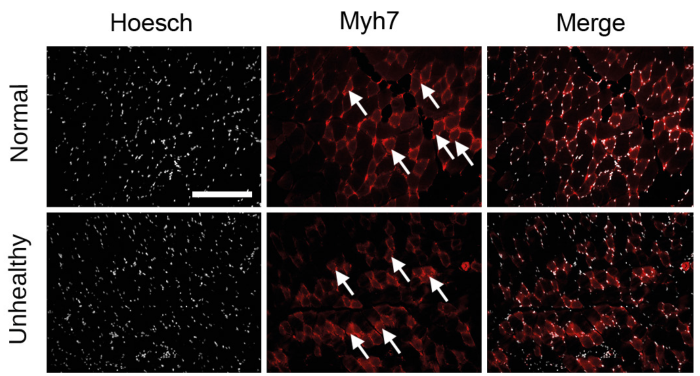


Figure 2. Analysis of gastrocnemius tissue in healthy and unhealthy mice. Myh7 immunostaining of gastrocnemius sections. Representative Myh7 expressing myofibers are marked with arrows. Scale bar: 250 µm.

Publication Highlights

Aguilera Y, Mellado-Damas N, Olmedo-Moreno L, López V, Panadero-Morón C, Benito M, Guerrero-Cázares H, Márquez-Vega C, Martín-Montalvo A, Capilla-González V. Preclinical Safety Evaluation of Intranasally Delivered Human Mesenchymal Stem Cells in Juvenile Mice. 2021. Cancers (Basel). Mar 9;13(5):1169.

Olmedo-Moreno L, Aguilera Y, Baliña-Sánchez C, Martín-Montalvo A, Capilla-González V. Heterogeneity of In Vitro Expanded Mesenchymal Stromal Cells and Strategies to Improve Their Therapeutic Actions. 2022. Pharmaceutics. May 23;14(5):1112.

The potential of the modulation of cysteine post-translational modifications in healthspan and lifespan.

We will determine the possible improvements at the cellular and physiological level produced by the modulation cysteine post-translational modifications. Using cell cultures isolated from patients suffering from Alzheimer's disease and healthy people, we will determine the possible improvements produced by the modulation cysteine post-translational modifications in processes associated with neurodegeneration. We will carry out longevity studies in rodents using wild type mice as well as experimental models of age-related diseases using this strategy. In this phase we will evaluate physical health, neurocognitive health and metabolic homeostasis at different ages. At mechanistic level we will carry out transcriptomic and proteomic studies focused to determine total proteomic changes and specific modulations on cysteine residues. We will also define the main master regulators of metabolism that coordinate the cellular responses.

Grants

- 2022-2024 PID2021-123965OB-I00. Ministerio de Ciencia e Innovación.
- 2022-2023 202220I059. CSIC.
- 2021-2022. P20_00480. Junta de Andalucía.
- 2019-2021 PI18/01590. Instituto de Salud Carlos III.

Principal Investigator

Dr. Francisco Javier Díaz-Corrales

Retinal Degeneration: from genetics to therapy

Emerging PI

Current position

- Since 2021: Staff Scientist/Nicolás Monardes Program.
- Fundación Pública Andaluza Progreso y Salud.

Group Members

Postdocs

- Berta De La Cerda Haynes.
- Álvaro Plaza Reyes.
- Estefanía Caballanos Infante.

PhD student

- Mohamad Mehdi Moshtaghion.

Technicians

- María Lourdes Valdés Sánchez.
- María José Marín Sainz.

Former Members (2021-2022)

- **Master students:** Félix Puerta, Rocío Mesa Sánchez.
- **Technicians:** Patricia Gallego Fernández.

Research Activity

Overview

The Retinal Degeneration Group was created in 2009 by Dr S. Bhattacharya, who retired in 2019. Since then, Dr Díaz-Corrales began to lead the laboratory, and in 2021, he formally became the group leader. The Retinal Degeneration Group's main goal is to perform translational medicine to treat and diagnose ophthalmological pathologies that cause blindness, such as retinitis pigmentosa (RP) and age-related macular degeneration (AMD). To meet the final purpose of this group, the research program has been divided into four strategic lines of interest. 1) The study of the molecular mechanisms of retinal degenerative diseases to identify new therapeutic targets using retinal organoids and mouse models. 2) Pre-clinical evaluation of new advanced cell and gene therapies based on developing non-viral vectors for gene delivery and replacing retinal cells derived from human iPS. 3) Development and pre-clinical evaluation of small retinal-protective molecules and epigenetic drugs that modulate the SIRT1/PARP1 axis. 4)

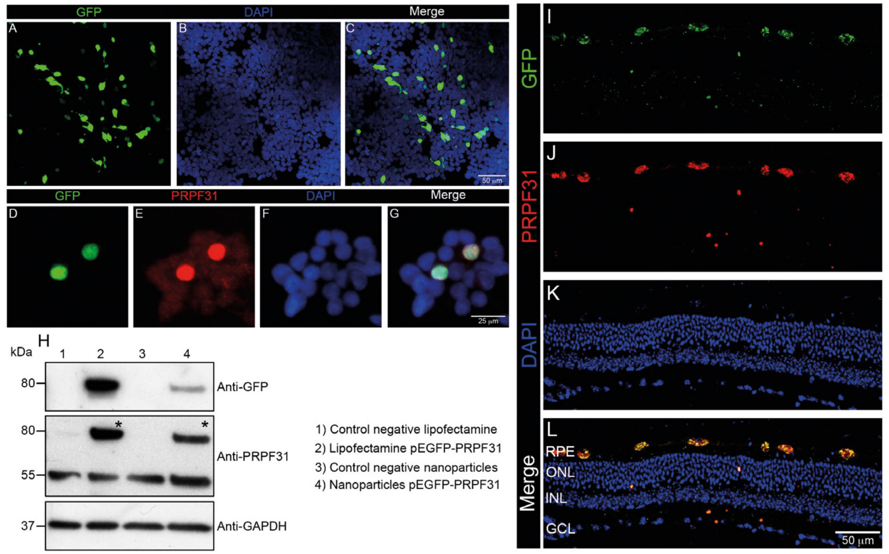


Figure 1. Transfection efficacy of PRPF31 gene using the nanoparticle delivery system PRPF31-GFP/N-MSiNPs in a human cell line and mouse retinas. Immunofluorescence images of HEK-293 cells transfected with N-MSiNPs loaded with the PRPF31-GFP plasmid (A-G). WB of HEK-293 transfected cells showing the expression levels of the PRPF31 transgene fused to GFP (H). Retinal section of PRPF31-GFP/N-MSiNP subretinally injected mice (I-L). PRPF31 signal co-localized mainly in retinal pigment epithelium (RPE) cells. ONL = outer nuclear layer, INL = inner nuclear layer, GCL = ganglion cell layer. Scale bars represent 25 or 50 µm.

Validation of new biomarkers present in tear fluid for early diagnosis of eye pathologies. In the frame of translational research, Dr Diaz-Corrales is co-founder of the Spin-off Limnopharma S.L., which would boost a future clinical trial for RP. Finally, the Retinal Degeneration Group also collaborate closely with the Ophthalmology Department of the University Hospital Virgen Macarena and regional and national patient associations.

Research Highlights

Development of a new non-viral vector for the PRPF31 gene therapy approach

During the 2021-2022 period, the Retinal Degeneration Group achieved several highlighting results. One of the most relevant was publishing one patent product of a collaborative work with the Materials Science Institute of Seville (ICMS) and CABIMER. Retinitis pigmentosa (RP) is the leading cause of inherited blindness in adults. RP begins with progressive rod photoreceptor degeneration, initially manifesting night blindness and tunnel vision. So far, mutations in more than 80 genes have been implicated in non-syndromic RP. Many of these genes encode retinal-specific proteins, but others, such as the pre-mRNA splicing factor *PRPF31*, are ubiquitously expressed. Mutations in the *PRPF31* gene commonly cause autosomal dominant retinitis pigmentosa. Gene therapy is a promising emerging treatment for degenerative retina pathologies, including RP caused by *PRPF31* mutations. Adeno-associated viral (AAV) vectors have been used to transduce ocular tissues efficiently. However, AAVs are challenging to handle, costly to produce and scale up, and it is debatable if neutralizing antibodies in treated patients would decrease the transfection efficiency in subsequent treatments. The development of alternative, non-viral delivery platforms like nanoparticles is of great interest to extend the application of gene therapy for RP. Amino-functionalized mesoporous silica-based nanoparticles (N-MSiNPs) were synthesized at the ICMS. Then the efficacy and safety of the N-MSiNPs were evaluated in CABIMER. Transgene expression was assessed by immunofluorescence and Western blot (WB) *in vitro* and *in vivo* (Fig. 1). The safety evaluation of mice subjected to subretinal injection was evaluated by ophthalmological tests (electroretinogram, funduscopy, tomography, and optokinetic test). N-MSiNPs delivered the therapeutic transgene *PRPF31* for RP, both *in vitro* and *in vivo*, showing no adverse effects. Developing new non-viral vectors such as N-MSiNPs might constitute a valuable alternative to AAVs (Valdés-Sánchez L, Borrego-González, et al. J Clin Med. 2022).

Grants

- 2021-2023, PI20/00043, Instituto de Salud Carlos III.
- 2022-2023, DTS21/00086, Instituto de Salud Carlos III.
- 2021-2023, Limnopharma S.L.
- 2019-2022, EIN2019-103021, Ministerio de Ciencia e Innovación. Acciones de dinamización «Europa Investigación».
- 2021-2023, ONCE, Ayudas a la investigación en visión 2020.

Publication Highlights

Garcia-Delgado AB, Valdes-Sanchez L, Morillo-Sanchez MJ, Ponte-Zuñiga B, Diaz-Corrales FJ, de la Cerda B. Dissecting the role of EYS in retinal degeneration: clinical and molecular aspects and its implications for future therapy. Orphanet J Rare Dis. 2021 May 17;16(1):222. doi:10.1186/s13023-021-01843-z.

Valdés-Sánchez L, Borrego-González S, Montero-Sánchez A, Massalini S, de la Cerda B, Díaz-Cuenca A, Díaz-Corrales FJ. Mesoporous Silica-Based Nanoparticles as Non-Viral Gene Delivery Platform for Treating Retinitis Pigmentosa. J Clin Med. 2022 Apr 13;11(8):2170. doi: 10.3390/jcm11082170.

Borrego-González S, de la Cerda B, Díaz-Corrales FJ, Díaz-Cuenca A. Nanofibrous Matrix of Defined Composition Sustains Human Induced Pluripotent Stem Cell Culture. ACS Appl Bio Mater. 2021 Apr 19;4(4):3035-3040. doi: 10.1021/acsabm.0c00425.

Cañibano-Hernández A, Valdes-Sanchez L, Garcia-Delgado AB, Ponte-Zúñiga B, Diaz-Corrales FJ, de la Cerda B. Generation of the human iPSC line ESi082-A from a patient with macular dystrophy associated to mutations in the CRB1 gene. Stem Cell Res. 2021 May;53:102301. doi: 10.1016/j.scr.2021.102301.

Buono L, Corbacho J, Naranjo S, Almuedo-Castillo M, Moreno-Marmol T, de la Cerda B, Sanabria-Reinoso E, Polvillo R, Díaz-Corrales FJ, Bogdanovic O, Bovolenta P, Martínez-Morales JR. Analysis of gene network bifurcation during optic cup morphogenesis in zebrafish. Nat Commun. 2021 Jun 23;12(1):3866. doi: 10.1038/s41467-021-24169-7.

Urbano-Gámez JD, Valdés-Sánchez L, Aracil C, de la Cerda B, Perdigones F, Plaza Reyes Á, Díaz-Corrales FJ, Relimpio López I, Quero JM. Biocompatibility Study of a Commercial Printed Circuit Board for Biomedical Applications: Lab-on-PCB for Organotypic Retina Cultures. Micromachines (Basel). 2021 Nov 29;12(12):1469. doi: 10.3390/mi12121469.

Ajana S, Cougnard-Grégoire A, Colijn JM, Merle BMJ, Verzijden T, de Jong PTVM, Hofman A, Vingerling JR, Hejblum BP, Korobelnik JF, Meester-Smoor MA, Ueffing M, Jacqmin-Gadda H, Klaver CCW, Delcourt C; EYE-RISK Consortium. Predicting Progression to Advanced Age-Related Macular Degeneration from Clinical, Genetic, and Lifestyle Factors Using Machine Learning. Ophthalmology. 2021 Apr;128(4):587-597. doi: 10.1016/j.ophtha.2020.08.031.

Díaz-Corrales, Francisco J.; Cerda, Berta de la ; Montero-Sánchez, Adoración ; Valdés-Sánchez, María Lourdes; Borrego-González, Sara; Díaz Cuenca, Aránzazu. Non-viral vectors from Mesoporous Nanoparticles for its application in gene therapy of degenerative pathologies of the retina. Patent Number: ES2915605 A1 Publication date: 2022/06/23

Principal Investigator

Dr. Vivian Capilla González

Stem Cells and Translational Neurology

Emerging PI

Current position

- Since 2020: Miguel Servet Researcher, CABIMER, Seville, Spain.
- Since 2021: Scientific coordinator of the Cytometry Unit of CABIMER.

Group Members

Postdoctoral
- Dr. Jesús María Sierra Párraga.

PhD Student
- Laura Olmedo Moreno.
- Concepción Panadero Morón.
- Caroline Stockwell.
- Carmen Baliña Sánchez.

Technicians
- Dr. Maria Norma Adán Castro.
- Rubén Bueno Fernández.
- Laura López Mangas.
- Carmen Burgos Cazorla.
- Laura Ruz Serván.
- Paula Juárez Blázquez.

Former Members (2021-2022)

- **Technicians:** Carlos Pinto Perea, Yolanda Aguilera, Nuria Mellado-Damas.
- **Master student:** Carmen Baliña Sánchez, Concepción Panadero Morón, Carmen Sánchez.
- **Erasmus+Students:** Adrianna Bilińska, Marta Jędrzejewska.

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. 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 burden raised to 18.1 million new cases and 9.6 million cancer deaths in 2018. However, the number of people that survive cancer is increasing due to advances in early detection and treatments for cancer. For this reason, more attention is being paid to the impact of cancer treatments on patients' health and quality of life. Radiotherapy is one of the most common treatments for cancer. Around 50% of all patients with cancer receive radiation at a given time. Unfortunately, radiotherapy comes with short and long term side effects. In particular, radiation for brain tumors produces neurofunctional sequelae, which may be progressive and permanent. The most frequently described neurological alterations of cranial radiation include learning and memory difficulties, problems in executive

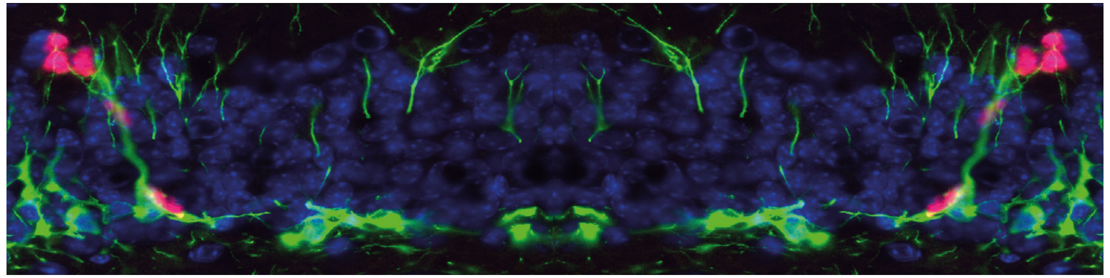


Figure 1. Hippocampus of a whole-brain irradiated mouse. Immunofluorescence against doublecortin (a marker for immature neurons; green) and Ki67 (a marker for proliferating cells; red), and DAPI counterstain in a mouse brain cryosection.

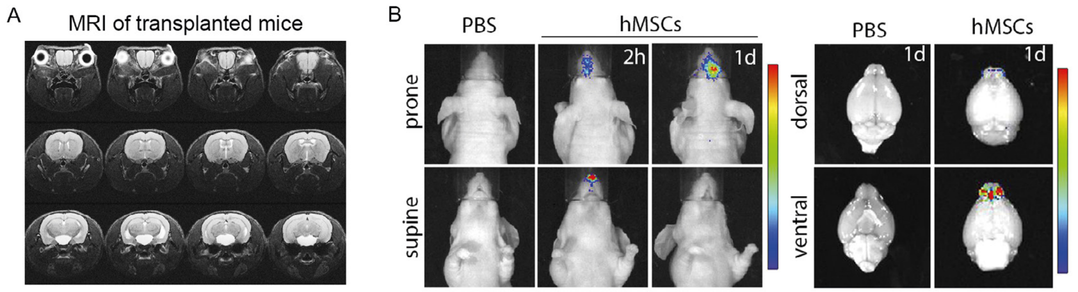


Figure 2. In vivo MRI and biodistribution of transplanted mice. (A) Magnetic resonance images (MRI) of mice that were transplanted with MSCs via the nasal route showing the absence of brain lesions. (B) Images showing the biodistribution of DiR-labelled MSCs that were intranasally administrated in mice.

functions, reduced processing speed, attention deficits, visual alterations and intellectual decline among others. These neurological sequelae are particularly relevant for pediatric patients because their developing brains are more radiosensitive. Therefore, there is an urgent need to develop new strategies to prevent radiation side effects and promote a healthy cancer-free life.

Our scientific program aims to investigates stem cell-based strategies to improve cancer treatments with the ultimate goal of improving the quality of life of cancer patients. We demonstrated for the first time that intranasally delivered mesenchymal stem cells (MSCs) exhibit therapeutic effect on radiation-related brain damages. In a recent report, we observed that commercial human MSCs migrate from the nasal cavity into the brain of adult mice. In particular, the day after administration, MSCs were observed in the olfactory bulbs and frontal lobes of dissected brains, suggesting efficient grafting. Furthermore, following whole-brain radiation, we found that intranasally delivered MSCs improve motor coordination, odor discrimination ability and cognition, as compared to non-transplanted animals. The molecular and cellular study of the brains revealed that MSCs reduce neuroinflammation, protect from oxidative stress and prevent neural cell loss in the irradiated mice, though beneficial effects in neurogenesis were not detected. At mechanistic level, we deciphered molecular pathways involved in neuroregeneration using transcriptomics and conventional western blots, which indicated that MSC administration reduces persistent activation of damage-induced c-AMP response element-binding (CREB) signaling in irradiated brains.

Our previous results uncover an unconventional approach to prevent sequelae of radiation using a non-invasive cell therapy. Now, we have focused on the application of this neuroprotective strategy for pediatric cancer. For this, we are evaluating the safety and efficacy of intranasally delivered patient-derived MSCs in a preclinical model of childhood brain cancer and radiation, which is mandatory before translation to clinical studies with pediatric patients. Furthermore, we are investigating the interaction mechanisms between MSCs and glioma cells to design safer MSC-based therapies for cancer.

Grants

- 2022-2024: POSTDOC/21/00424. PAIDI. Junta de Andalucía.
- 2022-2023: PY20/00481. PAIDI. Junta de Andalucía.
- 2022-2023: 1930-07/Garantía juvenil. POEJ. Junta de Andalucía.
- 2022-2023: 1950-01/Garantía juvenil. POEJ. Junta de Andalucía.
- 2021-2025: FPU19/04703. Ministerio de Universidades.
- 2021-2023: PI20/00341. Instituto de Salud Carlos III
- 2021-2022: JAEINT/21/02703. CSIC.
- 2020-2024: CP19/00046. Instituto de Salud Carlos III.
- 2020-2022: IDEAS20051CAPI. Asociación Española Contra el Cáncer.
- 2020-active: Asociación Pablo Ugarte (Proyecto +VIDA).

Publication Highlights

Capilla-González V*, Herranz-Pérez V, Sarabia-Estrada R, Kadri N, Moll G. **2022** Editorial: Mesenchymal Stromal Cell Therapy for Regenerative Medicine. **Front Cell Neurosci.** 26;16:932281.

Olmedo-Moreno L, Aguilera Y, Baliña-Sánchez C, Martín-Montalvo A, Capilla-González V*. **2022**. Heterogeneity of In Vitro Expanded Mesenchymal Stromal Cells and Strategies to Improve Their Therapeutic Actions. **Pharmaceutics.** 23;14(5):1112.

Aguilera Y, Mellado-Damas N, Olmedo-Moreno L, López V, Panadero-Morón C, Benito M, Guerrero-Cázares H, Márquez-Vega C, Martín-Montalvo A, Capilla-González V*. **2021**. Preclinical Safety Evaluation of Intranasally Delivered Human Mesenchymal Stem Cells in Juvenile Mice. **Cancers.** 9;13(5):1169.

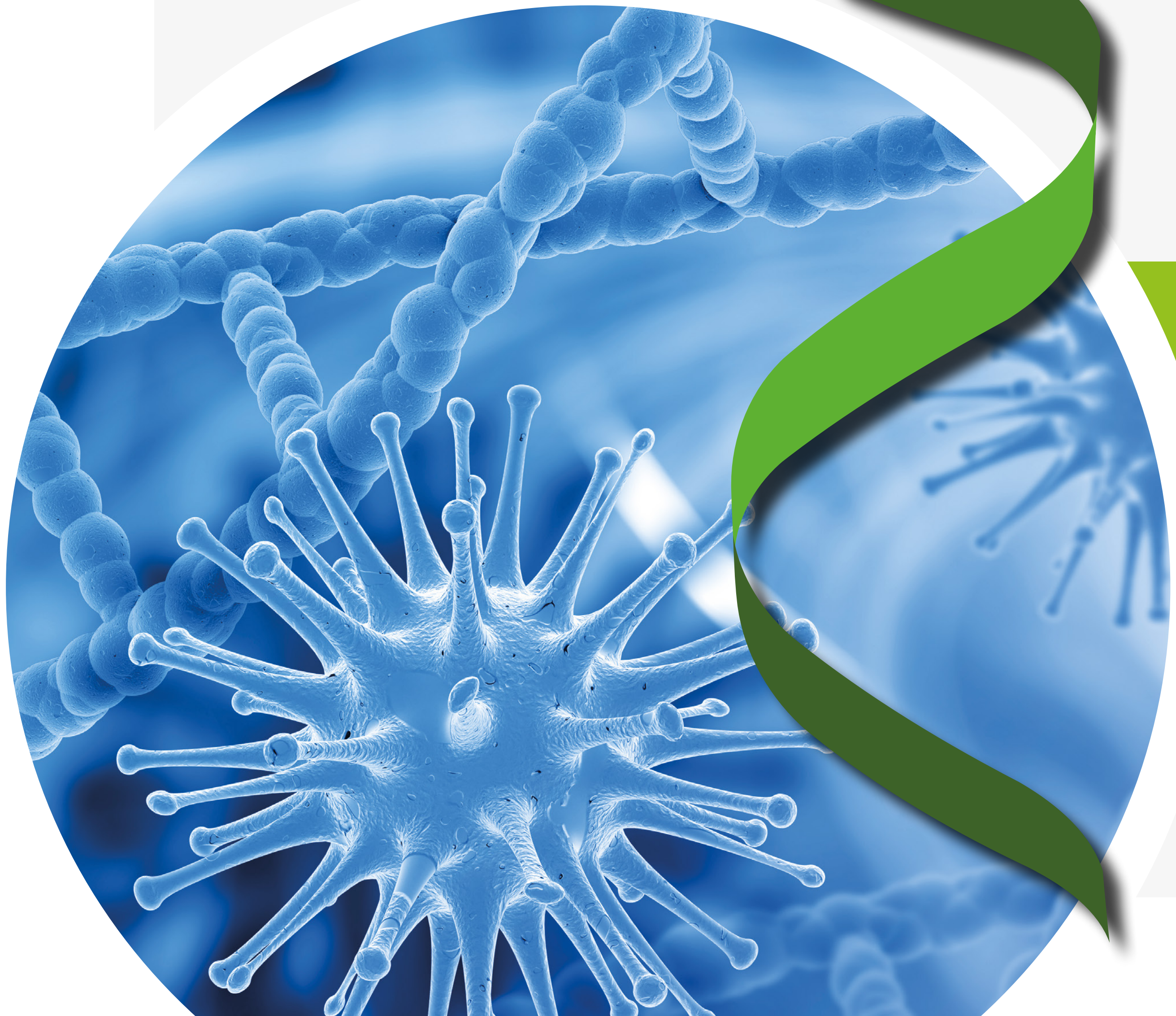
General Core Services

General Core Services

SCIENTIFIC CORE SERVICES

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

MANAGEMENT UNITS



Pilar Cebolla
Manager

Cabimer in Numbers

The *raison d'être* of the Management and General Services of a Research Center is to help their scientific community to keep their focus and effort on research.

CABIMER' annual running budget of an average of 2.7 million euros through the period 2021-2022, supported by the recently renewed partnership of the Spanish National Research Council (CSIC), the University of Seville, the University "Pablo de Olavide", "Consejería de Salud y Consumo" and "Consejeria de Universidad, Investigación e Innovación ", and also, thanks to the income from services offered by our advanced Genomic platform, covers the costs of regular operations (managing, technical services, maintenance, IT, security, etc) to provide support to our researchers.

Additionally, CABIMER has obtained during this period 1.6 million euros to finance new equipment to expand the science developed at CABIMER and undertaking dissemination activities such as the Second CABIMER International Workshop, CABIMER & Company (First CABIMER meeting to promote public-private collaboration between CABIMER research groups and pharmaceutical and biotechnological companies), and scientific seminar series, among others.

Concerning the infrastructure, a strong investment has been carried out to improve the technical capabilities of the core services, and consequently, to enhance the research conducted by CABIMER scientists, and also for many external users of our high-standard NGS services, which already account for half of the income obtained by the core units.

The valuable commitment of the technical and general services staff has helped to implement new equipment and services, keeping high-quality support for the research, facing a period of drastic rise of prices, especially the electricity costs, as well as the significant increase in the volume and complexity of administrative procedures.

We have continued increasing our efforts to communicate our work to society through our website, actively managing our Twitter account and, inaugurating CABIMER Youtube Channel, with videos of presentation, research groups and core services. Many guided visit has been organized in CABIMER and we welcomed more than 1.300 visitors through this period 2021-2022, mostly high-school students, patients associations and institutional representatives. We have coordinated our participation in 75 outreach events and conferences (Science Week, Science Fair, among others), and other events with companies such as BioSpain, Bio-Europe Spring, Transfiere, Medinbio, highlighting the organization of CABIMER&Company, the first meeting to promote public-private collaboration between CABIMER research groups and pharmaceutical and biotechnological companies, where we had the honor of having the participation of Farmaindustria, ASEBIO, Roche, Janssen, "Agencia Estatal de Investigación", and "Corporación Tecnológica de Andalucía". We have also organized the first conference in intellectual property and tech transfer for CABIMER researchers, with the participation of personnel from the transfer offices of the CABIMER holder institutions.

By the end of 2022, 193 persons worked at CABIMER, 28 Group Leader (including 7 emerging PIs), 11 senior researchers with stable positions, and a total of 79 PhDs researchers. In addition, CABIMER has master and last-year bachelor trainee students, which usually constitute a mass of ~20 persons distributed among the different groups.

CABIMER is proud of promoting the career of young researchers and technicians and it has become a training Center in a research environment that promotes gender equality. In 2022, we have organized the First CABIMER Gender Equality In Science Conference. By the end of 2022, women represented 65% of total workers but <30% of group leaders are women; although the ratio has improved in recent years from 10% to the current 27%, we still need to work harder along this line.

During this period, an average of 35 projects per year are undertaken and we have obtained resources for a total amount of 10.8 million euros including high-competitive grants from international institutions and collaborations with biotechnological companies such as Juvenile Diabetes Research Foundation (JDRF), the European Research Council and the H2020 Programme from EU, Spanish Cancer Association (AECC), Fundación CAIXA, among other entities.

We still have several goals to achieve, for instance, the recognition of National Excellence Research Centre, to increase private funding and companies collaborations… but also a wide spectrum of possibilities in the near future to fulfill CABIMER' mission: to transform the results of scientific work into direct improvements of health and quality of life.

I would like to conclude by expressing gratitude and recognition to the Director, Vice-director, and all the scientific, technical and, general services staff whose commitment and great work make it possible to achieve CABIMER's objectives and its enhancement.

Genomics

Executive Responsible
- Prof. Andrés Aguilera.

Scientific Coordinator
- Dr. Cristina González-Aguilera.

Technicians
- Dr. Eloísa Andújar
- Dr. Mónica Pérez
- Victoria Jiménez
- Dolores Pérez de Camino

The main aim of CABIMER Genomics Core Facility, established in 2007, is to provide internal and external investigators resources and services to support their research needs regarding High-throughput Functional Genomics. In recent years, NGS (next generation sequencing) and previously Microarray technologies have become essential in biology to perform studies of transcriptomes, epigenetics and genomes at a global scale. Nowadays, there are several technological platforms to carry out these studies.

The Facility is equipped with two platforms for Microarray analyses (Affymetrix and Agilent) able to provide services that include analyses on Molecular Cytogenetics, Expression profiles at the mRNA and Gene/Exon Level, Alternative Splicing, miRNA and Chip-on-Chip.

In addition, CABIMER is equipped with two NGS (Next Generation Sequencing) platforms, Ion-Torrent and Illumina technologies, with three NGS instruments: Ion- Torrent PGM sequencer, Illumina NextSeq500 and Illumina NOVASeq6000 sequencers. Furthermore, a microfluidics system for partitioning and barcoding single cells (sc 10xGenomics Chromium Controller) is extending this application. The Core Facility developed and standardized protocols for whole-genome sequencing, ChipSeq, DRIP-Seq, MNase-Seq, RNA-Seq, scRNA-Seq, scATAC-Seq and many others applications for different eukaryotic species using both platforms. The Core Facility also offers advice for experimental design and data analysis.

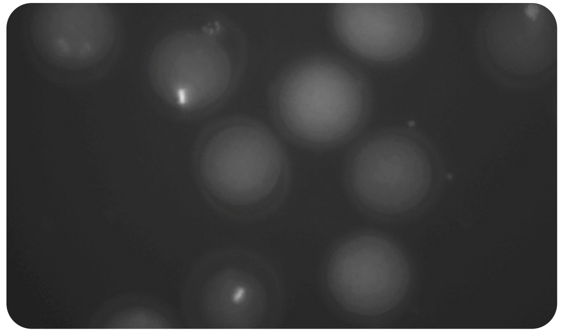
The manipulation of a vast amounts of samples processed in a reduced period of time, with accuracy and high reproducibility in the Core Facility, allows the researchers to move to a second stage in their studies on either a wide selection of genes or DNA elements as well as single ones. This is possible due to the diverse high content performance technologies that have been heavily improved in the Core Facility by the use of different instruments and professional people. The high standards and the use of the most modern technology has made this facility not only increasingly relevant for the research of the groups of the center, but also for the use of external laboratories from universities and research centers from all over Andalusia and a large part of Spain, with a relevant representation of studies performed for hospitals and the health system. We are certainly proud that our know-how and services have become a key part of the research of many labs in Andalusia, where we have become the public platform of reference for genome-wide sequencing.

Biological Resources

Scientific Coordinator
- Dr. Luis Sánchez Palazón

Veterinarian
- Dr. Itziar Benito Latasa de Aranibar

Technicians
- Andrea Fernández Reyes (until March 2022)
- María Gálvez Jiménez (from April 2022)
- Miriam González Fernández
- Flora Guerrero Iglesias
- Rosario Segarra Bermúdez



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. During this 2-year period, the unit has set up new equipment for in vivo imaging, Spectrum CT, and inhalational anesthetic. In addition, laboratory space and equipment is available for metabolic monitoring, behavioural test, stereotaxic surgery, electroretinography, optical coherence tomography and transgenesis, including microinjection of DNA into zygotes and rederivation of transgenic lines by embryo transfer.

Microscopy

Scientific Coordinator

- Dr. Pablo Huertas Sánchez

Technician

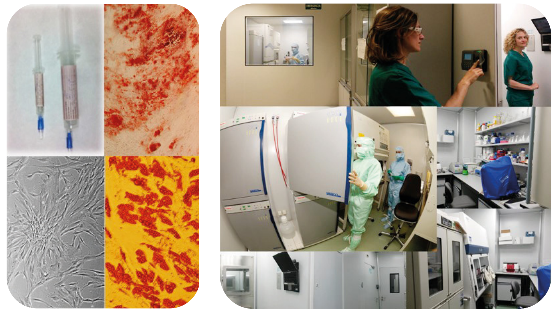
- Dr. Paloma Domínguez Giménez; Dr. Clara García Calderón

Microscopy is an invaluable tool to directly analyze events that take place in the cell or in a live organism. The diverse microscopy techniques facilitate the analysis of the function of proteins, their behavior in some structures, and the relationship among pathway components. These studies can be carried out in CABIMER with the support of our Microscopy Facility. This core service provides technical support to scientists from both CABIMER and external entities, helping them in all aspects regarding the preparation and development of microscopy experiments: from the experimental design and the use of the instruments to the processing of the data and the analysis of the images. The Microscopy Unit also assists scientists in interpreting and shaping final results. Finally, and in close collaboration with industry partners, the unit is also responsible for the maintenance of the microscopes, to provide the best possible service to our users. Due to the variety of biological models used in our institute, our Microscopy Unit is specially versatile, and have a great flexibility to adapt to new challenges and develop new strategies.

The Microscopy Facility presently counts with state-of-the-art equipment for the development of advanced microscopy techniques, including:

- Three Leica DM6000B vertical fluorescence microscopes for regular microscopy experiments.
- A Nikon Ni-E automated vertical fluorescence microscope, for the acquisition of compound big image that allows to either show a whole mount/ tissue section image at higher detail or to quantify cell phenotypes in large scale while maintaining high magnification /resolution.
- A Zeiss Axio-Imager2 vertical fluorescence microscope, equipped with an ApoTome that projects light in a structured way on the sample at different focal planes, creating several images. These subsequent images are further processed in real time, removing out-of-focus information before reconstructing them into a final optical section.
- An upgraded Leica DMI6000 inverted fluorescence microscope for fixed and live cell imaging optimized with a high resolution camera and a new workstation.
- A Leica Thunder Imager microscope for automatic acquisition of samples, with a high-speed stage, 8 different LEDs and a high resolution camera. Also, this system has been improved with incubation on stage for live cell imaging (see below).
- Two Leica TCS SP5 confocal microscopes, one of which is also capable of visualizing live cells in real time.

GMP



CABIMER's Good Manufacturing Practices (GMP) core facility is a Unit for ensuring that pharmaceutical products for human use are consistently manufactured, controlled and documented according to quality standards. GMP is designed to minimize the Risk and ensure the safety of patients enrolled in clinical trials (Regulation (EU) NO 536/2014). The GMP unit of CABIMER is a cell production core facility (UAPC-CABIMER) engaged in the manufacturing of investigational medicinal products "human cells" considered as Advanced Therapy Medicinal Products (ATMPs) in accordance with article 17 of Regulation (EC) nº 1394/2007. The production of ATMPs is carried out in accordance with GMP standards and handled with appropriate controls to ensure their safety, quality, and efficacy as a final medicinal product. UAPC-CABIMER was the first in Andalucía to obtain the Certification from the Spanish Agency of Medicines and Medical Devices (AEMPS, Agencia Española del Medicamento y Productos Sanitarios) to produce cellular medicaments (16 November 2009 and later on February 2012 and April 2015).

The UAPC-CABIMER facility is a fully equipped 57m² installation not being used at present, with 2 Grade B rooms (ISO 14644-1) for manufacturing ATMPs to use in Clinical Trials and Compassive Use and a fully equipped and independent Quality Control Laboratory. It follows the strict regulations established by Standard Operating Protocols (SOPs), which cover all aspects of ATMPs manufacturing, from the starting material, recordkeeping, premises, personnel qualifications, sanitation, cleanliness, equipment verification, process validation, and complaint handling to the training and personal hygiene of staff.

Regulation (EC) No 1394/2007 of the European Parliament and of the Council of 13 November 2007 on advanced therapy medicinal products and amending Directive 2001/83/EC and Regulation (EC) No 726/2004 (OJ L324, 10.12.2007, p.121).
Regulation (EU) No 536/2014 of the European Parliament and of the Council of 16 April 2014 on clinical trials on medicinal products for human use, and repealing Directive 2001/20/EC (OJ L158, 27.5.2014, p.1).

Flow Cytometry

Scientific Coordinator

- Dr. Vivian Capilla-González

Technicians

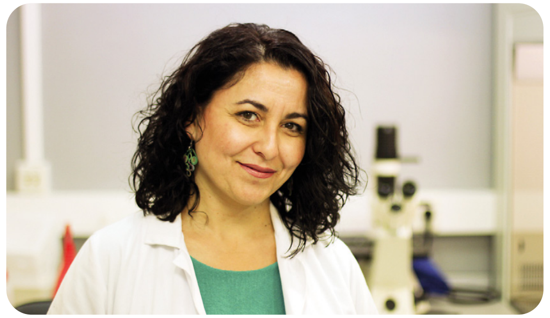
- Dr. María José Quintero Carrasco
- Dr. Clara B. García Calderón

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. Recently, three FlowJo and one FCS Express software licenses have been acquired, thus improving 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

- Dra. M. Mercedes Dana Jiménez

The Cell Culture Core Facility in CABIMER contains different restricted areas where primary and cell line cultures are carried out. Seven rooms are destined to established cell lines, one room to non-human primary cultures, a biosafety level II room for infecting cells with viruses, and a new room to carry out human primary cultures.

The Facility attends to the requirements from the researchers in order to facilitate the use of equipment, providing main reagents used in cell cultures such as serum, trypsin, antibiotics, glutamine and PBS. In addition, different fetal bovine serum (FBS) batches are tested in order to select one for common use.

Nowadays, the Facility is equipped with numerous normoxic and hypoxic incubators, safety cabinets, centrifuges, electroporation systems and microscopes. As more specific 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 adapt to the increasing number of users, either by incorporating new areas or by redistributing and optimizing the available space.

Model Organism

Scientific Coordinator

- Dr. Félix Prado

Technician

- Cristina Hernández

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 a 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, and the yeast Saccharomyces cerevisiae, which is required for in vivo assays for physical interactions between proteins, in vivo assays of gene expression, ectopic expression of recombinant proteins for purification, and vectors for cloning large human and mouse DNA fragments into yeast minichromosomes (YACs).

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 worm Caenorhabditis elegans.

The main objective of this Service is to provide specific facilities for a 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

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.

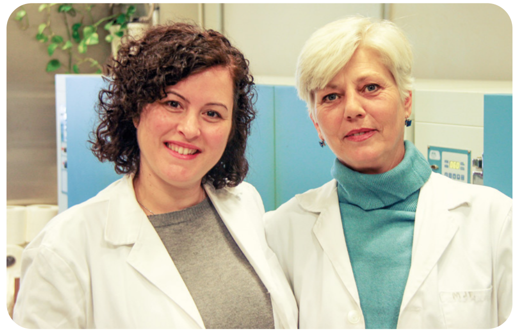
The Histology facility provides guidance, protocols and equipment allowing fixation techniques, sectioning of tissues and classical staining for easy viewing of samples. Specific protocols will be provided on demand and upon availability.

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 also offers staining techniques for various cellular structures. A Cytospin is also available for the processing of biological fluids and cell cultures at the facility.

Lab Material and Sterilization Unit

Scientific Coordinator
- Dr. Ralf Wellinger

Technician
- 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 handles 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, in order 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 have to 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, a Biosafety level 2 laboratory (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 28 Tm in 2020-2022.

Scientific Core Services: Management Units

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
- Paula Mauri

Purchasing and supplies
- Francisco J. Dorantes
- María Isabel Tovaruela
- Jennifer Chiguano
- Esperanza Muñoz
- Lucía Díaz

IT Service
- Arturo Fernández
- Modesto Jurado

Maintenance
- Rafael León

Scientific Publications

2021

- Aguilera Y, Mellado-Damas N, Olmedo-Moreno L, López V, Panadero-Morón C, Benito M, Guerrero-Cázares H, Márquez-Vega C, Martín-Montalvo A, Capilla-González V. Preclinical Safety Evaluation of Intranasally Delivered Human Mesenchymal Stem Cells in Juvenile Mice. **Cancers (Basel).** 13(5):1169

- Álvarez-Amor L, Sierra AL, Cárdenas A, López-Bermudo L, López-Beas J, Andújar E, Pérez-Alegre M, Gallego-Durán R, Varela LM, Martin-Montalvo A, Berná G, Rojas A, Robles-Frías MJ, Hmadcha A, Romero-Gómez M, Kleemann R, Martín F. Extra virgin olive oil improved body weight and insulin sensitivity in high fat diet-induced obese LDLr-/-Leiden mice without attenuation of steatohepatitis. **Sci Rep.** 11(1):8250

- Arroyo N, Villamayor L, Díaz I, Carmona R, Ramos-Rodríguez M, Muñoz-Chápuli R, Pasquali L, Toscano MG, Martín F, Cano DA, Rojas A. GATA4 induces liver fibrosis regression by deactivating hepatic stellate cells. **JCI Insight**. (23):e150059

- Balestra FR, Domínguez-Calvo A, Wolf B, Busso C, Buff A, Averink T, Lipsanen-Nyman M, Huertas P, Ríos RM, Gönczy P. TRIM37 prevents formation of centriolar protein assemblies by regulating Centrobin. **Elife.** 25;10:e62640

- Bayona-Feliu A, Aguilera A. The role of chromatin at transcription-replication conflicts as a genome safeguard. **Biochem Soc Trans.** 49(6):2727-2736

- Bayona-Feliu A, Aguilera A. The SWI/SNF complex, transcription-replication conflicts and cancer: a connection with high therapeutic potential. **Mol Cell Oncol.** 8(4):1976582

- Bayona-Feliu A, Barroso S, Muñoz S, Aguilera A. The SWI/SNF chromatin remodeling complex helps resolve R-loop-mediated transcription-replication conflicts. **Nat Genet.** 53(7):1050-1063

- Bodineau C, Tomé M, Courtois S, Costa ASH, Sciacovelli M, Rousseau B, Richard E, Vacher P, Parejo-Pérez C, Bessede E, Varon C, Soubeyran P, Frezza C, Murdoch PDS, Villar VH, Durán RV. Two parallel pathways connect glutamine metabolism and mTORC1 activity to regulate glutamoptosis. **Nat Commun.** 12(1):4814

- Buono L, Corbacho J, Naranjo S, Almuedo-Castillo M, Moreno-Marmol T, de la Cerda B, Sanbria-Reinoso E, Polvillo R, Díaz-Corrales FJ, Bogdanovic O, Bovolenta P, Martínez-Morales JR. Analysis of gene network bifurcation during optic cup morphogenesis in zebrafish. **Nat Commun.** 12(1):3866

- Caballano-Infantes E, Díaz I, Hitos AB, Cahuana GM, Martínez-Ruiz A, Soria-Juan B, Rodríguez-Griñolo R, Hmadcha A, Martín F, Soria B, Tejedo JR, Bedoya FJ. Stemness of Human Pluripotent Cells: Hypoxia-Like Response Induced by Low Nitric Oxide. **Antioxidants (Basel).** 10(9):1408

- Cabello-Lobato MJ, González-Garrido C, Cano-Linares MI, Wong RP, Yáñez-Vílchez A, Morillo-Huesca M, Roldán-Romero JM, Vicioso M, González-Prieto R, Ulrich HD, Prado F. Physical interactions between MCM and Rad51 facilitate replication fork lesion bypass and ssDNA gap filling by non-recombinogenic functions. **Cell Reports.** 36(4):109440

- Camarillo R, Jimeno S, Huertas P. The Effect of Atypical Nucleic Acids Structures in DNA Double Strand Break Repair: A Tale of R-loops and G-Quadruplexes. **Front Genet.** 12:742434

- Cañibano-Hernández A, Valdes-Sanchez L, Garcia-Delgado AB, Ponte-Zúñiga B, Diaz-Corrales FJ, de la Cerda B. Generation of the human iPSC line ESi082-A from a patient with macular dystrophy associated to mutations in the CRB1 gene. **Stem Cell Res.** 53:102301

- Cano-Linares MI, Yáñez-Vilches A, García-Rodríguez N, Barrientos-Moreno M, González-Prieto R, San-Segundo P, Ulrich HD, Prado F. Non-recombinogenic roles for Rad52 in translesion synthesis during DNA damage tolerance. **EMBO Reports.** 22(1):e50410

- Chacin E, Bansal P, Reusswig KU, Diaz-Santin LM, Ortega P, Vizjak P, Gómez-González B, Müller-Planitz F, Aguilera A, Pfander B, Cheung ACM, Kurat CF. A CDK-regulated chromatin segregase promoting chromosome replication. **Nat Commun.** 12(1):5224

- Correa-Vázquez JF, Juárez-Vicente F, García-Gutiérrez P, Barysch SV, Melchior F, García-Domínguez M. The Sumo proteome of proliferating and neuronal-differentiating cells reveals Utf1 among key Sumo targets involved in neurogenesis. **Cell Death Dis.** 12(4):305

- Courtois S, Haykal M, Bodineau C, Sifré E, Azzi-Martin L, Ménard A, Mégraud F, Lehours P, Durán RV, Varon C, Bessède E. Autophagy induced by Helicobacter pylori infection is necessary for gastric cancer stem cell emergence. **Gastric Cancer.** 24(1):133-144

- Dagg RA, Zonderland G, Lombardi EP, Rossetti GG, Groelly FJ, Barroso S, Tacconi EMC, Wright B, Lockstone H, Aguilera A, Halazonetis TD, Tarsounas M. A transcription-based mechanism for oncogenic?-catenin-induced lethality in BRCA1/2-deficient cells. **Nat Commun.** 13;12(1):4919

- Domínguez-Calvo A, Gönczy P, Holland AJ, Balestra FR. TRIM37: a critical orchestrator of centrosome function. **Cell Cycle.** (23):2443-2451

- Gabasa M, Radisky ES, Ikemori R, Bertolini G, Arshakyan M, Hockla A, Duch P, Rondinone O, Llorente A, Maqueda M, Davalos A, Gavilán E, Perera A, Ramírez J, Gascón P, Reguart N, Roz L, Radisky DC, Alcaraz J. MMP1 drives tumor progression in large cell carcinoma of the lung through fibroblast senescence. **Cancer Lett.** S0304-3835(21)00052-5

- Galati E, Bosio MC, Novarina D, Chiara M, Bernini GM, Mozzarelli AM, García-Rubio ML, Gómez-González B, Aguilera A, Carzaniga T, Todisco M, Bellini T, Nava GM, Frigè G, Sertic S, Horner DS, Baryshnikova A, Manzari C, D'Erchia AM, Pesole G, Brown GW, Muzi-Falconi M, Lazzaro F. VID22 counteracts G-quadruplex-induced genome instability. **Nucleic Acids Res.** 8(4):1976582

- Gallego P, Luque-Sierra A, Falcon G, Carbonero P, Grande L, Bautista JD, Martín F, Del Campo JA. White Button Mushroom Extracts Modulate Hepatic Fibrosis Progression, Inflammation, and Oxidative Stress In Vitro and in LDLR-/- Mice. **Foods.** 10(8):1788

- Garcia-Delgado AB, Valdes-Sanchez L, Morillo-Sanchez MJ, Ponte-Zuñiga B, Diaz-Corrales FJ, de la Cerda B. Dissecting the role of EYS in retinal degeneration: clinical and molecular aspects and its implications for future therapy. **Orphanet J Rare Dis.** 16(1):222

- García-García R, Martín-Herrero L, Blanca-Pariente L, Pérez-Cabello J,Roodveldt C. Immune Signaling Kinases in Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal Dementia (FTD). **Int J Mol Sci.** 22(24):13280

- García-Gutiérrez P, García-Domínguez M. SUMO control of nervous system development. **Semin Cell Dev Biol.** 8(4):1976582

- García-Gutiérrez P, García-Domínguez M. BETting on a Transcriptional Deficit as the Main Cause for Cornelia de Lange Syndrome. **Front Mol Biosci.** 8:709232

- Garcia-Jimenez MJ, Gil-Caballero S, Maza S, Corzana F, Juarez-Vicente F, Miles JR, Sakamoto K, Kadomatsu K, Dominguez-García M, de Paz JL, Nieto PM. Midkine Interaction with Chondroitin Sulfate Model Synthetic Tetrasaccharides and Their Mimetics: The Role of Aromatic Interactions. **CHEMISTRY-A EUROPEAN JOURNAL.** 27(48):12395-12409

- Gauthier BR, Comaills V. Nuclear Envelope Integrity in Health and Disease: Consequences on Genome Instability and Inflammation. **Int J Mol Sci.** 22(14):7281

- Gauthier, Benoit R, Petra I Lorenzo, and Valentine Comaills. Physical Forces and Transient Nuclear Envelope Rupture during Metastasis: The Key for Success. **Cancers.** 14, no. 1: 83

- Gea-Botella S, Agulló L, Martí N, Martínez-Madrid MC, Lizama V, Martín-Bermudo F, Berná G, Saura D, Valero M. Carotenoids from persimmon juice processing. **Food Res Int.** 141:109882

- Gómez-González B, Aguilera A. Origin matters: spontaneous DNA-RNA hybrids do not form in trans as a source of genome instability. **Current Genet.** 63: 93?97

- Gómez-González B, Sessa G, Carreira A, Aguilera A. A new interaction between BRCA2 and DDX5 promotes the repair of DNA breaks at transcribed chromatin. **Mol Cell Oncol.** 8(3):1910474

- González-Mariscal I, Pozo Morales M, Romero-Zerbo SY, Espinosa-Jimenez V, Escamilla-Sánchez A, Sánchez-Salido L, Cobo-Vuilleumier N, Gauthier BR, Bermúdez-Silva FJ. Abnormal cannabidiol ameliorates inflammation preserving pancreatic beta cells in mouse models of experimental type 1 diabetes and beta cell damage. **Biomed Pharmacother.** 145:112361

- Herrero-Ruiz A, Martínez-García PM, Terrón-Bautista J, Millán-Zambrano G, Lieberman JA, Jimeno-González S, Cortés-Ledesma F. Topoisomerase IIa represses transcription by enforcing promoter-proximal pausing. **Cell Reports.** 35(2):108977

- Jimeno S, Balestra FR, Huertas P. 2021. The Emerging Role of RNA Modifications in DNA Double-Strand Break Repair. **Front Mol Biosci.** 8:664872

- Jimeno S, Prados-Carvajal R, Fernández-Ávila MJ, Silva S, Silvestris DA, Endara-Coll M, Rodríguez-Real G, Domingo-Prim J, Mejías-Navarro F, Romero-Franco A, Jimeno-González S, Barroso S, Cesarini V, Aguilera A, Gallo A, Visa N, Huertas P. ADAR-mediated RNA editing of DNA:RNA hybrids is required for DNA double strand break repair. **Nat Commun.** 12(1):5512

- Katherine S Ruth et al. Genetic insights into biological mechanisms governing human ovarian ageing. **NATURE.** 596(7872):393-397

- Klionsky DJ, Abdel-Aziz AK, Abdelfatah S, Abdellatif M, Abdoli A, Abel S, Abeliovich H, Abildgaard MH, Abudu YP, Acevedo-Arozena A, Adamopoulos IE, Adeli K, Adolph TE, Adornetto A, Aflaki E, Agam G, Agarwal A, Aggarwal BB, Agnello M, Agostinis P, Agrewala JN, Agrotis A, Aguilar PV, Ahmad ST, Ahmed ZM, Ahumada-Castro U, Aits S, Aizawa S, Akkoc Y, et al. Guidelines for the use and interpretation of assays for monitoring autophagy (4th edition) 1. **Autophagy.** 17(1):1-382

- Kosar M, Giannattasio M, Piccini D, Maya-Mendoza A, García-Benítez F, Bartkova J, Barroso SI, Gaillard H, Martini E, Restuccia U, Ramirez-Otero MA, Garre M, Verga E, Andújar-Sánchez M, Maynard S, Hodny Z, Costanzo V, Kumar A, Bachi A, Aguilera A, Bartek J, Foiani M. The human nucleoporin Tpr protects cells from RNA-mediated replication stress. **Nat Commun.** 12(1):3937

- Lara-Ureña N, García-Domínguez M. Relevance of BET Family Proteins in SARS-CoV-2 Infection. **Biomolecules.** 11(8):1126
- Leal-Lasarte M, Mannini B, Chiti F, Vendruscolo M, Dobson CM, Roodveldt C, Pozo D. Distinct responses of human peripheral blood cells to different misfolded protein oligomers. **Immunology.** 164(2):358-371
- Lebrón JA, López-Cornejo P, García-Dionisio E, Huertas P, García-Calderón M, Moyá ML, Ostos FJ, López-López M. Cationic Single-Chained Surfactants with a Functional Group at the End of the Hydrophobic Tail DNA Compacting Efficiency. **Pharmaceutics.** 13(4):589
- Lebrón JA, López-López M, García-Calderón CB, V Rosado I, Balestra FR, Huertas P, Rodik RV, Kalchenko VI, Bernal E, Moyá ML, López-Cornejo P, Ostos FJ. Multivalent Calixarene-Based Liposomes as Platforms for Gene and Drug Delivery. **Pharmaceutics.** 13(8):1250
- López-Bermudo L, Gómez-Landero LA. Healthy Habits in Firefighters: Assessment in Shift Days Versus Days Off. **J Occup Environ Med.** 63(9):e580-e585
- Lorenzo PI, Cobo-Vuilleumier N, Martín-Vázquez E, López-Noriega L, Gauthier BR. Harnessing the Endogenous Plasticity of Pancreatic Islets: A Feasible Regenerative Medicine Therapy for Diabetes?. **Int J Mol Sci.** 22(8):4239
- Martínez-García PM, García-Torres M, Divina F, Terrón-Bautista J, Delgado-Sainz I, Gómez-Vela F, Cortés-Ledesma F. Genome-wide prediction of topoisomerase IIB binding by architectural factors and chromatin accessibility. **PLoS Comput Biol.** 17(1):e1007814
- Nabi D, Drechsler H, Pschirer J, Korn F, Schuler N, Diez S, Jessberger R, Chacón M. CENP-V is required for proper chromosome segregation through interaction with spindle microtubules in mouse oocytes. **Nat Commun.** 12(1):6547
- Nguyen TL, Nokin MJ, Terés S, Tomé M, Bodineau C, Galmar O, Pasquet JM, Rousseau B, van Liempd S, Falcon-Perez JM, Richard E, Muzotte E, Rezvani HR, Priault M, Bouchecareilh M, Redonnet-Vernhet I, Calvo J, Uzan B, Pflumio F, Fuentes P, Toribio ML, Khatib AM, Soubeyran P, Murdoch PDS, Durán RV. Downregulation of Glutamine Synthetase, not glutaminolysis, is responsible for glutamine addiction in Notch1-driven acute lymphoblastic leukemia. **Molecular Oncology.** 15(5):1412-1431
- Olivieri M, Cho T, Álvarez-Quilón A, Li K, Schellenberg MJ, Zimmermann M, Hustedt N, Rossi SE, Adam S, Melo H, Heijink AM, Sastre-Moreno G, Moatti N, Szilard RK, McEwan A, Ling AK, Serrano-Benitez A, Ubhi T, Feng S, Pawling J, Delgado-Sainz I, Ferguson MW, Dennis JW, Brown GW, Cortés-Ledesma F, Williams RS, Martin A, Xu D, Durocher D. A Genetic Map of the Response to DNA Damage in Human Cells. **CELL.** 182(2):481-496.e21
- Ortega P, Gómez-González B, Aguilera A. Heterogeneity of DNA damage incidence and repair in different chromatin contexts. **DNA Repair (Amst).** 107:103210
- Ortega P, Mérida-Cerro JA, Rondón AG, Gómez-González B, Aguilera A. DNA-RNA hybrids at DSBs interfere with repair by homologous recombination. **Elife.** e69881
- Payán-Bravo L, Fontalva S, Peñate X, Cases I, Guerrero-Martínez JA, Pareja-Sánchez Y, Odriozola-Gil Y, Lara E, Jimeno-González S, Suñé C, Muñoz-Centeno MC, Reyes JC, Chávez S. Human prefoldin modulates co-transcriptional pre-mRNA splicing. **Nucleic Acids Res.** 49(11):6267-6280
- Petra I Lorenzo, Eugenia Martin Vazquez, Livia López-Noriega, Esther Fuente-Martín, José M Mellado-Gil, Jaime M Franco, Nadia Cobo-Vuilleumier, José A Guerrero Martínez, Silvana Y Romero-Zerbo, Jesús A Perez-Cabello, Sabrina Rivero Canalejo, Antonio Campos-Caro, Christian Claude Lachaud, Alejandra Crespo Barreda, Manuel Aguilar-Diosdado, Eduardo García Fuentes, Alejandro Martin-Montalvo, Manuel Álvarez Dolado, Franz Martin, Gemma Rojo-Martinez, David Pozo, Francisco J Bérmudez-Silva, Valentine Comaills, José C Reyes , Benoit R Gauthier. The metabesity factor HMG20A potentiates astrocyte survival and reactive astrogliosis preserving neuronal integrity. **Theranostics.** 11(14):6983-7004
- Petryk N, Reverón-Gómez N, González-Aguilera C, Dalby M, Andersson R, Groth A. Genome-wide and sister chromatid-resolved profiling of protein occupancy in replicated chromatin with ChOR-seq and SCAR-seq. **Nat protocol.** 16(9):4446-4493
- Prado, Felix. Non-Recombinogenic Functions of Rad51, BRCA2, and Rad52 in DNA Damage Tolerance. **Genes.** 12,155
- Rivera-Molina FE, Xi Z, Reales E, Wang B, Toomre D. Exocyst complex mediates recycling of internal cilia. **Curr Biol.** S0960-9822(21)01323-3
- Rivero S, Rodríguez-Real G, Marín I, Huertas P. MRGBP, a member of the NuA4 complex, inhibits DNA double-strand break repair. **FEBS Open Bio.** 11(3):622-632
- Rivero-Rodríguez F, Díaz-Quintana A, Velázquez-Cruz A, González-Arzola K, Gavilan MP, Velázquez-Campoy A, Ríos RM, De la Rosa MA, Díaz-Moreno I. Inhibition of the PP2A activity by the histone chaperone ANP32B is long-range allosterically regulated by respiratory cytochrome c. **Redox Biol.** 43:101967
- Rosell-Valle C, Martínez-Losa M, Matas-Rico E, Castilla-Ortega E, Zambrana-Infantes E, Gómez-Conde AI, Sánchez-Salido L, Ladrón de Guevara-Miranda D, Pedraza C, Serrano-Castro PJ, Chun J, Rodríguez de Fonseca F, Álvarez-Dolado M, Santín LJ, Estivill-Torrús G. GABAergic deficits in absence of LPA 1 receptor, associated anxiety-like and coping behaviors, and amelioration by interneuron precursor transplants into the dorsal hippocampus. **Brain Struct Funct.** 226(5):1479-1495

- Ruth KS, Day FR, Hussain J, Martínez-Marchal A, Aiken CE, Azad A, Thompson DJ, Knoblochova L, Abe H, Tarry-Adkins JL, Gonzalez JM, Fontanillas P, Claringbould A, Bakker OB, Sulem P, Walters RG, Terao C, Turon S, Horikoshi M, Lin K, Onland-Moret NC, Sankar A, Hertz EPT, Timshel PN, Shukla V, Borup R, Olsen KW, Aguilera P, Ferrer-Roda M, et all. Genetic insights into biological mechanisms governing human ovarian. **Nature.** 596(7872):393-397

- Sabido-Bozo S, Perez-Linero AM, Manzano-Lopez J, Rodriguez-Gallardo S, Aguilera-Romero A, Cortes-Gomez A, Lopez S, Wellinger RE, Muñiz M. The p24 Complex Contributes to Specify Arf1 for COPI Coat Selection. **Int J Mol Sci.** 22(1):E423

- Safari M, Litman T, Robey R, Aguilera A, Chakraborty AR, Reinhold W, Basseville A, Petrukhin L, Scotto L, O'Connor OA, Pommier Y, Fojo AT, Bates SE. R-loop-Mediated ssDNA Breaks Accumulate Following Short-Term Exposure to the HDAC Inhibitor Romidepsin. **Mol Cancer Res.** 19(8):1361-1374

- San Martin-Alonso M, Soler-Oliva ME, García-Rubio M, García-Muse T, Aguilera A. Harmful R-loops are prevented via different cell cycle-specific mechanisms. **Nat Commun.** 12(1):4451

- Sanchez-Castro EE, Pajuelo-Reyes C, Tejedo R, Soria-Juan B, Tapia-Limonchi R, Andreu E, Hitos AB, Martin F, Cahuana GM, Guerra-Duarte C, de Assis TCS, Bedoya FJ, Soria B, Chávez-Olórtegui C, Tejedo JR. Mesenchymal Stromal Cell-Based Therapies as Promising Treatments for Muscle Regeneration After Snakebite Envenoming. **Front Immunol.** 11:609961

- Santos-Rosa H, Millán-Zambrano G, Han N, Leonardi T, Klimontova M, Nasiscionyte S, Pandolfini L, Tzelepis K, Bartke T, Kouzarides T. Methylation of histone H3 at lysine 37 by Set1 and Set2 prevents spurious DNA replication. **Moll cell.** 81(13):2793-2807.e8

- Sessa G, Gómez-González B, Silva S, Pérez-Calero C, Beaurepere R, Barroso S, Martineau S, Martin C, Ehlén Å, Martínez JS, Lombard B, Loew D, Vagner S, Aguilera A, Carreira A. BRCA2 promotes R-loop resolution by DDX5 helicase at DNA breaks to facilitate their repair by homologous recombination. **EMBO JOURNAL.** 40(7):e106018

- Simón J, Goikoetxea-Usandizaga N, Serrano-Maciá M, Fernández-Ramos D, Sáenz de Urturi D, Gruskos JJ, Fernández-Tussy P, Lachiondo-Ortega S, González-Recio I, Rodríguez-Agudo R, Gutiérrez-de-Juan V, Rodríguez-Iruretagoyena B, Varela-Rey M, Gimenez-Mascarell P, Mercado-Gomez M, Gómez-Santos B, Fernandez-Rodriguez C, Lopitz-Otsoa F, Bizkarguenaga M, Dames S, Schaeper U, Martin F, Sabio G, Iruzubieta P, Crespo J, Aspichueta P, Chu KH, Buccella D, Martín C, Delgado TC, Martínez-Cruz LA, Martínez-Chantar ML. Magnesium accumulation upon cyclin M4 silencing activates microsomal triglyceride transfer protein improving NASH Downregulation of Glutamine Synthetase, not glutaminolysis, is responsible for glutamine addiction in Notch1-driven acute lymphoblastic leuk. **J Hepatol.** 75(1):34-45

- Solaguren-Beascoa M, Bujakowska KM, Méjécase C, Emmenegger L, Orhan E, Neuillé M, Mohand-Saïd S, Condroyer C, Lancelot ME, Michiels C, Demontant V, Antonio A, Letexier M, Saraiva JP, Lonjou C, Carpentier W, Léveillard T, Pierce EA, Dollfus H, Sahel JA, Bhattacharya SS, Audo I, Zeitz C. WDR34, a candidate gene for non-syndromic rod-cone dystrophy. **Clin Genet.** 99(2):298-302

- Vakilian M, Ghaedi K. A new hypothetical model for pancreatic development based on change in the cell division orientation. **Gene.** 785:145607

- Zheleva A, Camino LP, Fernández-Fernández N, García-Rubio M, Askjaer P, García-Muse T, Aguilera A. C elegans THSC/TREX-2 deficiency causes replication stress and genome instability. **J Cell Sci.** 134(20):jcs258435

2022

- Benoit R Gauthier , Diana Rubio-Contreras , Juan Carlos Gómez-Rosado , Luis Cristobal Capitán-Morales , Abdelkrim Hmadcha, Bernat Soria, Christian Claude Lachaud. Human Omental Mesothelial Cells Impart an Immunomodulatory Landscape Impeding B- and T-Cell Activation. **Int J Mol Sci.** 3(11):5924

- Blessing C, Apelt K, van den Heuvel D, Gonzalez-Leal C, Rother MB, van der Woude M, González-Prieto R, Yifrach A, Parnas A, Shah RG, Kuo TT, Boer DEC, Cai J, Kragten A, Kim HS, Schärer OD, Vertegaal ACO, Shah GM, Adar S, Lans H, van Attikum H, Ladurner AG, Luijsterburg MS. XPC-PARP complexes engage the chromatin remodeler ALC1 to catalyze global genome DNA damage repair. **Nat Commun.** 13(1):4762

- Bodineau C, Tomé M, Murdoch PDS, Durán RV. Glutamine, MTOR and autophagy: a multiconnection relationship. **Autophagy (Review).** 18(11):2749-2750

- Bruxel MA, da Silva FN, da Silva RA, Zimath PL, Rojas A, Moreira ELG, Quesada I, Rafacho A. 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

- Caballano-Infantes E, Cahuana GM, Bedoya FJ, Salguero-Aranda C, Tejedo JR. The Role of Nitric Oxide in Stem Cell Biology. **Antioxidants (Basel).** 11(3):497

- Cañas JC, García-Rubio ML, García A, Antequera F, Gómez-González B, Aguilera A. A role for the Saccharomyces cerevisiae Rtt109 histone acetyltransferase in R-loop homeostasis and associated genome instability. **Genetics.** 222(1):iyac108

- Castañé A, Riga MS, Cano M, Ruiz-Avila L, Miquel-Rio L, Celada P, Artigas F. The dual 5-HT3 and 5-HT6 receptor antagonist FPPQ normalizes phencyclidine-induced disruption of brain oscillatory activity in rats. **Int J Neuropsychopharmacol.** 25(5):425-431

- Delgado-Chaves FM, Martínez-García PM, Herrero-Ruiz A, Gómez-Vela F, Divina F, Jimeno-González S, Cortés-Ledesma F. Data of transcriptional effects of the merbarone-mediated inhibition of TOP2. **Data Brief.** 44:108499

- Gallardo-Chamizo F, Lara-Ureña N, Correa-Vázquez JF, Reyes JC, Gauthier BR, García-Domínguez M. SENP7 overexpression protects cancer cells from oxygen and glucose deprivation and associates with poor prognosis in colon cancer. **Genes Dis.** 9(6):1419-1422

- Gallego-Durán R, Albillos A, Ampuero J, Arechederra M, Bañares R, Blas-García A, Berná G, Caparrós E, Delgado TC, Falcón-Pérez JM, Francés R, Fernández-Barrena MG, Graupera I, Iruzubieta P, Nevzorova YA, Nogueiras R, Macías RI, Marín F, Sabio G, Soriano G, Vaquero J, Cubero FJ, Gracia-Sancho J. Metabolic-associated fatty liver disease: from simple steatosis towards liver cirrhosis and potential complications Proceedings of the Third Translational Hepatology Meeting, endorsed by the Spanish Association for the Study of the Liver (AEEH). **Gastroenterol Hepatol.** S0210-5705(22)00079-6

- Gallego-Durán R, Albillos A, Ampuero J, Arechederra M, Bañares R, Blas-García A, Berná G, Caparrós E, Delgado TC, Falcón-Pérez JM, Francés R, Fernández-Barrena MG, Graupera I, Iruzubieta P, Nevzorova YA, Nogueiras R, Macías RIR, Martín F, Sabio G, Soriano G, Vaquero J, Cubero FJ, Gracia-Sancho J. Metabolic-associated fatty liver disease: From simple steatosis toward liver cirrhosis and potential complications Proceedings of the Third Translational Hepatology Meeting, organized by the Spanish Association for the Study of the Liver (AEEH). **J GASTROENTEROL Hepatology.** 45(9):724-734

- Gallego-Durán R, Ampuero J, Pastor-Ramírez H, Álvarez-Amor L, Del Campo JA, Maya-Miles D, Montero-Vallejo R, Cárdenas-García A, Pareja MJ, Gato-Zambrano S, Millán R, Del Carmen Rico M, Luque-Sierra A, Gil-Gómez A, Rojas Á, Muñoz-Hernández R, García-Lozano M, Aller R, Andrade RJ, García-Monzón C, Andreola F, Martín F, Jalan R, Romero-Gómez M. Liver injury in non-alcoholic fatty liver disease is associated with urea cycle enzyme dysregulation. **Sci Rep.** 12(1):3418

- Garcia-Dominguez M. Proper progression of neurogenesis relies on a defined pattern of SUMO-modified proteins. **Neural Regen Res.** 17(8):1731-1732

- García-Gutiérrez P, García-Domínguez M. SUMO control of nervous system development. **Semin Cell Dev Biol.** 132:203-212

- García-Rodríguez N, Manzano-López J, Muñoz-Bravo M, Fernández-García E, Muñiz M, Wellinger RE. Manganese redistribution by calcium-stimulated vesicle trafficking bypasses the need for P-type ATPase function. **J Biol Chem.** 290(15):9335-47

- Gómez-Marín E, Posavec-Marjanovi? M, Zarzuela L, Basurto-Cayuela L, Guerrero-Martínez JA, Arribas G, Yerbes R, Ceballos-Chávez M, Rodríguez-Paredes M, Tomé M, Durán RV, Buschbeck M, Reyes JC. The high mobility group protein HMG20A cooperates with the histone reader PHF14 to modulate TGF? and Hippo pathways. **Nucleic Acid RES.** 50(17):9838-9857

- González-Arzola K, Díaz-Quintana A, Bernardo-García N, Martínez-Fábregas J, Rivero-Rodríguez F, Casado-Combreras MÁ, Elena-Real CA, Velázquez-Cruz A, Gil-Caballero S, Velázquez-Campoy A, Szulc E, Gavilán MP, Ayala I, Arranz R, Ríos RM, Salvatella X, Valpuesta JM, Hermoso JA, De la Rosa MA, Díaz-Moreno I. Nucleus-translocated mitochondrial cytochrome c liberates nucleophosmin-sequestered ARF tumor suppressor by changing nucleolar liquid-liquid phase separation. **Nat Struct Mol Biol.** 29(10):1024-1036

- González-Garrido C, Prado F. Novel insights into the roles of Cdc7 in response to replication stress. **FEBS J.** Online ahead of print

- González-Mariscal I, Pozo Morales M, Romero-Zerbo SY, Espinosa-Jimenez V, Escamilla-Sánchez A, Sánchez-Salido L, Cobo-Vuilleumier N, Gauthier BR, Bermúdez-Silva FJ. Abnormal cannabidiol ameliorates inflammation preserving pancreatic beta cells in mouse models of experimental type 1 diabetes and beta cell damage. **Biomed Pharmacother.** 145:112361

- González-Sánchez Z, Areal-Quecuty V, Jimenez-Guerra A, Cabanillas-Balsera D, Gil FJ, Velasco-Ortega E, Pozo D. Titanium Surface Characteristics Induce the Specific Reprogramming of Toll-like Receptor Signaling in Macrophages. **Int J Mol Sci.** 23(8):4285

- Han SS, Wen KK, García-Rubio ML, Wold MS, Aguilera A, Niedzwiedz W, Vyas YM. WASp modulates RPA function on single-stranded DNA in response to replication stress and DNA damage. **Nat Commun.** 13(1):3743

- Lara-Ureña N, Jafari V, García-Domínguez M. Cancer-Associated Dysregulation of Sumo Regulators: Proteases and Ligases. **Int J Mol Sci.** 23(14):8012

- López-Bermudo L, Luque-Sierra A, Maya-Miles D, Gallego-Durán R, Ampuero J, Romero-Gómez M, Berná G, Martín F. Contribution of Liver and Pancreatic Islet Crosstalk to ?-Cell Function/Dysfunction in the Presence of Fatty Liver. **Front Endocrinol (Lausanne).** 13:892672

- López-Jiménez E; González-Aguilera C. Role of Chromatin Replication in Transcriptional Plasticity, Cell Differentiation and Disease. **GENES.** 13(6):1002

- López-Terrones E, Celada P, Riga MS, Artigas F. Preferential In Vivo Inhibitory Action of Serotonin in Rat Infralimbic versus Prelimbic Cortex: Relevance for Antidepressant Treatments. **Cereb Cortex.** 32(14):3000-3013

- Martin Vázquez E, Cobo-Vuilleumier N, Araujo Legido R, Marín-Cañas S, Nola E, Dorronsoro A, López Bermudo L, Crespo A, Romero-Zerbo SY, García-Fernández M, Martin Montalvo A, Rojas A, Comaills V, Bérmudez-Silva FJ, Gannon M, Martin F, Eizirik D, Lorenzo PI, Gauthier BR. NR5A2/LRH-1 regulates the PTGS2-PGE 2-PTGER1 pathway contributing to pancreatic islet survival and function. **Iscience.** 25(5):104345

- Martínez-García MÁ, Sierra-Párraga JM, Garcia-Ortega A A. Obstructive sleep apnea in women: We can do more and better. **Sleep Med Rev.** 64:101645

- Martinez-Garcia MA, Sierra-Párraga JM, Quintana E, López-Campos JL. 2022. CFTR dysfunction and targeted therapies: A vision from non-cystic fibrosis bronchiectasis and COPD. **J Cyst Fibros.** 1569-1993(22)00107-2
- Mayoral I, Bevilacqua E, Gómez G, Hmadcha A, González-Loscertales I, Reina E, Sotelo J, Domínguez A, Pérez-Alcántara P, Smani Y, González-Puertas P, Mendez A, Uribe S, Smani T, Ordoñez A, Valverde I. Tissue engineered in-vitro vascular patch fabrication using hybrid 3D printing and electrospinning. **Mater Today Bio.** 14:100292
- Millán-Zambrano G, Burton A, Bannister AJ, Schneider R. Histone post-translational modifications - cause and consequence of genome function. **Nat Rev Genet.** 23(9):563-580
- Mora-Molina R and Lopez-Rivas A. Restoring TRAILR2/DR5-Mediated Activation of Apoptosis upon Endoplasmic Reticulum Stress as a Therapeutic Strategy in Cancer. **Int J Mol Sci.** 23(16):8987
- Mora-Molina R, Stöhr D, Rehm M, López-Rivas A. cFLIP downregulation is an early event required for endoplasmic reticulum stress-induced apoptosis in tumor cells. **Cell Death Dis.** 13(2):111
- Olmedo-Moreno L, Aguilera Y, Baliña-Sánchez C, Martín-Montalvo A, Capilla-González V. Heterogeneity of In Vitro Expanded Mesenchymal Stromal Cells and Strategies to Improve Their Therapeutic Actions. **Pharmaceutics.** 14(5):1112
- Peña-Gómez MJ, Moreno-Gordillo P, Narmont? M, García-Calderón CB, Ruk??nait? A, Klima?auskas S, Rosado IV. FANCD2 maintains replication fork stability during misincorporation of the DNA demethylation products 5-hydroxymethyl-2'-deoxycytidine and 5-hydroxymethyl-2'-deoxyuridine. **Cell Death Dis.** 13(5):503
- Peña-Gómez MJ, Suárez-Pizarro M, Rosado IV. XRCC1 Prevents Replication Fork Instability during Misincorporation of the DNA Demethylation Bases. **Int J Mol Sci.** 14;23(2):893
- Pladevall-Morera D, Castejón-Griñán M, Aguilera P, Gaardahl K, Ingham A, Brosnan-Cashman JA, Meeker AK, Lopez-Contreras AJ. ATRX-Deficient High-Grade Glioma Cells Exhibit Increased Sensitivity to RTK and PDGFR Inhibitors. **Cancers (Basel).** 14(7):1790
- Polo-Generelo S, Torres B, Guerrero-Martínez JA, Camafeita E, Vázquez J, Reyes JC, Pintor-Toro JA. TGF-?-Upregulated Lnc-Nr6a1 Acts as a Reservoir of miR-181 and Mediates Assembly of a Glycolytic Complex. **Noncoding RNA.** 8(5):62
- Ravoityt? B, Luk?a J, Wellinger RE, Serva S, Servien? E. Adaptive Response of Saccharomyces Hosts to Totiviridae L-A dsRNA Viruses Is Achieved through Intrinsically Balanced Action of Targeted Transcription Factors. **J Fungi (Basel).** 8(4):381
- Romero-Gómez M, Aller R, Martín-Bermudo F. Dietary Recommendations for the Management of Non-alcoholic Fatty Liver Disease (NAFLD): A Nutritional Geometry Perspective. **Semin Liver Dis.** 22(1):63
- Sakellariou D, Bak ST, Isik E, Barroso SI, Porro A, Aguilera A, Bartek J, Janscak P, Peña-Diaz J. MutS? regulates G4-associated telomeric R-loops to maintain telomere integrity in ALT cancer cells. **Cell Rep.** 39(1):110602
- Salas-Lloret D, González-Prieto R. Insights in Post-Translational Modifications: Ubiquitin and SUMO. **Int J Mol Sci.** 23(6):3281
- Salguero-Aranda C, Beltran-Povea A, Postigo-Corrales F, Hitos AB, Díaz I, Caballano-Infantes E, Fraga MF, Hmadcha A, Martín F, Soria B, Tapia-Limonchi R, Bedoya FJ, Tejedo JR, Cahuana GM. Pdx1 Is Transcriptionally Regulated by EGR-1 during Nitric Oxide-Induced Endoderm Differentiation of Mouse Embryonic Stem Cells. **Int J Mol Sci.** 23(7):3920
- Sánchez-Escabias E, Guerrero-Martínez JA, Reyes JC. Co-transcriptional splicing efficiency is a gene-specific feature that can be regulated by TGF. **Commun Biol.** 5(1):277
- Sarmiento A, Durán-Díaz I, Fondón I, Tomé M, Bodineau C, Durán RV. A Method for Unsupervised Semi-Quantification of Inmunohistochemical Staining with Beta Divergences. **Entropy (Basel).** 24(4):546
- Sarni D, Barroso S, Shtrikman A, Irony-Tur Sinai M, Oren YS, Aguilera A, Kerem B. Topoisomerase 1-dependent R-loop deficiency drives accelerated replication and genomic instability. **Cell Rep.** 40(13):111397
- Tessier S, Ferhi O, Geoffroy MC, González-Prieto R, Canat A, Quentin S, Pla M, Niwa-Kawakita M, Bercier P, Rérolle D, Therizols P, Fabre E, Vertegaal ACO, de Thé H, Lallemand-Breitenbach V. Exploration of nuclear body-enhanced sumoylation reveals that PML represses 2-cell features of embryonic stem cells. **Nat Commun.** 13(1):5726
- Valdés-Sánchez L, Borrego-González S, Montero-Sánchez A, Massalini S, de la Cerda B, Díaz-Cuenca A, Díaz-Corrales FJ. Mesoporous Silica-Based Nanoparticles as Non-Viral Gene Delivery Platform for Treating Retinitis Pigmentosa. **J Clin Med.** 11(8):2170
- Wellinger RE, Aguilar-Ruiz JS. A new challenge for data analytics: transposons. **BioData Min.** 15(1):9
- Yerbes R, Mora-Molina R, Fernández-Farrán FJ, Hiraldo L, López-Rivas A, Palacios C. 2022. Limiting glutamine utilization activates a GCN2/TRAIL-R2/Caspase-8 apoptotic pathway in glutamine-addicted tumor cells. **Cell Death Dis.** 27;13(10):906

Book Chapters

2021

- Bayona-Feliu A, Aguilera A. The role of chromatin at transcription-replication conflicts as a genome safeguard. Biochem Soc Trans. 17;49(6):2727-2736.
- González-Prieto R, Cabello-Lobato MJ, Prado F. In Vivo Binding of Recombination Proteins to Non-DSB DNA Lesions and to Replication Forks. Methods Mol Biol. 2153:447-458.
- Tumini E, Aguilera A. The Sister-Chromatid Exchange Assay in Human Cells. Methods Mol Biol. 2153:383-393.
- García-Muse T. Detection of DSBs in C. elegans Meiosis. Methods Mol Biol. 2153:287-293.
- Barroso SI, Aguilera A. Detection of DNA Double-Strand Breaks by γ-H2AX Immunodetection. Methods Mol Biol. 2153:1-8.

2022

- García-Rubio M, Soler-Oliva ME, Aguilera A. Genome-Wide Analysis of DNA-RNA Hybrids in Yeast by DRIPc-Seq and DRIP-Seq. Methods Mol Biol. 2528:429-443.
- Silva S, Guillén-Mendoza C, Aguilera A. RNase H1 Hybrid-Binding Domain-Based Tools for Cellular Biology Studies of DNA-RNA Hybrids in Mammalian Cells. Methods Mol Biol. 2528:115-125.
- Cañas JC, Aguilera A, Gómez-González B. Detection of R-Loops by In Vivo and In Vitro Cytosine Deamination in Saccharomyces cerevisiae. Methods Mol Biol. 2528:39-53.

Patents

2021

- de La Cerda, B, Diaz Corrales, F, García, A, Díaz, A, Borrego, S. Composición Biopolimérica, Procedimiento para su Preparación y Uso de la Misma. 2021. ES2823398B2. CSIC y Fundación Pública Andaluza Progreso y Salud.

2022

- Capilla-González V, Aguilera Y, Olmedo-Moreno L, Mellado-Damas N, Martín-Montalvo A. Células madre mesenquimales con potencial terapéutico aumentado para el tratamiento del cáncer.2022. PCT/ES2022/070346. Fundación Pública Andaluza Progreso y Salud.
- Capilla González, V, Martín-Montalvo, A. Inhibidores de la ATP-citrato liasa como agentes geroprotectores.2022. P202230801. Fundación Pública Andaluza Progreso y Salud.

Doctoral Theses

2021

Inés García de Oya
"Mecanismos que regulan la salida de mitosis para el control de la correcta ploidía celular" Thesis Supervisor: Dr. Fernando Monje. Universidad de Sevilla.

José Antonio Guerrero Martínez
"Identificación y caracterización de elementos regulatorios distales controlados por TGFB." Thesis Supervisor: Dr. Jose Carlos Reyes Rosa. Universidad de Sevilla

Ana Belén García Delgado
"Terapias avanzadas en enfermedades degenerativas de la retina". Thesis Supervisors: Dr. Francisco Javier Díaz Corrales and Dr. Shom Shanker Bhattacharya. Universidad de Sevilla

Pedro Ortega Moreno
"Influence of chromatin factors and DNA-RNA hybrids in the repair of replication-born DSBs". Thesis Supervisors: Prof. Andrés Aguilera López and Dr. Belén Gómez González. Universidad de Sevilla

Hayat Heluani Gahete
"Identification and characterization of factors involved in sodium selenite toxicity." Thesis Supervisor: Dr. Ralf-Erik Wellinger. Universidad de Sevilla

Juan Francisco Correa Vázquez
"Función de SUMO en el control de la neurogenesis". Thesis Supervisor: Dr. Mario García Domínguez. Universidad de Sevilla

Esther Marchena Cruz
"DDX47 and MECP2 two novel human functions controlling R-loop-mediated genome integrity". Thesis Supervisors: Prof. Andrés Aguilera López and Dra. Rosa Mª Luna Varo. Universidad de Sevilla

2022

José Terrón Bautista
"Global Dynamics of Topoisomerase II Activity". Thesis Supervisors: Dr. Felipe Cortés Ledesma and Prof. Andrés Aguilera López. Universidad de Sevilla

Salvador Polo Generelo
"Identificación y análisis funcional del Inc-Nr6a1 SERPINE1 como reguladores tempranos de la transición epitelio-mesénquima". Thesis supervisor: Dr. José Antonio Pintor Toro. Universidad de Sevilla

Aurora Yáñez Vilches
"Descripción y caracterización de la interacción física entre los complejos MCM y RNR en respuesta a daños en el ADN". Thesis Supervisor: Dr. Félix Prado Velasco. Universidad de Sevilla

Rocío Mora Molina
"Role of FLIP and REDD1 in the control of tumor cell fate under endoplasmic reticulum stress". Thesis supervisors: Dr. Abelardo López Rivas and Dra. María del Carmen Palacios Casanova. Universidad de Sevilla

Jesús Roca García
"Contribución de y-tubulina a la nucleación de microtúbulos desde los centros organizadores de microtúbulos de células animales". Thesis supervisors: Dra. Rosa María Ríos Sánchez and Dra. María de la Paz Gavilán Dorronzoro. Universidad de Sevilla

Seminar Speakers

2021

January 2021

“Alternative Lengthening of Telomeres in cancer: triggers and alleviators” January 15th. Claus Azzalin. Instituto de Medicina Molecular, Lisboa, Portugal

“Genome-wide analysis of DNA repair at single-nucleotide resolution” January 22nd. Andrés Clemente. Instituto de Biología Funcional y Genómica (IBFG), Salamanca, Spain

“Mitochondrial dynamics and metabolic disorders” January 29th. Antonio Zorzano. Institute for Research in Biomedicine (IRB), Barcelona, Spain

February 2021

“Can melatonin reduce the severity of COVID-19 pandemic?” February 5th. Germaine Escames. Centro de investigación Biomédica de la Universidad de Granada, Granada, Spain

“Understanding beta cell heterogeneity from the single protein to the intact islet” February 19th. David Hodson. Institute of Metabolism and Systems Research (IMSR), and Centre of Membrane Proteins and Receptors (COMPARE), University of Birmingham (UK)

“Emerging immunomodulatory roles of galectin-3 under conditions of neurodegenerations” February 26th. José Luis Venero. Instituto de Biomedicina de Sevilla (IBIS), Sevilla, Spain

March 2021

“Redox control of protein aggregation through the autophagy master regulator TFEB/HLH-30” March 5th. Antonio Miranda. Instituto de Biomedicina de Sevilla (IBIS), Sevilla, Spain

“Physiology of the adult carotid germinal niche” March 12th. Ricardo Pardal. Instituto de Biomedicina de Sevilla (IBIS), Sevilla, Spain

May 2021

“Targeting the DNA-damage response in cancer treatment” May 7th. Josep Vicent Forment. DNA Damage Response biology Oncology R&D AstraZeneca, Cambridge, UK

“Unravelin the Dynamic nano- and meso-scale architecture of the living cell membrane” May 14th. María García Parajo. The institute of Photonic Sciences (ICFO), Barcelona, Spain

“Cytoskeleton meets Chromatin: the role of Myosin VI in DSB repair” May 21st. Hans-Peter Wollsheid. Institute of Molecular Biology (IMB), Mainz, Germany

“Pathogenic mechanisms of CoQ deficiency” May 28th. Plácido Navas. Centro Andaluz de Biología del Desarrollo (CABD), Sevilla, Spain

June 2021

“A CRIPR view of transcriptional termination” June 4th. Steven West. University of Exeter, Exeter, UK

“Impact of the subventricular zone on glioblastoma aggressiveness” June 25th. Hugo Guerrero-Cazares. Clínica Mayo, Florida, USA

July 2021

“Principles and consequences of spatial organization of the genome” July 9th. Peter Askjaer. Centro Andaluz de Biología del Desarrollo (CABD), Sevilla, Spain

November 2021

“Protein kinases and phosphatases regulating autophagy” November 26th. Jörn Dengjel. Department of Biology, University of Fribourg, Fribourg, Switzerland

December 2021

“A new function of the TRESLIN-MTBP complex in human cells: regulation of the S/G2 transition” December 3rd. Luis Toledo. Center of Chromosome Stability, University of Copenhagen, Copenhagen, Denmark

2022

January 2022

“SEACing to understand TORC1 regulation” January 21st. Robbie Loewith. University of Geneva, Geneva, Switzerland

“Use of functional genomics for the study of the role of SWI/SNF in lung cancer” January 28th. Ignacio Varela. Instituto de Biomedicina y Biotecnología de Cantabria, Universidad de Cantabria, Cantabria, Spain

February 2022

“Modulation of microglía and macrophage phenotype to mediate repair after central nervous system injury” February 11th. Rubén López-Vales. Instituto de Neurociencias, Universidad Autónoma de Barcelona, Barcelona, Spain

“Mechanisms of DNA crosslink repair” February 18th. Julien Duxin. Novo Nordisk Foundation center for protein Research (CPR), University of Copenhagen, Copenhagen, Denmark

March 2022

“Chromatin replication and epigenome Stability” March 4th. Anja Groth. Novo Nordisk Foundation center for protein Research (CPR), University of Copenhagen, Copenhagen, Denmark

“Insights into KRAS biology to identify nove therapeutic strategies for cancer” March 11th. Chiara Ambrogio. University of Torino, Torino, Italy

“Novel players in chromatin” March 18th. Rob Schneider. Institute of Functional Epigenetics, Helmholtz Center, Munich, Germany

“LHR-1 regulated extra-adrenal glucocorticoid synthesis as an immune scape mechanism of colorectal tumors” March 22nd. Thomi Brunner. University of Konstanz, Konstanz, Germany

April 2022

“Establishment and function of Chromatin Structure around Eukaryotic Chromosome Replication Origins” April 21st. Christoph Kurat. Biomedical Center Munich (BMC), Ludwig-Maximilians University (LMU), Munich, Germany

“Centriole architecture: from molecular assembly to diseases” April 29th. Virginie Hamel. University of Geneva, Department of Cell Biology, Faculty of Science, Geneva, Switzerland

May 2022

“Multi-omics integration and long read sequencing at the forefront of transcriptome research” May 13th. Ana Conesa. Institute for Integrative Systems Biology (I2SysBio), CSIC, Valencia, Spain

“Chromosome tracing” May 20th. Marc Martí-Renom. The National center for Genomic Analysis-Center for Genomic Regulation (CNAG-CRG), Barcelona, Spain

“New roles of autophagy in the nervous system” May 27th. Patricia Boya. Centro de Investigaciones Biológicas Margarita Salas, CSIC, Madrid, Spain

June 2022

“The distinct role of Polycomb and MLL proteins in stem cell biology and cancer” June 10th. Luciano Di Croce. Center for Genomic Regulation (CRG)-ICREA, Barcelona, Spain

October 2022

“Harnessing the healing power of macrophages: a journey aided by epigenomic and high-dimentional biology approaches” October 13th. Laszlo Nagy. John Hopkins All Children’s Hospital, Florida, USA

“HnRNPA1 and G-quadruplexes regulatory role in KRAS expression” October 20th. Gilmar Salgado. Université de Bourdeaux, Bordeaux, France

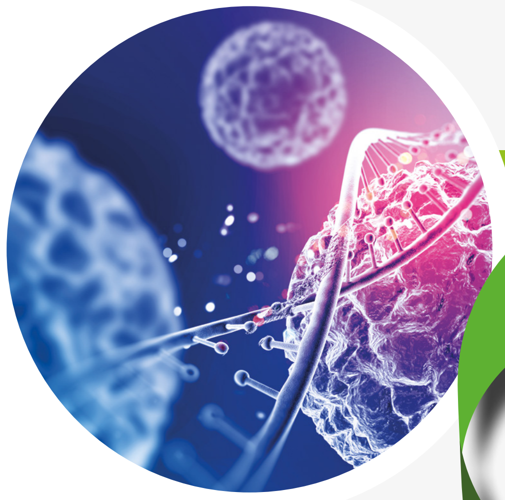
“Cohesin and Chromosome Segregation in Oocytes: a Goldilocks scenario” October 21st. Neil Hunter. University of California, David, USA

“Non-coding RNA roles in coordinating DNA replication and its associated stress response” October 28th. Maite Huarte. CIMA Universidad de Navarra, Navarra, Spain

November 2022

“Metabolism in the single cell era: approaches to sharpen the cutting edge of the metabolism field” November 4th. Rafael Arguello. Centre d’Immunologie de Marseille-Luminy, Marseille, France

Characterization of bursts of structural genomic variation in budding yeast” November 11th. Juan Lucas Argueso. Colorado State University, Colorado, USA



Workshops, Retreats & Seminars

International Workshops

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ANDALUSIAN CENTER FOR MOLECULAR BIOLOGY AND REGENERATIVE MEDICINE

II CABIMER International Workshop

Trends in Cancer Biology & Advanced Therapies

February 23rd - 25th, 2022. CABIMER, Sevilla, Spain

Organizers:

Raúl V. Durán (ES)
Abelardo López-Rivas (ES)

Speakers:

Anna Bigas (ES)
Sandra Blanco (ES)
Xosé Bustelo (ES)
David Cameron (UK)
Arkaitz Carracedo (ES)
Serge Evrard (FR)
Christian Frezza (DE)
Roger Gomis (ES)
Eyal Gottlieb (IL)
Michael N. Hall (CH)
Massimiliano Mazzone (NL)
Simón Méndez Ferrer (UK)
Ángela Nieto (ES)
Javier Oliver (ES)
Kevin Ryan (UK)
Owen Sanson (UK)
Marisol Soengas (ES)

Registration deadline:
November 30th, 2021
www.cabimer.es/workshop-2022/

Proyecto SOMM17-6112-USE
MEJORA DE INFRAESTRUCTURA CIENTÍFICA Y ACTIVIDADES DE TRANSFERENCIA Y DIFUSIÓN DE CABIMER PARA LA ADQUISICIÓN DEL SELLO "MARÍA DE MAEZTU"
Actividad cofinanciada con el Fondo Europeo de Desarrollo Regional.

Andalucía se mueve con Europa

Junta de Andalucía

UNIÓN EUROPEA

Predoc and Postdoc Retreats

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CSIC

IV Predoc & Junior Postdoc Retreat

10.12.2021
Cortijo del Alamillo

9:00 – 9:15 Arrival and Presentation

9:30 Rocío Mora Molina: *Role of cFLIP in endoplasmic reticulum stress-induced apoptosis in tumor cells.*

9:50 Lucía López Bermudo: *Maternal high fat diets based on olive oil protect offspring against MAFLD features through epigenetic changes.*

10:10 Alejandra Álvarez Llamas: *Defects in meiosis associated to alterations in Aurora B kinase expression.*

10:30 Paula Aguilera Aguilera: *CRISPR activation screen to define strategies for cancer prevention.*

10:50 Federica Bruno*: ChOR-seq: a new technology to study chromatin replication in mammalian cells.*

11:10-11:45 Coffee break

11:45 Alejandra Crespo Barreda: *Human placental MSCs and their exosomes as vehicles for the Na/I symporter (hNIS): a new theragnostic agent.*

12:05 Amador Romero Franco: *Regulation of DNA repair by the circadian clock.*

12:25 Alejandro Sola García: *The ACLY inhibitor SB204990 does not alter lysine histone acetylation in mouse liver.*

12:45 Cristina Guillen Mendoza: *Exploring epigenetic compounds that induce genetic instability.*

13:05 Nuria Fernández Fernández: *Generating phospho-mutants in C. elegans by CRISPR-Cas9.*

13:25 Álvaro Plaza Reyes: *Modeling Retinitis Pigmentosa using iPSC-derived Retinal Organoids.*

13:45 Lunch

15:30 Team-building games

17:00 End of the Retreat

Organizers:
Noelia Arroyo de Alba
Laura Olmedo Moreno
Concepción Panadero Morón

Retreat Cabimer

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CENTRO ANDALUZ DE BIOLOGÍA MOLECULAR
& MEDICINA REGENERATIVA

Retreat CABIMER 14-15 November 2022

MONDAY, November 14th

13:45H Departure from CABIMER by bus
14:30H Arrival to Alcazar de la Reina Hotel

Chair: Hèléne Gaillard
15:30H Fernando Monje Casas
15:55H Abelardo López Rivas
16:20H José Carlos Reyes Rosa
16:45H Andrés Aguilera López
17:10H Tatiana García Muse

17:30H COFFEE BREAK - Patio de las Columnas

Chair: Ana García Rondón
18:00H Benoit Gauthier
18:25H Mario García Domínguez
18:50H Pablo Huertas Sánchez
19:15H Raúl Durán Díaz
19:40H Silvia Jimeno González
20:00H Cristina Gonzállez Aguilera

20:30H DINNER - Hotel

SPONSORED BY:

TUESDAY, November 15th

Chair: Berta de la Cerda
9:00H Anabel Rojas
9:25H Féliz Prado
9:50H Andrés López Contreras
10:15H Iván Valle Rosado
10:40H Román González Prieto

11:00H COFFEE BREAK - Patio de las Columnas

Chair: Belén Gómez-González
11:30H Franz Martín Bermudo
11:55H Francisco Díaz Corrales
12:20H Inés Pineda Torra
12:45H David Pozo Pérez / Cintia Roodveldt
13:10H Manuel Álvarez Dolado
13:35H Vivian Capilla González

14:00 LUNCH - Patio de las Columnas

Chair: Sonia Jimeno González
15:30H Alejandro Martín Montalvo
15:55H Ralf Wellinger
16:20H Gonzalo Millán Zambrano

16:40H END
17:15H Departure by bus
18:00H Arrival to CABIMER

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Internal Workshops

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ANDALUSIAN CENTER FOR MOLECULAR BIOLOGY
AND REGENERATIVE MEDICINE

CABIMER WORKSHOP
December, 2021

Tuesday, December 21

09:15-09:30	Introductory remarks
09:30-10:00	Benoit Gauthier: "Coupling immunity to regeneration: harnessing wound healing to treat autoimmune diseases"
10:00-10:30	Fernando Romero Balestra: "TRIM37: an orchestrator of centrosome assembly"
10:30-11:00	Socorro Murdoch: "Practical approach to metabolit by LC-MS/MS"
11:00-11:30	Coffee break
11:30-12:00	Tatiana García Muse: "*C. elegans* BLOOM is a target
12:00-12:30	Rosario Yerbes Cadenas: "ECM stiffness confers resi by the YAP/TAZ-mediated control of TRAIL-R2/DR5
12:30-13:00	Ana Rincón Romero: "A new role of the cell wall int distribution of the spindle microtubule organizing

Wec

09:30-10:00	Hélène Gaillard: "Manganese is a physiologically re and mammals"
10:00-10:30	María Castejón Griñán: "PICH as a potential therape
10:30-11:00	Mariola Rodríguez Chacón: "CENP-V is required for through interaction with spindle microtubules in m
11:00-11:30	Coffee break
11:30-12:00	Noelia Arroyo de Alba: "Modulation of hepatic stell liver fibrosis regression"
12:00-12:30	Román González Prieto: "Genome (Ubiquitin-like) P
12:30-12:45	Concluding remarks

Junta de Andaluci

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CENTRO ANDALUZ DE BIOLOGÍA MOLECULAR
& MEDICINA REGENERATIVA

WORKSHOP
December 2022

Thursday 22nd

9:15 Welcome

9:30 Franz Martín Bermudo
Boosting mitochondrial activity in metabolic liver diseases is organ-dependent

10:00 Amador Romero Franco
Human Cryptochrome1 dampens
at nightfall

10:30 Laura Olmedo Moreno
Cross-talk between mesenchyma

11:00 Coffee Break

11:30 Zaira González Sánchez
The versatile role of glutamate ca
MA) and its potential as a smart delivery
tion

12:00 Belén Gómez González
A new activity for the resolution o

12:30 Manuel Álvarez Dolado
A shared Cell-therapy for Alzheim

13:00 Concluding remarks
Workshop Program Coordinator
Ana G. Rondón

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AND REGENERATIVE MEDICINE

CABIMER WORKSHOP
April, 2022

Thursday, April 7th

09:15-09:30	Introductory remarks
09:30-10:00	Mª Eugenia Martín-Vázquez García: "Induced-pluripotent stem cell-derived human islet organoids: the next generation model to study anti-diabetic therapeutic targets"
10:00-10:30	Mariola Rodríguez Chacón: "CENP-V is required for proper chromosome segregation through interaction with spindle microtubules in mouse oocytes"
10:30-11:00	Socorro Murdoch: "Practical approach to metabolite characterization and quantification by LC-MS/MS"
11:00-11:30	Coffee break
11:30-12:00	María Castejón Griñán: "PICH as a potential therapeutic target in lymphoma"
12:00-12:30	Rosario Yerbes Cadenas: "ECM stiffness confers resistance to ER stress-induced apoptosis by the YAP/TAZ-mediated control of TRAIL-R2/DR5 activation"
12:30-13:00	Ana Rincón Romero: "A new role of the cell wall integrity pathway in regulating the distribution of the spindle microtubule organizing centers"

Friday, April 8th

09:30-10:00	Hélène Gaillard: "Manganese is a physiologically relevant TORC1 activator in yeast and mammals"
10:00-10:30	Tatiana García Muse: "*C. elegans* BLOOM is a target of the DDR"
10:30-11:00	Fernando Romero Balestra: "TRIM37: an orchestrator of centrosome assembly"
11:00-11:30	Coffee break
11:30-12:00	Cristina González Aguilera: "Contribution of chromatin replication on transcriptional plasticity"
12:00-12:30	Román González Prieto: "Genome (Ubiquitin-like) Proteomics"
12:30-12:45	Concluding remarks

Workshop Program Coordinator:
Fernando Monje-Casas

Junta de Andalucía

Conference Series

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ANDALUSIAN CENTER FOR MOLECULAR BIOLOGY AND REGENERATIVE MEDICINE

CONFERENCE SERIES
2020 - 2021

CABIMER hosts invited Speakers every Friday at 12:00 noon
Please check our website for updates
www.cabimer.es

December

4th *The Eukaryotic Replisome: Structure and Mechanism*
Joseph Yeeles
MRC Laboratory of Molecular Biology, Cambridge (UK)

January

15th *Alternative Lengthening of Telomeres in cancer: triggers and alleviators*
Claus Azzalin
Instituto de Medicina Molecular, Lisboa, (Portugal)

22nd *Genome-wide analysis of DNA repair at single-nucleotide resolution*
Andrés Clemente
Instituto de Biología Funcional y Genómica (IBFG), Salamanca, (Spain)

29th *Mitochondrial dynamics and metabolic disorders*
Antonio Zorzano
Institute for Research in Biomedicine (IRB), Barcelona, (Spain)

February

5th *Can melatonin reduce the severity of COVID-19 pandemic?*
Germaine Escames
Centro de Investigación Biomédica de la Universidad de Granada, Granada (Spain)

19nth *Understanding beta cell heterogeneity from the single protein to the intact islet*
David Hodson
Institute of Metabolism and Systems Research (IMSR), and Centre of Membrane Proteins and Receptors (COMPARE), University of Birmingham, Birmingham (UK)

26th *Emerging immunomodulatory roles of galectin-3 under conditions of neurodegeneration*
José Luis Venero
Instituto de Biomedicina de Sevilla (IBIS), Sevilla (Spain)

March

5th *Redox control of protein aggregation through the autophagy master regulator TFEB/HLH-30*
Antonio Miranda
Instituto de Biomedicina de Sevilla (IBIS), Sevilla (Spain)

March

12th *Physiology of the adult carotid body germinal niche*
Ricardo Pardal
Instituto de Biom

9th *Pathogenic mec*
Plácido Navas
Centro Andaluz
Sevilla (Spain)

14th *Unraveling the c*
ture of the living
María García P
The Institute of
(Spain)

21st *Cytoskeleton me*
DSB repair
Hans-Peter Wo
Institute of Mole

4th *A CRISPR view*
Steven West
University of Ex

18th *Targeting the D*
treatment
Josep Vicent
DNA Damage R
AstraZeneca, C

25th *Impact of the su*
aggressiveness
Hugo Guerrero
Clínica Mayo, Flo

9th *Principles and c*
of the genome
Peter Askjaer
Centro Andaluz
Sevilla (Spain)

This season´s conferences can be attended through an invitation link t

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CONFERENCE SERIES
2021-2022

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November

26th *Protein kinases and phosphatases regulating autophagy*
Jörn Dengjel
Department of Biology, University of Fribourg, Fribourg, Switzerland

December

3rd *A new function for the TRESLIN-MTBP complex in human cells: regulation of the S/G2 transition*
Luis Toledo
Center for Chromosome Stability, University of Copenhagen, Copenhagen, Denmark

January

21st *SEACing to understand TORC1 regulation*
Robbie Loewith
University of Geneva, Geneva, Switzerland

28th *Use of functional genomics for the study of the role of SWI/SNF in lung cancer*
Ignacio Varela
Instituto de Biomedicina y Biotecnología de Cantabria, Universidad de Cantabria, Cantabria, Spain

February

11th *Modulation of microglia and macrophage phenotype to mediate repair after central nervous system injury*
Rubén López-Vales
Instituto de Neurociencias. Universidad Autónoma de Barcelona

18th *Mechanisms of DNA crosslink repair*
Julien Duxin
Novo Nordisk Foundation Center for protein Research (CPR), University of Copenhagen, Copenhagen, Denmark

March

4th *Chromatin Replication and Epigenome Stability*
Anja Groth
Novo Nordisk Foundation Center for protein Research (CPR), University of Copenhagen, Copenhagen, Denmark

11th *Insights into KRAS biology to identify novel therapeutic strategies for cáncer*
Chiara Ambrogio
University of Torino, Torino, Italy

18th *Novel play*
Rob Schn
Institute of
Munich, G

22nd *LRH-1-reg*
as an imm
Thomi Br
University

21st *Establishn*
around Eu
Christoph
Biomedica
Ludwig-Ma

29th *Centriole a*
Virginie H
University
Science, C

13th *Multi-omic*
forefront o
Ana Cone
Institute fo
CSIC, Val

20th *Chromoso*
Marc Mar
The Natio
Genomic

27th *New roles*
Patricia B
Centro de
CSIC, Mad

10th *The distin*
cell biolog
Luciano D
Centre fo
Spain

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CONFERENCE SERIES
2022-2023

CABIMER hosts invited Speakers on Fridays at 12:00 noon
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October

13th *Harnessing the healing power of macrophages: a journey aided by epigenomic and high-dimentional biology approaches*
Laszlo Nagy
Johns Hopkins All Children's Hospital, Florida, USA

20th *HnRNPA1 and G-quadruplexes regulatory role in KRAS expression*
Gilmar Salgado
Université de Bourdeaux, Bordeaux, France

21st *Cohesin and Chromosome Segregation in Oocytes: a Goldilocks scenario*
Neil Hunter
University of California, Davis, USA

28th *Non-coding RNA roles in coordinating DNA replication and its associated stress response*
Maite Huarte
CIMA Universidad de Navarra, Navarra, Spain

November

4th *Metabolism in the single cell era:approaches to sharpen the cutting edge of the metabolism field*
Rafael Arguello
Centre d'Immunologie de Marseille-Luminy, Marseille, France

11th *Characterization of bursts of structural genomic variation in budding yeast*
Juan Lucas Argueso
Colorado State University, Colorado, USA

January

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

27th *Dual functional role of Gasdermin B in breast cancer*
Gema Moreno Bueno
IIIB, Madrid, Spain

February

3rd *Targeted protein degradation: a novel paradigm in drug development*
Cristina Mayor-Ruiz
IRB Barcelona, Barcelona, Spain

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

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

March

3rd *SLX4: playing with nucleases, helicases and beyond*
Pierre Henri Gaillard
CRCM - Marseille, Marseille, France

March

10th *Replication fork remodelling in cancer and stem cells*
Massimo Lopes
ETH Institute of Biochemistry, Zurich, Switzerland

17th *Controlling nucleases and helicases that determine our genetic make-up*
Joao Matos
Max Perutz Labs, Viena, Austria

24th *Unravelling the genetic pathways underlying ageing in mice*
Colin Selman
University of Gasglow, Glasgow, United Kingdom

April

14th *Advancing knowledge in the biology of the oval cell and its role in liver pathology*
Ángela Martínez Valverde
Instituto de Investigaciones Biomédicas Alberto Sols (IIBM), Madrid, Spain

21st *Role of neuroinflammation in ALS and FTD pathogenesis*
Robert Baloh
Novartis Institutes for Biomedical Research (NIBR), Basel, Switzerland

May

5th *SUMO rules!*
Andrea Pichler
ETH Institute of Biochemistry, Zurich, Switzerland
Max Planck Institute of Immunobiology and Epigenetics, Freiburg, Germany

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

19th *Exploring the role of Polycomb and MLL complexes on chromatin topology, stem cell differentiation, and cancer*
Luciano Di Croce
CRG-ICREA, Barcelona, Spain

29th *Causes and Consequences of Replication Stress*
Karlene Cimprich
Stanford University, Stanford, USA

June

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

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

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

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Conference on Equality in Science

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1ª Jornada sobre Igualdad en Ciencia de CABIMER

Jueves 27 de octubre 2022, CABIMER, Sevilla

Organizadores: Unidad de Igualdad* CABIMER

Programa

9:30 Inicio
9:40 "Matemáticas con M de mujer"
Clara Grima *(Profesora Titular de Matemática Aplicada, Universidad de Sevilla y divulgadora)*
10:20 "La física, ¿un mundo masculino?"
MªIsabel Gallardo *(Catedrática jubilada de Física Atómica, Molecular y Nuclear. Dpto. de Física Atómica, Molecular y Nuclear, Universidad de Sevilla)*
11:00 Coffee break
11:30 "Percepciones y experiencias personales como mujer en ciencia"
Maite Huarte *(Investigadora principal. CIMA, Universidad de Navarra)*
12:10 Mesa redonda
13:20 Conclusiones

Registro hasta completar aforo.
Actividad válida para los cursos de doctorado.

*Carmen Ramos, Cristina González, Fernando Romero, Macarena Morillo, Sonia Jimeno y Tatiana García

XXXIV Night Race of Guadalquivir

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Jornada
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05/11/2021

		Intervención
		Prof. Andrés Aguilera. Director de CABIMER.
		Dña. Rosa María Ríos Sánchez. Secretaria General de Universidades, Investigación y Tecnología. Consejería de Transformación Económica, Industria, Conocimiento y Universidades. Junta de Andalucía.
		Dña. Amelia Martín Uranga. Directora Asociada de Investigación clínica y traslacional. FARMAINDUSTRIA.
		D. Ion Arocena. Director General. ASEBIO.
10:15 11:30	Presentaciones de grupos de investigación de CABIMER y empresas	**Dr. Francisco Javier Díaz-Corrales.** *Modelos experimentales para el estudio de terapias avanzadas y nuevos fármacos en patologías degenerativas de la retina*
		Dr. Manuel A. Dolado. *Oportunidades Tecnológicas del Lab. de Terapia Celular en Neuropatologías*
		Dr. David Pozo. *Presentación de las líneas de trabajo del Laboratorio de Neuroinmunología Celular y Molecular*
		Dra. Mariola Chacón. *CENP-V en el envejecimiento del óvulo de ratón. Investigación traslacional en el ovocito humano*
		Dra. Belén Gómez-González. *Marcadores y dianas de inestabilidad genética para diagnóstico y estudios de fármacos antitumorales*
		D. José María Gómez. Regional HCS Leader. Roche Farma. **Dña. Rosana Cabello**. Therapeutic Area Leader (Rare Conditions). Roche Farma.
11:30 12:00	Pausa café	
12:00 13:30	Presentaciones de grupos de investigación de CABIMER y empresas	**Dr. Iván Valle**. *Estudio de las bases moleculares de la patología Humana Anemia de Fanconi*
		Dr. Andrés J López-Contreras. *La translocasa de ADN PICH como potencial diana terapéutica en Linfoma de Burkitt*
		Dra. Eloísa Andújar. Unidad de Genómica de CABIMER.
		D. Antonio Gómez. Alliance Manager. The Janssen Pharmaceutical Companies of Johnson & Johnson.
		Dr. Raúl V. Durán. *Desarrollo de una nueva generación de inhibidores de mTORC1 con capacidad citotóxica en células tumorales contra el cáncer de colon*
		Dr. Mario García-Domínguez. *Péptidos derivados de proteínas BET contra el cáncer y otras patologías*
		Dr. Benoit Gauthier. *New chemotherapies for Diabetes/autoimmune diseases*
13:30 14:30	Financiación y apoyo a la Innovación	**D. Juan Luis Romera.** Departamento de Promoción Institucional y Cooperación Territorial. CDTI.
		Dña. Marian Ferre. Subdivisión de Programas Temáticos Científico-Técnicos. AGENCIA ESTATAL DE INVESTIGACIÓN.
		Dña. Carmen Ronchel. Responsable Técnico Sector Biotecnología. CORPORACIÓN TECNOLÓGICA DE ANDALUCÍA.
14:30 15:00	Cierre de la jornada	**D. Isaac Túnez Fiñana**. Secretario General de Investigación, Desarrollo e Innovación. Consejería de Salud y Familias. Junta de Andalucía.
15:00 16:30	Networking – Establecimiento de Contactos y Reuniones	

Participan:

Science Week in Andalusia

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Semana de la Ciencia en Andalucía

Del 7 al 19 de noviembre de 2022

www.fundaciondescubre.es

Semana de la Ciencia en Andalucía

Del 2 al 14 de noviembre de 2021

www.fundaciondescubre.es

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European Night of Researchers

Investigadores de CABIMER, comprometidos con acercar la ciencia a la sociedad, participaron en la

Noche Europea de los Investigadores

¡GRACIAS!

a todos los implicados en esta iniciativa

Scientific Advisory Board

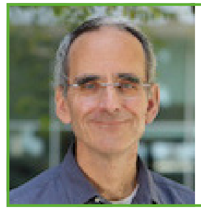
Dr. Pier Paolo Di Fiore
IFOM- FIRC Institute of Molecular Oncology.
Milan (Italy)

Dr. Susan M. Gasser
Friedrich Miescher Institute for Biomedical
Research. Basel (Switzerland)

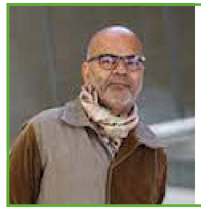
Dr. Simon Boulton
The Francis Crick Institute.
London (UK)

Dr. Maria Carmo Fonseca
Molecular Medicine Institute-iMM
University of Lisbon (Portugal)

Dr. Manuel Serrano (ended 2022)
Spanish National Cancer Research Center-CNIO
Madrid (Spain).



Dr. Juan Valcárcel
ICREA Research Professor at the Centre de
Regulació Genòmica-CRG Barcelona (Spain)



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

Dr. Purificación Muñoz (ended 2022)
ICREA and Pompeu Fabra University (UPF)
Barcelona (Spain)

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

Dr. Marisol Soengas
Spanish National Cancer Research Center-CNIO
Madrid (Spain)

Where we are

Centro Andaluz de Biología Molecular y Medicina Regenerativa

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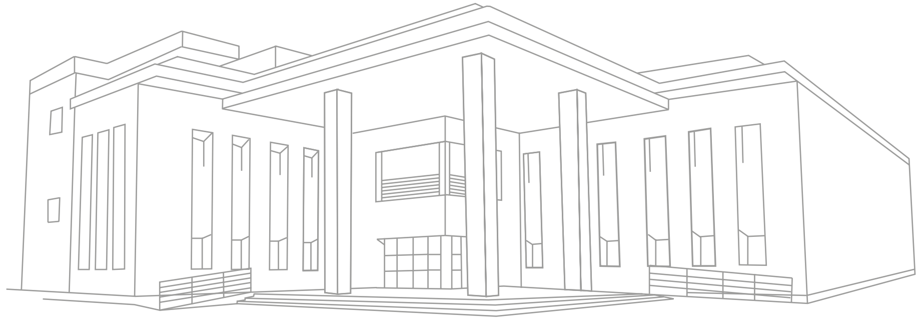

GPS Coordinates:
Latitude: 37.4112 or 37° 24'6720 N
Longitude: -6.0066 or 6° 0'3960 W

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