

Granada, 12 y 13 de diciembre de 2024

XII Jornadas Científicas



CENTRO PFIZER-UNIVERSIDAD DE GRANADA-JUNTA DE ANDALUCÍA
DE **GENÓMICA** E **INVESTIGACIÓN ONCOLÓGICA**



Libro de resúmenes/ abstract

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Comité Organizador

Álvarez Cubero, Maria Jesús
García Salcedo, Jose Antonio
Granados Principal, Sergio
Ramírez Macías, Inmaculada

Colaboradores

Nuñez Millan, Jose Angel
Ramírez Serrano, Eduardo

Comité Científico

Alcázar Fabra, María
Carmona Sáez, Pedro
Castro González, Sergio
García Salcedo, José Antonio
Marañón Lizana, Concepción
Martínez González, Luis Javier
Montes Lorenzo, Rosa María
Ortega Liébana, María del Carmen
Ortega Sánchez, Gabriel
Ramírez Macías, Inmaculada
Widmann, Thomas

Libro de abstract – Comunicación oral

Antonio José Cabrera-Serrano*, Giulia Peduzzi*, Cosmeri Rizzato*, José Manuel Sánchez-Maldonado, Rob Ter Horst, Laura Fernández, Yang Li, Aleksandra Butrym, Miguel Ángel López-Nevot, Mihai Netea, Federico Canzian, Daniele Campa#, Juan Sainz#

* These authors equally contribute to this work.

A meta-analysis of genome-wide association studies discovers new loci for myeloproliferative neoplasms

Myeloproliferative neoplasms (MPNs) are a heterogeneous group of hematologic malignancies characterized by an abnormal growth of blood-forming cells in the bone marrow. We investigated the genetic risk associated with Philadelphia-positive and Philadelphia-negative MPNs including the largest cohort of cases investigated to date, 4,272 MPNs cases and 724,567 controls from MIRACLe consortium and the UKBioBank and FinnGen initiatives. The study aimed to identify novel risk loci linked to MPN risk and explored their functional impact.

Araceli López-Tejada^{1,2,3,*}, José L. Blaya-Cánovas^{2,3,4,*}, Francisca E. Carac, Jesús Calahorra^{2,3,4}, César Ramírez-Tortosa^{3,5}, Isabel Blancas^{3,6,7}, Violeta Delgado-Almenta², Marta Ávalos-Moreno², Ana Sánchez², Adrián González-González², Juan A. Marchal^{3,4,8,9}, Carmen Griñán-Lisón^{1,2,3,10,#}, Sergio Granados-Principal^{1,2,3,#}.

* These authors equally contribute to this work

1. Department of Biochemistry and Molecular Biology; 2. Faculty of Pharmacy, University of Granada, Campus de Cartuja s/n, 18071, Granada, Spain bGENYO, Centre for Genomics and Oncological Research, Pfizer/University of Granada/Andalusian Regional Government, 18016, Granada, Spain; 3. Instituto de Investigación Biosanitaria ibs.GRANADA, University Hospitals of Granada-University of Granada, 18100, Granada, Spain; 4. UGC de Oncología Médica, Hospital Universitario de Jaén, 23007, Jaén, Spain; 5. UGC de Anatomía Patológica Hospital San Cecilio de Granada, 18016, Granada, Spain; 6. UGC de Oncología, Hospital Universitario "San Cecilio", 18016, Granada, Spain; 7. Department of Medicine, University of Granada, 18016, Granada, Spain 8. Biopathology and Regenerative Medicine Institute (IBIMER), Centre for Biomedical Research, (CIBM) University of Granada, 18100, Granada, Spain; 9. Department of Human Anatomy and Embryology, Faculty of Medicine, University of Granada, 18016, Granada, Spain; 10. Excellence Research Unit "Modeling Nature" (MNat), University of Granada, 18100, Granada, Spain

Signature-based repurposed drugs resemble the inhibition of TGFβ-induced NDRG1 in triple-negative breast cancer by targeting AKT.

There is an urgent need for new therapeutic strategies against aggressive triple-negative breast cancer (TNBC), and drug repurposing offers a promising, time- and cost-effective solution. We previously reported that TGFβ leads the tumorigenic role of NDRG1 in TNBC. Here, we aimed to identify drugs that mimic the transcriptomic signature obtained after the inhibition of TGFβ-induced NDRG1 and to determine their antitumor properties. Using Connectivity Map, we selected efavirenz, ouabain, and vinburnine as the repurposed drug candidates to further evaluate their antiproliferative effects in TNBC as monotherapies and pairwise combinations. The candidate drugs significantly reduced tumor cell proliferation, cancer stem cells, self-renewal, clonogenic properties, and migration abilities. We

determined that none of the repurposed drugs inhibited NDRG1 expression, circumventing possible negative effects derived from the inhibition of the tumor-suppressive version of NDRG1, but exerted their activity through the blockade of AKT. We validated their translational potential in organoid cultures from a TNBC patient-derived xenograft model. The repurposed drugs effectively reduced cell viability, both alone and in combination with docetaxel by decreasing p-AKT and Ki67. In conclusion, our comprehensive study highlights the promise of these repurposed drugs against TNBC and supports the new era in the development of new treatments.

Francisco Pérez-Cózar ¹; María Palomeque ¹; Nieves Varela ¹; Daniel Díaz ¹; Cristina Moral ¹; Concepción Fernández-Roldán ²; Inmaculada Jiménez-Moleón ³; José-Luis Callejas ²; Norberto Ortego ^{2,4}; Adoración Martínez ⁵; Enrique Raya ^{3,4}; Bartosz Foronczewicz ⁶; Marta E. Alarcón-Riquelme ¹; Concepción Maraño ^{1#}

1. Pfizer-University of Granada-Junta de Andalucía Centre for Genomics and Oncological Research (GENYO), Granada, Spain; 2. Department of Internal Medicine. Systemic Autoimmune Diseases Unit. San Cecilio University Hospital, Granada, Spain; 3. Rheumatology Department. San Cecilio University Hospital, Granada, Spain; 4. Department of Medicine. University of Granada, Granada, Spain; 5. Nephrology Unit. Poniente Hospital, Almería, Spain; 6. Department of Immunology, Transplantology and Internal Diseases. Warsaw Medical University, Warsaw, Poland

Urinary anti-SSA as biomarker of renal involvement in Sjögren's syndrome in a cohort of patients with systemic autoimmune diseases

Background: Renal involvement is one of the most severe manifestations in systemic autoimmune diseases (SADs), such as systemic lupus erythematosus (SLE), Sjögren's syndrome (SJS), and others. The current gold standard for diagnosing renal pathology is biopsy, but its invasiveness and cost highlight the need for non-invasive biomarkers. Urinary biomarkers, directly reflecting renal status, are promising candidates for early detection and monitoring of kidney disease in SADs. *Methods:* A total of 504 participants with systemic autoimmune diseases (SADs) and age and gender-matched healthy donors (CTRL) were recruited. For each patient, clinical information and urine and serum samples were collected. A Luminex-based kit was used to study the levels of 9 different autoantibodies. Commercial anti-Ro52 and Ro-60 ELISAs were also used. *Results:* SJS patients showed significantly elevated urinary anti-SSA levels compared to healthy controls. Among SJS patients, those with renal involvement had higher urinary anti-SSA than those without and healthy controls. Importantly, urinary anti-SSA levels in SJS patients with renal pathology were higher than in lupus nephritis (LN) patients. No significant differences in urinary anti-SSA levels were found between LN, SJS without renal pathology, and controls. In contrast, serum anti-SSA levels were elevated in SJS patients but did not correlate with renal involvement or urinary levels. Additionally, urinary anti-SSA was not associated with extra-renal symptoms. *Conclusions:* Urinary anti-SSA autoantibodies may exhibit specificity for SJS among other SADs. Unlike serum anti-SSA, urinary anti-SSA provides insights into renal activity, highlighting its potential as a non-invasive diagnostic tool for SJS-associated kidney damage.

Ivan Ellson ¹, Pedro Carmona-Sáez ¹, Verónica Ramos-Mejía ¹.

1. GENYO. Centre for Genomics and Oncological Research Pfizer, University of Granada, Andalusian Regional Government, PTS, 18016, Granada, Spain

A transcriptomics-based drug repositioning approach to identify therapeutic candidates for KMT2A-rearranged pediatric AML patients

KMT2A rearrangement (*KMT2A-r*) is the most common structural aberration in pediatric acute myeloid leukemia (AML) and plays an important role in the risk stratification and treatment selection of patients since it is associated with poor outcome. However, there are few targeted therapies for this gene fusion in pediatric patients and the functioning mechanisms involving this alteration are still poorly understood. Here we performed a gene expression-based characterization and in silico drug repurposing for the *KMT2A-r* using novel bioinformatic tools and transcriptomic data from a large cohort of pediatric AML patients with *KMT2A-r* and healthy controls. The repurposed drugs were then validated in vitro and in vivo. We found that pediatric AML patients with *KMT2A-r* had down-regulated expression of *HOXA* genes compared to healthy controls, along with increased *MYC* transcription factor activity and overall suppression of immune response functions. We identified several potentially therapeutic compounds that were capable of reverse the transcriptomic signature associated to *KMT2A*-rearranged pediatric AML. Among them we found that BI-2536 and KF-38789 reduced cell viability and induced apoptosis in AML cell lines with *KMT2A-r*, specifically, reducing tumor content also in vivo. Furthermore, BI-2536 reduced colony-forming potential in *KMT2A-r* cells and had a high synergistic effect with the standard chemotherapeutic agent Cytarabine, allowing its dose to be reduced. These findings may improve the understanding of *KMT2A-r* and provide new personalized treatments in pediatric AML patients.

Jordi Martorell-Marugán ^{1,2}, Raúl López-Domínguez ¹, Juan Antonio Villatoro-García ^{1,3}, Daniel Toro-Domínguez ⁴, Marco Chierici ⁵, Giuseppe Jurman ⁵ and Pedro Carmona-Sáez ^{1,3}.

1. GENYO. Centre for Genomics and Oncological Research: Pfizer / University of Granada / Andalusian Regional Government, PTS Granada, Granada, 18016, Spain; 2. Fundación para la Investigación Biosanitaria de Andalucía Oriental-Alejandro Otero (FIBAO), Granada, 18012, Spain; 3. Department of Statistics and Operational Research, University of Granada, Granada, 18071, Spain; 4. Unit of Inflammatory Diseases, Department of Environmental Medicine, Karolinska Institutet, Solna, 171 77, Sweden; 5. Data Science for Health Research Unit, Fondazione Bruno Kessler, Trento, 38123, Italy.

Explainable Artificial Intelligence for predicting sample phenotypes and extracting biological insights from single-cell transcriptomics

Advancements in single-cell RNA sequencing (scRNA-Seq) have transformed our understanding of molecular phenotypes, particularly in disease contexts, at the individual cell level. Analyzing scRNA-Seq data is challenging due to its sparsity and volume, necessitating specialized statistical methods for effective information extraction. Standard gene expression classification methods fall short for scRNA-Seq, highlighting the need for novel algorithms. We present singleDeep, an end-to-end pipeline leveraging deep neural networks to streamline scRNA-Seq data analysis, enabling robust phenotype prediction and characterization. Validation across datasets from systemic lupus erythematosus, COVID-19, and Alzheimer's disease showcased singleDeep's superior diagnostic performance over traditional machine learning methods applied to pseudobulk data. Besides high prediction accuracy, singleDeep offers insights into cell type significance and gene importance for phenotypic characterization, effectively addressing false positives from highly expressed genes. Thus, singleDeep represents a significant advancement in scRNA-Seq data analysis, providing accurate predictions and enhancing our understanding of complex diseases through detailed cell and gene analysis.

Martín-López, P ^{1,2}, Castro-González, S ^{1,2}, Montenegro-Elvira, F ^{1,2}, Patiño-Mercau, JR ³, Cuadros-Celorrio, M ^{4,5}, Medina-Vico, P ^{2,5}

1. Pfizer-University of Granada-Junta de Andalucía Centre for Genomics and Oncological Research (GENYO), Granada, Spain; 2. Department of Biochemistry and Molecular Biology I, University of Granada, Granada, Spain; 3. Beth Israel Deaconess Medical Center, Harvard University, Boston, USA;

4. Department of Biochemistry and Molecular Biology III and Immunology, University of Granada, Granada, Spain; 5. Institute for Biomedical Research IBS. Granada, University Hospital Complex of Granada/ University of Granada, Granada, Spain.

BCL7A tumor suppressor role in germinal center derived-lymphomas involves two functionally distinct residues

Massive sequencing techniques in the last decade have emphasized the significance of epigenetic factors in tumor development. Notably, the SWI/SNF chromatin remodeling complexes, containing a high prevalence of mutations, play a crucial role. This complex modifies DNA-histone interactions, influencing DNA accessibility for transcriptional regulation. Comprising a catalytic subunit (SMARCA4/2) and associated proteins (BAFS), BCL7A is a recently discovered BAF subunit. Previous data from different studies demonstrated that BCL7A is highly mutated in hematological malignancies, including germinal center-derived lymphomas. Moreover, BCL7A expression peaks in GC cells, highlighting its relevance within this structure. Furthermore, our previous research identified a high mutation frequency within exon 1 in diffuse large B cell lymphoma (DLBCL), a germinal center-derived lymphoma, with the most prevalent mutation being a splicing alteration ($\Delta 27$ mutation) that results in a truncated, non-functional protein, underscoring the importance of the first exon of BCL7A in its tumor suppressor role. In this study, we employed in vivo Bcl7a knock-out (KO) models to investigate its role in lymphomagenesis. Bcl7a KO mice exhibited aberrant germinal center formation. Under oncogenic stimulus, Bcl7a KO accelerated lymphomagenesis in vivo, reinforcing BCL7A's tumor suppressive role. Moreover, our study of the mutations within BCL7A first exon identified two critical amino acids essential for its tumor suppressor function, which are involved in binding to the SWI/SNF complex and chromatin affinity. In conclusion, this work confirms that BCL7A plays a crucial role in the correct germinal center reaction. Moreover the loss of this tumor suppressor accelerated lymphomagenesis in vivo. Finally, we demonstrated that this tumor suppressor role requires of two distinct functions, which involves two key amino acids within BCL7A amino terminal domain.

Ana Colomer-Boronat ^{1,2}, Lissanne I. Knol ³, Guillermo Peris ^{4,2}, Laura Sánchez¹, Silvia Peluso ⁶, Pablo Tristan-Ramos ^{1,2}, Ana Gázquez-Gutiérrez ^{1,2}, Priscilla Chin ³, Katrina Gordon ³, Guillermo Barturen ^{2,6}, Robert E. Hill ⁵, Jose Luis García-Pérez ², Alasdair Ivens ³, Sara Macías ³, Sara R. Heras ^{1,2}

1. Dept. of Biochemistry and Molecular Biology II, Faculty of Pharmacy, University of Granada.; 2. GENYO. Centre for Genomics and Oncological Research, Pfizer University of Granada, Andalusian Regional Government.; 3. Institute of Immunology and Infection Research, School of Biological Sciences, University of Edinburgh; 4. Dept. of Computer Languages and Systems, Universitat Jaume I; 5. MRC Human Genetics Unit, Institute of Genetics and Cancer, University of Edinburgh; 6. Dept. of Genetics, Faculty of Science, University of Granada

DGCR8 haploinsufficiency leads to primate-specific RNA dysregulation and pluripotency defects

The 22q11.2 deletion syndrome (22qDS) is caused by a microdeletion in chromosome 22, including DGCR8, an essential gene for miRNA production. The contribution of human DGCR8 hemizyosity to the disease is still unclear. In this study, we generated two human pluripotent cell models containing a single functional DGCR8 allele to elucidate its role on 22qDS. DGCR8+/- cells show increased apoptosis as well as self-renewal and differentiation defects in both the naïve and primed states. The expression of primate-specific miRNAs was largely affected, due to impaired miRNA processing and chromatin accessibility. DGCR8+/-

cells also displayed a pronounced reduction in human endogenous retrovirus class H (HERVH) expression, a primate-specific retroelement essential for pluripotency maintenance. Importantly, the reintroduction of miRNAs belonging to the primate-specific C19MC cluster as well as the miR-371-3 cluster rescued the cellular and molecular phenotypes of DGCR8+/- cells. Our results suggest that DGCR8 is haploinsufficient in humans and that miRNAs and transposable elements may have co-evolved in primates as part of an essential regulatory network to maintain stem cell identity.

Monica Rodriguez Segura ^{1,2,3*}, Jose Francisco López Delgado ^{4*}, Maria Victoria Cano-Cortes ^{1,2,3}, Juan Jose Diaz-Mochon ^{1,2,3} and Rosario M. Sanchez-Martin ^{1,2,3#}

* These authors equally contribute to this work

1 GENYO, Centre for Genomics and Oncological Research, Pfizer/University of Granada/Andalusian Regional Government, PTS Granada, Avda. Ilustración 114, 18016 Granada, Spain 2 Department of Medicinal & Organic Chemistry and Excellence Research Unit of “Chemistry applied to Biomedicine and the Environment”, Faculty of Pharmacy, University of Granada, Campus de Cartuja s/n, Granada, Spain 3 Instituto de investigación biosanitaria de Granada (ibs.GRANADA), University Hospitals of Granada-University of Granada, Granada, Spain 4 DestINA Genómica SL, PTS Granada, Av. de la Innovación, 1, 18100 Granada, Spain ‡ These authors have contributed equally to this work

Nanocatalyst-Mediated Intracellular Activation of Therapeutics

Click chemistry reactions were introduced in the early 20th century by Sharpless, and in the early 2000s Bertozzi took the concept a step further by developing a new field called bioorthogonal chemistry. The most important of these reactions is the copper-catalysed azide-alkyne cycloaddition (CuAAC), for which Sharpless, Meldal and Bertozzi were awarded the Nobel Prize in Chemistry in 2022. However, the use of copper in biological systems presents significant challenges. The structure and oxidation state of copper complexes can influence their bioavailability, ligand transfer, cellular uptake and toxicity. In addition, reactive oxygen species (ROS) generated by copper catalysts can make the CuAAC reaction less suitable for biological applications. To overcome these obstacles, Bertozzi introduced the copper-free bioorthogonal reaction for in vivo applications, using the cyclooctyne ring to favour the reaction and achieve suitable click chemistry without copper. Despite this success, there are still many open-alkyne precursors that would benefit from CuAAC reactions in living systems, highlighting the need for copper compounds with reduced biotoxicity. In this context, heterogeneous copper catalysts have emerged as a less toxic version compared to homogeneous copper catalysts used in azide-alkyne cycloaddition processes in biological systems. In this work, we have developed a nanocatalyst based on copper metallofluorescent nanoparticles capable of catalysing cytoplasmic in vitro CuAAC reactions, providing a new tool in the set of non-biotoxic heterogeneous copper catalysts.

Patricia Porras-Quesada ^{1,2}, Verónica Arenas-Rodríguez ^{1,2}, Ana Pozo-Agundo ², Luis Javier Martínez-González ^{1,2} and María Jesús Álvarez-Cubero ^{1,2,3}.

1. Department of Biochemistry and Molecular Biology III and Immunology, Faculty of Medicine, University of Granada, Granada, Spain.; 2. GENYO, Centre for Genomics and Oncological Research, Pfizer, University of Granada, Andalusian Regional Government, PTS, Granada, Spain.; 3. Biosanitary Research Institute, ibs.GRANADA, Granada, Spain.

Exploring the role of miR-93-5p in prostate cancer tumorigenesis and prognosis

Ramos-Hernández I ^{1, 2}, Lozano M L ³, C Fuster-Garcia ⁴, Bueren J A ³, Guenechea G ³, T Cathomen ^{4,5, 6}; Muñoz P ^{1, 8, 10*}, Molina-Estévez F J ^{1, 2, 10*}, Martín F ^{1, 9, 10}

* These authors equally contribute to this work

1. Centro Pfizer – Universidad de Granada – Junta de Andalucía de Genómica e Investigación Oncológica (Genyo). Parque Tecnológico Ciencias de la Salud, Av. de la Ilustración 114, 18016 Granada; Spain; 2. Fundación Pública Andaluza para la Investigación Biosanitaria en Andalucía Oriental Alejandro Otero (FIBAO), Granada, Spain; 3. Unit of Telomeropathies, Centro de Investigaciones Energéticas, Medioambientales y Tecnológicas (CIEMAT), Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER) e Instituto de Investigación Sanitaria Fundación Jiménez Díaz (IIS-FJD). Madrid, 28040, Spain.; 4. Center for Chronic Immunodeficiency (CCI), Medical Center—University of Freiburg, 79106 Freiburg, Germany 5. Institute for Transfusion Medicine and Gene Therapy, Medical Center—University of Freiburg, 79106 Freiburg, Germany 6. Institute for Experimental Hematology, Hannover Medical School, 30625 Hannover, Germany 7. Departamento de Biología Celular, University of Granada, Science Faculty, 18071, Spain. 8. Departamento de Bioquímica, Biología Molecular III en Inmunología. University of Granada, Medicine Faculty, Granada, 18016, Spain. ; 9 Instituto de Investigación Biosanitaria (IBS) Granada, University of Granada, Granada, Spain.

Donor insertion into CX3CR1 allows epigenetic modulation of a constitutive promoter on HSCPs and its activation upon myeloid differentiation

Hematopoietic stem and progenitor cells (HSPCs) represent a reliable source for paracrine cross-correction strategies and are often modified using lentiviral vectors for therapeutic purposes. However, concerns regarding insertional mutagenesis and genotoxicity urge exploring alternative gene modification approaches. To address these concerns, we developed a novel knock-in (KI) strategy, which exploits local epigenome regulation (named KI-Ep) at myeloid-modulated genomic loci.

Our previous works demonstrated the behavior of transgene cassettes inserted into the fourth intron of the CX3CR1 gene in primitive and differentiated HSPCs in vitro. Using an optimized protocol, we achieved approximately 90% indel formation in HSPCs when targeting the fourth intron of CX3CR1, taking advantage of homologous-mediated end-joining (HMEJ) for enhanced donor template efficiency. To further evaluate the ability of the KI-Ep strategy to modulate transgene expression and assess its safety, we transplanted KI-Ep-modified HSPCs into human-permissive NSG mice. The modified HSPCs maintained viability, and no reduction in chimerism was observed four months post-transplantation. Additionally, the fitness of KI-Ep-modified HSPCs was comparable to that of unedited cells, with similarly high levels of chimerism observed in the bone marrow.

Biodistribution studies showed increased expression of our cassette in myeloid populations, particularly CD11b-positive cells, where CX3CR1 is naturally expressed. Furthermore, CAST-seq analysis confirmed the safety of the editing process by identifying minimal off-target effects and alternative DNA repair outcomes. To better understand the downregulation of the KI-Ep cassette in primitive HSPCs, as well as the skewed expression pattern towards myeloid populations (with peak expression in M1 macrophages in vitro), we sorted GFP-negative cells from stem or lineage-committed populations and differentiated them into M1 macrophages. This process revealed a "switch-on" phenomenon, suggesting that silent targeted integrations were activated upon differentiation. A comparison between lentiviral vector integrations and KI-Ep-modified HSPCs ruled out cassette design as the cause of this modulation, and we are currently investigating CIS-epigenome modifications as contributors to the KI-Ep expression profile.

Based on these findings, we propose the fourth intron of CX3CR1 as a promising "safe harbor" KI-Ep locus for potent paracrine cross-correction cell therapies, offering the potential for therapeutic benefit without inducing genotoxicity in HSPCs.

Rita Caracuel-Peramos ¹, Francisco J. Rodríguez-Baena ¹, Silvia Redondo-García ¹, Juan A. Villatoro-García ^{1,2}, Ana García-Muñoz ¹, Carlos Peris-Torres ¹, María C. Plaza-Calonge ¹, Belén López-Millán ^{1,3}, Carmela Ricciardelli ⁴, Darryl L. Russell ⁵, Pedro Carmona-Sáez ^{1,2}, and Juan C. Rodríguez-Manzaneque^{1#}

1. GENYO. Centre for Genomics and Oncological Research: Pfizer/Universidad de Granada/Junta de Andalucía, Avda. de la Ilustración, 114, Granada 18016, Spain.; 2. Department of Statistics, University of Granada, Granada, Spain.; 3. Department of Physiology, University of Granada, Granada, Spain.; 4. Robinson Research Institute, Adelaide Medical School, University of Adelaide, Australia.; 5. Robinson Research Institute, School of Biomedicine, University of Adelaide, Australia.

Loss of the extracellular protease ADAMTS1 reveals an antitumorigenic program involving the action of NIDOGEN-1 on macrophage polarization

The tumorigenesis process involves a dynamic and continuous remodeling of the extracellular matrix (ECM), driven by intricate interactions with tumor and stromal cells, including fibroblasts, immune and endothelial cells. Within this complex scenario, the extracellular protease ADAMTS1 has been involved in tumorigenesis, with recent findings highlighting its immunomodulatory role. Our work with melanoma and mammary tumor models revealed that tumor blockade induced by the lack of Adamts1 led to an increased vascular deposition of its substrate, the basement membrane glycoprotein NIDOGEN-1. Significantly, the overexpression of NID1 in the melanoma syngeneic model also blocked tumor progression, disclosing an overlapping phenotype with the absence of Adamts1. These tumors showed important alterations in their immune infiltrates, emphasizing an enhanced presence of antitumorigenic macrophages and a global inflammatory landscape. We corroborated in vitro that full length NID1, but not its proteolyzed fragments, promoted an M1-like macrophage polarization, mainly mediated by the $\alpha v\beta 3$ integrin. Significantly, the projection of RNA-seq from our tumor models to two large human melanoma datasets allowed us to discover a new gene signature associated with good prognosis and the abundance of M1-like macrophages. These results support NID1 as a novel tumor suppressor with immunomodulatory properties, and unveiled the existence of key oncological drivers in the extracellular scenario.

Sanchez-Mañas JM ^{1,2}, Perales S ^{1,2,3}, Martínez-Galan J ^{3,4}, Torres C ^{1,3,5}, Real PJ ^{1,2,3}.

1. GENyO, Centre for Genomics and Oncological Research, Pfizer-University of Granada-Andalusian Regional Government, Gene Regulation, Stem Cells & Development Lab, PTS Granada, Avenida de la Ilustracion 114, 18016 Granada, Spain.; 2. Department of Biochemistry and Molecular Biology I, Faculty of Science, University of Granada, Avenida Fuentenueva s/n, 18071 Granada, Spain.; 3. Instituto de Investigación Biosanitaria (ibs.GRANADA), Personalized Oncology Group, Avenida de las Fuerzas Armadas 2, 18014 Granada, Spain.; 4. Department of Medical Oncology, Hospital Universitario Virgen de las Nieves, Granada, Spain.; 5. Department of Biochemistry and Molecular Biology III and Immunology, Faculty of Sciences, University of Granada, Granada 18071, Spain.

“Silent messengers: the role of microvesicles and exosomes in platelet-pancreatic cancer communication.”

Introduction: Pancreatic cancer (PDAC) is a disease characterized by its lack of specific symptoms. Despite its low incidence, it has a mortality rate of 80%, with an overall 5-year survival of only 12.5% (2024) [1]. Distinctive associated factors include a persistent hypercoagulable state in all patients, which is associated with a constant interaction between platelets (PLTs) and pancreatic tumor cells (TC) [2]. The bidirectional and indirect communication between PLTs and TCs involves extracellular vesicles (EVs), both larger ones known as microvesicles (MVs) and smaller ones known as exosomes (EXOs). EVs act as biomolecule transporters, facilitating signal exchange in early stages of cancer, and promoting hypercoagulation, tumor progression, angiogenesis and metastasis in PDAC [3,4,5]. We aim

to study the mechanisms of indirect communication mediated by EVs between PLTs and TCs to understand how they contribute to carcinogenesis and employ them as a potential source of unique and non-invasive biomarkers for early diagnosis, treatment and monitoring of PDAC [6]. Methodology: Characterization of EVs will be performed by NTA, TEM, Zeta potential quantification, flow cytometry (FACS) or imaging (ImageStream). Bidirectional cell communication between PLTs from healthy donors and established TCs (BxPC3 and PANC-1) will be performed by in vitro cocultures. In the TC->PLTs direction we address activation and biomolecule transfer studies by FACS or adhesion by xCELLigence. In the PLTs->TC direction, we studied proliferation by xCELLigence, biomolecule transfer by FACS, ImageStream and gene expression by qPCR. Results and discussion: In TC->PLTs direction, we have verified how EVs originating from CT induce a differential activation and adhesion state dependent on the vesicle type (MVs and EXO) and on the type of line from which the EVs originate on PLTs. In addition, we observed the ability to transfer biomolecules of different nature (lipid or protein) to PLTs. In the PLTs->TC direction, we found that EVs derived from PLTs and plasma have the capacity to induce TC proliferation. Also, these EVs transfer their content to TCs, suggesting an active role in the regulation of tumor malignancy. Conclusions: EVs participate in PLTs-TC communication, demonstrating their potential as a source of non-invasive biomarkers for the diagnosis and monitoring of PDAC, as well as possible therapeutic targets for its treatment.

Thomas Widmann, Victoria Cano Cortés, Iván Ellson Lancho, Lucía Rodríguez Ruíz, Jose Laz Ruiz, Paula Sacristán, Laura Rodríguez Rusillo, Rosa Montes Lorenzo, Rosario Sánchez Martín, Verónica Ramos Mejia.

GENYO. Centre for Genomics and Oncological Research, Pfizer University of Granada, Andalusian Regional Government

Towards a more personalized medicine: Zebrafish xenografts as human cancer models for fast therapy testing in vivo

Blood cancers, like Acute Myeloid Leukemia (AML) and Non-Hodgkin Lymphomas (NHL), are still a highly mortal diseases with 5-year survival rates between only 29% and 70% in Europe and the US. Despite progress, actual one-fits-all therapies are often insufficient to cure blood cancer. Personalized, molecularly guided treatments extend patient life in 30-40% of the cases, according to a clinical study of the author. The objective of the research project "MY-PERSONAL-AVATAR" is to combine molecular tumor analysis with direct tumor therapy testing in rapid zebrafish avatars, in order to improve treatment decisions for each patient. The project will predict tumor neoantigens and highly expressed surface makers from molecular variant and expression data and use them later for targeted therapy via antibody-guided nanoparticles carrying chemotherapy drugs right to the tumor. The most effective targeted treatment for each tumor will be selected. In a first approach, in vivo cancer models have been validated. To this aim, zebrafish xenografts with patient-derived AML and NHL cell lines have been generated for in vivo testing of new therapies. I show here our results of successful repurposing of chemotherapy compounds leading to a strong reduction in tumor cell number in pediatric AML. Co-treatment of new compounds allows strong dosage reduction of an established chemotherapeutical agent, considerably lowering side effects and long-term sequelae to young patients. In a second approach, nanoparticle-based directed drug delivery systems against a tumor surface marker (CD3) successfully targeted NHL xenografts in vivo. When finally using xenografts from patient biopsies, MY-PERSONAL-AVATAR aims to improve treatment decisions, lower side effects and prolong tumor-free patient life in more patients.

V Ronco ¹, K Pavlovic ¹, C Camacho ², A Rodríguez ¹, P Heredia ¹, L Algeciras ¹, A Millán ^{3,4}, A Ballesteros ^{3,4}, H Boulaiz ², M D Carmona ^{3,4,*}, E Atilla ^{5,*} – I C Herrera ^{3,4,*} – K Benabdellah ^{1,*}

* These authors equally contribute to this work

1. Genomic Editing applied to Advanced Therapies (eGATA) Group. Pfizer-University of Granada-Junta de Andalucía Center for Genomics and Oncological Research (GENYO), Granada, Spain. 2. Department of Anatomy and Human Embryology, Faculty of Medicine, University of Granada, Granada, Spain; Biopathology and Regenerative Medicine Institute (IBIMER), Granada, Spain. 3. Cellular Therapy Group. Maimonides Biomedical Research Institute of Córdoba (IMIBIC), Córdoba, Spain. 4. Reina Sofía University Hospital, Cordoba, Spain. 5. Fred Hutchinson Cancer Research Center, Clinical Research Division, Seattle, USA.

Evaluation of second-generation CARs with CD28 and 4-1BB co-stimulatory domains targeting CLL-1 for AML therapy

Chimeric antigen receptor T cell (CAR-T) therapy has significantly advanced the treatment of blood cancers. Nonetheless, it faces substantial obstacles in treating acute myeloid leukemia (AML). Our approach aims to create an optimized CAR-T therapy that targets CLL-1. We analyzed widely used AML research cell lines, including Raji, K-562, MOLM-13, HL-60, THP-1, and U-937. Expression analysis was performed to study AML markers, encompassing CLL-1. Cell lines were transduced with a lentiviral vector carrying GFP-NanoLuc to facilitate in vitro and in vivo tracking. We used CRISPR-Cas9 to generate a negative control cell line lacking CLL-1 expression. Anti-CLL-1 CAR lentiviral vectors were produced to transduce PBMCs to generate anti-CLL-1 CAR-T cells. The efficacy of anti-CLL-1 CAR transduced T-cells was tested on the cell lines. Our preliminary results demonstrated that transduction and sorting achieved 100% expression of GFP-NanoLuc in the cell lines. Each cell line exhibited a characteristic expression profile of markers, with several significant differences, particularly in CLL-1. We successfully generated a U-937 GFP-NanoLuc CLL-1 knock-out (KO) cell line as specific negative control. Finally, we demonstrated that our CAR constructs were capable of lysing this CLL-1 expressing AML cell line. In conclusion, we achieved 100% GFP-NanoLuc expression in the cell lines for in vitro and in vivo studies. In addition, our study revealed distinct expression patterns of the studied markers in these cell lines with a particular emphasis on CLL-1. Also, we created a 100% U-937 CLL-1 knock-out cell line as a negative control. In summary, these cell lines are excellent models for mimicry of markers observed in AML patients, making them ideal for in vitro and in vivo studies. Finally, we have successfully designed and produced two anti-CLL-1 CARs with 4-1BB and CD28 co-stimulatory domains, demonstrating specific lytic capacity against CLL-1 expression in the U-937 cell line.

Virginia Pérez-Carrasco ^{1,2,3}, Ana Soriano-Lerma ^{1,3,5}, Ángel Linde-Rodríguez ^{1,2,3}, Inmaculada Ramírez-Macías ^{1,2,3}, María J Tello ^{1,4}, Miguel Soriano ^{1,4}, José Gutiérrez-Fernández ^{2,3*}, José A. García-Salcedo ^{1,2,3*}

*These authors have contributed equally: José Gutiérrez-Fernández and Jose A. García- Salcedo

1. GENYO. Centre for Genomics and Oncological Research: Pfizer, University of Granada, Andalusian Regional Government, PTS Granada-Avenida de la Ilustración, 18016 Granada, Spain.; 2. Servicio de Microbiología, Hospital Universitario Virgen de las Nieves, Granada, Spain.; 3. Instituto de Investigación Biosanitaria ibs.GRANADA, Granada, Spain.; 4. Center for Intensive Mediterranean Agrosystems and Agri-Food Biotechnology (CIAIMBITAL), University of Almeria, 04001 Almería, Spain.; 5. Department of Physiology (Faculty of Pharmacy, Cartuja University Campus), Institute of Nutrition and Food Technology “José Mataix”, University of Granada, 18071 Granada, Spain.

Deciphering the involvement of the urobiome in urinary tract infection: pathogenesis and diagnosis.

Urinary tract infection (UTI) is one of the most common bacterial infections. Its diagnosis remains challenging due to limitations of conventional uroculture methods, which may yield negative results despite the presence of clinical symptoms. This study explores the role of the urobiome—the microbial community of the urinary tract—in patients with UTI symptoms, focusing on both positive and negative

uroculture cases. Using advanced sequencing techniques, we have characterized the diversity and microbial composition of the urobiome in symptomatic individuals, identifying distinctive microbial signatures associated with UTI patients. Furthermore, we identified potential bacterial gene-based biomarkers that could enable accurate UTI diagnosis independent of uroculture results. These biomarkers demonstrated robust performance in distinguishing symptomatic UTI cases from healthy controls. Our findings provide new insights into the urobiome's role in UTI pathogenesis and offer a foundation for developing novel diagnostic tools that overcome the limitations of traditional culture-based methods improving patient care and antibiotic stewardship.

Libro de abstract – Comunicación poster

Adrián Castillo Linde ^{1,2,3}, Andrea Serrano Prados ^{1,3,4}, Lourdes López Onieva ^{1,2,3}, David Landeira Frías ^{1,3,4}

1 GENYO, 2. Departamento de Bioquímica y Biología Molecular I, Facultad de Ciencias, Universidad de Granada. 3. Instituto de Investigación Biosanitaria ibs.GRANADA. 4. Departamento de Bioquímica y Biología molecular II, Facultad de Farmacia, Universidad de Granada.

Setup of a model to study cancer epigenetic deprogramming using acute myeloid leukemia cells

Cancers represent the second leading of deaths occurring worldwide, accounting for up to 10 million deceases just in 2020. Despite these numbers though, current therapeutic approaches still rely on traditional antiproliferative strategies like radio and chemotherapy, which although effective, have their utility limited by their high toxicity. It is in this scenario that epigenetic therapy could offer an interesting alternative. Emerging evidence is revealing epigenetics plays a significant role in cancer behavior, with epigenetic regulators being important controllers behind tumor-related processes or even acting as major drivers of disease. Therefore, and although epigenetic therapy was first designed seeking cytotoxic activity, we believe it has the potential to, through changes in cancer epigenome, deprogram malignant cells and change disease evolution through disruption of tumor function. To study this possibility, we have set up a model through which we will be able to perturbate Polycomb Repressive Complex 2 (PRC2) activity and dissect the long-term effects caused in the epigenome of acute myeloid leukemia cells. Combining treatment with a specific EZH2 inhibitor, transcriptomic analyses and in vivo xenograft assays our results suggest that treatment with epigenetic drugs could induce the deprogramming of leukemia cells independently of their conventional cytotoxicity and impair disease progression.

Aguilar-González A ^{1,2,3,4}, Martos-Jamai I ^{1,2,3}, Molina-Estévez FJ ¹, Puig-Serra P ⁵, Rodríguez-Perales S ⁵, Torres R ^{5,6}, Sánchez-Martín RM ^{1,2,3}, Díaz-Mochón JJ ^{1,2,3}, Martín F ^{1,7}

1. GENYO. Centre for Genomics and Oncological Research Granada (Spain); 2. Department of Medicinal & Organic Chemistry and Excellence Research Unit of “Chemistry applied to Bio-medicine and the Environment”, University of Granada,(Spain); 3. Instituto de Investigación Biosanitaria ibs.GRANADA (Spain); 4. CRISPNA Bio S.L (Spain); 5. Centro Nacional de Investigaciones Oncológicas (CNIO), Madrid (Spain); 6. Centro de Investigación Energéticas Medioambientales y Tecnológicas (CIEMAT), Madrid (Spain); 7. Department of Biochemistry and Immunology III, University of Granada (Spain).

CRISPNA, a new tool for genome editing and diagnosis

CRISPR/Cas systems have emerged as transformative tools for scientists engaged in fundamental biological research, therapeutic development, and diagnostic applications. These systems rely on RNA molecules (*crRNAs* or *sgRNAs*) to guide Cas proteins to their intended DNA or RNA targets. While potent and specific, RNA molecules may exhibit instability in certain conditions and permit occasional mismatches during target binding. Peptide Nucleic Acids (PNAs), synthetically engineered oligonucleotides, offer superior affinity and specificity for binding complementary DNA and RNA when compared to conventional oligonucleotides. Also, their uncharged backbone endows PNAs with exceptional stability in biological fluids, as they resist degradation by proteases and nucleases. In this study, we introduce CRISPNA, a novel tool that combines the versatility of CRISPR-associated enzymes (Cas) with the robustness, stability, and specificity of PNAs. Our findings demonstrate that PNAs effectively guide different Cas proteins, such as Cas9 and Cas13, to their respective targets. Further analysis reveals that, in our initial designs, CRISPNA/Cas9's enzymatic activity is compromised, while CRISPNA/Cas13 functions optimally. Additionally, the CRISPNA/Cas13 complex exhibits sequence-specific *cis*- and *trans*-RNase activity *in vitro*. Notably, the *in-vitro* sensitivity of the CRISPNA/Cas13 system in its current configurations is 100-1000 times lower than that of the CRISPR/Cas13 counterpart. Remarkably, when electroporated, the CRISPNA/Cas13 ribonucleoprotein (RNP) demonstrates comparable efficiency to CRISPR/Cas13 in selectively degrading transcripts containing SARS-CoV-2 in HEK293T cells. In summary, we introduce here CRISPNA, a novel tool with a great potential, although PNAs designs for CRISPNA needs to be improved.

Alba Hidalgo-Ramirez ^{1,2}; Mar I. Reyes-Alcalde ^{1,2}; Giselle Xavier-Reis ^{1,2}; Whitney J Sisso ^{3,4}; Despina Siolas ⁵; Lukas E Dow ^{3,6}; Alfonso Rubio-Navarro ^{1,2,7,9}; Juan A Marchal ^{1,2,7,8,9}; Maria Paz Zafra ^{1,2,7,8,9}

1. Instituto de Investigación Biosanitaria ibs.GRANADA, University Hospitals of Granada- University of Granada, Granada, Spain 2. Centre for Biomedical Research, (CIBM), University of Granada, Granada, Spain 3. Meyer Cancer Center, Weill Cornell Medicine, New York, NY, USA 4. Weill Cornell Graduate School of Medical Science, Weill Cornell Medicine, New York, NY, USA. 5. Department of Oncology/Hematology, New York Presbyterian/Weill Cornell Medicine New York, New York, NY, USA 6. Department of Medicine, Weill Cornell Medicine, New York, NY, USA. 7. Excellence Research Unit "Modeling Nature" (MNat), University of Granada, Granada, Spain. 8. Department of Human Anatomy and Embryology, Faculty of Medicine, University of Granada, Granada, Spain. 9. BioFab i3D Lab-Biofabrication and 3D (bio)printing Singular Laboratory, University of Granada, Granada, Spain.

Phenotypic characterization of FIRINOX resistance in pancreatic organoid lines with common KRAS mutations in PDAC.

The majority of pancreatic cancer patients undergo chemotherapy regimens such as FOLFIRINOX. Regrettably, resistance mechanisms are common recurrent causes of treatment failure. Kras is an oncogene mutated in over 95% of patients with pancreatic ductal adenocarcinoma (PDAC), and mechanisms of chemoresistance have been linked to KRAS activation. Therefore, the main objective of the study was to evaluate the impact of FIRINOX resistance in PDAC in the context of different types of Kras mutations.

To achieve this goal, we have set up two types of models: 1. Simultaneous induction of resistance to 5-Fluorouracil, SN-38, and Oxaliplatin (FIRINOX) in different pancreatic tumor organoid (KPO) lines from mice carrying prevalent PDAC mutations. KPOs were previously generated by transfecting LSL-KrasMUT organoids (G12D, G12R and G12C) with a plasmid expressing Cre recombinase as well as a sgRNA targeting Tp53. Importantly, we have chosen two prevalent mutations in PDAC as KRAS-G12D (45%) and KRAS-G12R (18%), and a less common mutation as KRAS-G12C (4%). To assess the acquisition of chemoresistance (ch), cell viability were measured. 2. Orthotopic transplantation of chemoresistant KPOs (ch-KPOs) and control KPOs into the pancreas of syngeneic mice. Harvested tumors were characterized using immunohistochemistry and immunofluorescence.

We have induced chemoresistance to FIRINOX in 3 KPO-KRASG12D, 3 KPO-KRASG12R and 1 KPO-KRASG12C lines. Preliminary data has shown that ch-KPO lines tolerate higher concentrations of FIRINOX regimen. Initial tumor characterization revealed differences in the number and diameter of intraepithelial lesions between chemoresistant and non-chemoresistant tumors.

This preliminary data is the seed to continue our research strategy, that seeks to unravel the connection between specific-KRAS mutations, tumor heterogeneity and treatment resistance by performing single cell transcriptional analysis. In the near future we hope to identify new vulnerable targets to design new therapy approaches depending on the specific KRAS mutation in the context of chemoresistance.

Alba Rubio-Gayarre ^{1,2,3}, Meritxell Vinyoles ², Rita Caracuel-Peramos ¹, Narcís Fernandez-Fuentes ², Juan Ramón Tejedor ^{4,5}, María C Plaza Calonge ¹, Mercedes Guerrero-Murillo ², Ronald W Stam ⁶, Juan Carlos Rodríguez-Manzaneque ¹, Pablo Menendez ^{2,7,8,9}, Clara Bueno ^{2,7,8}, and Belén Lopez-Millan ^{1,2,3}

1. GENYO. Centre for Genomics and Oncological Research: Pfizer / University of Granada / Andalusian Regional Government, PTS Granada, Granada, Spain. 2. Josep Carreras Leukemia Research Institute-Campus Clinic, Department of Biomedicine, School of Medicine, University of Barcelona, Barcelona, Spain. 3. Department of Physiology. University of Granada. Granada, Spain. 4. Fundación para la Investigación Biosanitaria de Asturias (FINBA), Instituto de Investigación Sanitaria del Principado de Asturias (ISPA), Instituto Universitario de Oncología de Asturias (IUOPA), Hospital Universitario Central de Asturias (HUCA), Universidad de Oviedo, Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), ISCIII, Asturias, Spain. 5. Nanomaterials and Nanotechnology Research Center (CINN-CSIC), University of Oviedo, Asturias, Spain. 6. Department of Pediatric Oncology/Hematology, Erasmus MC-Sophia Children's Hospital, Rotterdam, The Netherlands. 7. Centro de Investigación Biomédica en Red de Cáncer (CIBERONC), ISIII, Barcelona, Spain. 8. RICORS-TERAV Network, ISCIII, Madrid, Spain. 9. Institutò Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain.

Innovative therapeutic strategies for B cell Acute Lymphoblastic Leukemia patients harboring KMT2A rearrangements

B-cell Acute Lymphoblastic Leukemia (B-ALL) is the most common pediatric cancer reaching overall survival rates up to 80%. However, B-ALL patients carrying KMT2A/MLL gene rearrangements (KMT2A-r B-ALL) still have dismal prognosis with overall survival below 40%. This subtype of leukemia is commonly in infants younger than 1 year who present an aggressive phenotype with therapy resistance and high relapse rates. Therefore, better understanding of the mechanisms involved in the leukemia aggressiveness is crucial for improving patients outcomes. Remarkably, Neuron-gial antigen 2 (NG2), which is barely expressed in normal hematopoietic cells, is found in around 90% of KMT2A-r B-ALL leukemic cells. Our group has provided key insights into the pathogenesis of KMT2A-r B-ALL, correlating NG2 expression with poor prognosis, CNS infiltration and showing that anti-NG2 immunotherapy strongly impairs leukemogenesis in a xenograft model of KMT2A-r B-ALL. Importantly, NG2 has also been associated with aggressiveness in solid tumors through its ability to modulate the intracellular signalling activation of integrins. However, the mechanism by which NG2 contributes in KMT2A-r leukemia aggressiveness remains unclear.

In this study, transcriptomic analysis demonstrated the correlation of NG2 with $\alpha 4$ integrin (ITGA4), which has been widely identified as a key player in relapse and poor prognosis in ALL. Furthermore, both proteins colocalize in the membrane of leukemic cells, triggering a NG2-dependent activation of the integrin.

Our in vivo models revealed a longer delay in the development of the leukemia in mice transplanted cells NG2(KO)ITGA4(KO) CRISPR-edited B-ALL cells, resulting in significantly improved survival. Taken together, our results identify NG2 and ITGA4 as promising therapeutic targets for KMT2A-r B-ALL

patients. This was further confirmed with a preclinical *in vivo* model, in which mice were treated with FDA-approved monoclonal antibody anti-ITGA4, Natalizumab, leading to higher survival rates and bringing us closer to new therapeutic strategies for these patients.

Allinson Olaechea ^{1,2} *, Pablo Espejo-Hijano ^{2*}, Cristina Camacho-Rubio ⁴, Victor Ronco ², Sara Gomez-Melero ³, Pablo Galindo-Moreno ^{1#}, Karim Benabdellah ².

* These authors equally contribute to this work

1. Oral Surgery and Implant Dentistry Department, School of Dentistry, University of Granada, Granada-Spain 2. Department of Genomic Medicine, Pfizer- University of Granada-Andalusian Regional Government Centre of Genomics and Oncological Research (GENYO), Granada- Spain. 3. Maimónides Biomedical Research Institute of Córdoba (IMIBIC), Reina Sofia University Hospital, University of Córdoba, Spain. 4. Department of human anatomy and embryology faculty of medicine (UGR), IBS Granada

Personalized Bone Healing Through Oral MSCs-Derived Exosomes Delivery of hBMP-2

Introduction

Traditional bone regeneration methods have long relied on bone substitutes and biomaterial scaffolds to facilitate the intricate process of new bone formation. This process involves a series of orchestrated events, including progenitor cell recruitment, angiogenesis, and dynamic microenvironment changes. Currently mesenchymal stem cells (MSCs), with their self-renewal and multi-differentiation potential are used in regenerative therapies. The latest research on MSCs has highlighted their ability to differentiate into adipogenic, chondrogenic, osteogenic, endothelial, neural, and epithelial lineages both in vivo and in vitro.

MSCs have been found to produce growth factors and other bioactive molecules stored in exosomes, which are small particles surrounded by a bilayer-lipid membrane. These exosomes contain specific proteins and RNA that facilitate cellular and tissue regeneration and proliferation. Isolated MSCs-exosomes have shown properties similar to their parent cells and have been successfully utilized in research settings.

This study aims to pioneer a novel bone regeneration strategy by harnessing the synergistic potential of (hMSCs genetically modified with lentiviral vectors expressing bone morphogenetic protein 2 (BMP-2), in conjunction with their therapeutic exosomes derived from hMSCs-BMP-2.

Materials and Methods

MSCs were isolated from patients requiring implants. The cells were tested by flow cytometry for positive MSCs-associated surface markers (CD73, CD90 and CD105) and negative markers (CD34 and CD45). These cells were transduced with a lentiviral vector containing BMP-2-GFP, for the overexpression of BMP-2 and GFP cells were cultured in conditioned medium (osteogenic differentiation medium) and compared with cells cultured in non-conditioned medium. RNA extraction was performed at 7-, 15-, 21-, and 28-days post-transduction, followed by reverse transcription to obtain cDNA. Quantitative PCR (qPCR) was carried out to measure the expression of BMP2, OCN, ALP, and RUNX2. Western blot analysis was performed to determine the BMP2 protein levels in different conditions. ELISA was used to determine the BMP2 protein levels in the cell culture supernatants at various time points.

Results

Flow cytometry analysis confirmed the expression of predicted markers, with CD73, CD90, and CD105 being positive and CD34 and CD45 being negative throughout the 28-day period. Additionally, differentiation into three lineages (osteogenic, adipogenic, and chondrogenic) was confirmed through staining with Alizarin Red, Oil Red, and Alcian Blue. The ELISA and Western blot assays demonstrated the overexpression of BMP2 by the transduced cells. The qPCR assays also show that MSCs-BMP-2 cells have a higher expression of osteogenesis genes (RUNX2, ALP) compared to WT cells, with this expression being even greater when the cells were cultured in conditioned medium.

Conclusions

The MSCs-BMP-2 cells obtained here and cultured in conditioned medium exhibit the highest levels of

BMP-2 protein in both cell supernatants (ELISA) and cell lysates (Western blot). The overexpression of BMP-2 enhances the expression of osteogenic genes, as evidenced by qPCR. The BMP-2 overloaded exosomes derived from these cells might serve as an appealing tool for tissue engineering and regenerative medicine, potentially making a significant impact on the successful clinical application of these exosomes for bone regeneration.

Ana Utrilla-Maestre ^{1,2,3*}, Ana M. Matia-González ^{1,2,3*}, Paola Peinado ^{1,2,3,4*},
Maria Angeles Becerra-Rodriguez ^{1,2}, Maria S. Benitez-Cantos ^{2,3,5}, José Carlos
González-Álvarez ^{1,2,5,6}, Marta Cuadros ^{2,3,5}, Pedro P. Medina ^{1,2,3#}

* These authors equally contribute to this work

1. Department of Biochemistry and Molecular Biology I, University of Granada, Granada, Spain. 2. GENYO, Centre for Genomics and Oncological Research: Pfizer/University of Granada/Andalusian Regional Government, PTS Granada, Granada, Spain. 3. Instituto de Investigación Biosanitaria de Granada (ibs.GRANADA), Granada, Spain 4. Present address: The Francis Crick Institute, London, United Kingdom. 5. Department of Biochemistry and Molecular Biology III, University of Granada, Granada, Spain. 6. Present address: Centro Nacional de Investigaciones Oncológicas, Madrid, Spain.

Backsplicing modulation by RBM10 in lung adenocarcinoma

Although RBM10 exerts a tumor suppressor role in lung adenocarcinoma (LUAD), its molecular mechanism is still unclear. Many RNA-binding proteins, such as RBM10, participate in backsplicing, a type of splicing that generates circular RNAs (circRNAs) whose abnormal expression have been associated with different types of malignancies including cancer. Therefore, we hypothesize that RBM10 could execute part of its tumor suppressor function by regulating the formation of circRNAs in LUAD. To identify circRNAs regulated by RBM10, we conducted an RNA sequencing of two isogenic LUAD lines with and without RBM10 expression and performed an in silico analysis with the circEXPLORER2 tool. By RT-qPCR, top candidates were validated in two different RBM10 restoration cell models and the correlation of their expression and RBM10 in a cohort of LUAD patients was examined. Subcellular localization was investigated by cell fractionation and the direct interaction between RBM10 and these circRNAs or their flanking regions was assessed by analyzing PAR-CLIP data and by in vitro RNA-pulldown assays. The phenotype after the modulation of circRNAs was monitored by viability and clonogenic assays in vitro and by xenograft assays in vivo. Numerous circRNAs were differentially expressed during RBM10 restoration, some of which were experimentally validated and confirmed in a cohort of LUAD patients. While most circRNAs are expressed in the cytoplasm, RBM10 is found in the nucleus pointing to a role of this protein in the biogenesis of these species. Furthermore, we observed that RBM10 directly interacts with its flanking regions. Finally, modulation of validated circRNAs phenocopied the tumor suppressor effect of RBM10 in vitro and in vivo. This work shows evidence for the role of RBM10 in circRNA biogenesis and identifies potential targets to develop therapeutic tools for LUAD patients with mutations in RBM10.

Ángel Linde-Rodríguez ^{1,2}, Inmaculada Ramírez-Macías ^{1,3}, Virginia Pérez-Carrasco ^{1,2}, Ana Soriano-Lerma ¹, Victoria Sánchez-Martín ¹, Matilde Ortiz González ¹, Miguel Soriano ^{1,4}, Jose María Navarro Marí ², José Antonio García-Salcedo ^{1,2}.

1. GENYO. Centre for Genomics and Oncological Research: Pfizer/University of Granada/Andalusian Regional Government, Granada, Spain. 2. Microbiology Unit, University Hospital Virgen de las Nieves, Granada, Spain. Instituto de Investigación Biosanitaria ibs. GRANADA 3. Department of Parasitology. Instituto de Investigación Biosanitaria ibs. GRANADA. University of Granada, Granada, Spain. 4. Center for Intensive Mediterranean Agrosystems and Agri-food Biotechnology (CIAMBITAL), University of Almeria, Almeria, Spain.

DEVELOPMENT OF A THERAPY BASED ON NANOBODY AGAINST THE HERPES SIMPLEX VIRUS.

Herpesviruses of the Simplexvirus genus (HSV-1 and HSV-2) are highly prevalent pathogens that cause lytic infections and establish latency in the nervous system, with a risk of reactivation and more serious complications such as encephalitis. To address the need for new therapies against increasing resistance to classic antivirals such as acyclovir, a neutralizing nanoantibody (NbHSV69B) was generated against a conformational epitope in the HSV-1 glycoprotein gB, identified by immunoassays. This nanoantibody demonstrated statistically significant antiviral activity against acyclovir at 2.5 μ M. In order to treat central nervous system infections, a variant of the nanoantibody was designed by fusing a transferrin receptor ligand (NbHSV69B-TfRL) to facilitate its passage through the blood-brain barrier by transcytosis. NbHSV69B-TfRL showed statistically significant antiviral activity against acyclovir and NbHSV69B at 2.5 and 1.25 μ M. Immunofluorescence assays confirmed recognition of conserved epitopes on the membrane of Vero cells infected with HSV-1 and HSV-2. These results suggest that NbHSV69B and NbHSV69B-TfRL nanoantibodies are promising candidates for the development of targeted therapies against HSV infections, including those of the central nervous system.

C Blanco-Benitez ^{1 2}, P Fernandez-Coca ¹, P Garcia-Tirado ¹, P Justicia-Lirio ², M Tristan-Manzano ³, J Martin-Campos ², V Ayllon ^{1 2}, F Martin ^{2 4}

1. Department of Cellular Biology, Faculty of Science, University of Granada, Granada, Spain 2. Department of Genomic Medicine, Pfizer-University of Granada-Andalusian Regional Government Centre for Genomics and Oncological Research (GENYO), PTS, Granada, Spain 3. LentiStem Biotech 4. Department of Biochemistry and Molecular Biology III and Immunology, Faculty of Medicine, University of Granada, Granada, Spain

Development of activation-inducible promoters for the improvement of CAR-T therapy

Tumor immunotherapy aims to enhance the immune system to target and destroy tumor cells effectively. Among its most successful approaches is CAR-T cell therapy, where T cells are genetically engineered to express Chimeric Antigen Receptors (CARs) targeting specific antigens like CD19. Despite its success in treating refractory B-cell malignancies, CAR-T therapy faces challenges due to severe side effects such as Cytokine Release Syndrome and neurotoxicity caused by uncontrolled T cell activation. Improving safety requires precise control of transgene expression, turning it on and off at optimal times. To address this, the study focuses on developing inducible promoters for CAR-T cells that regulate the expression of bioactive molecules like IL12 or IL18 only after T cell activation. Researchers designed three chimeric promoters incorporating transcription factor binding sites linked to T cell activation. These promoters, inserted into lentiviral backbones expressing GFP as a reporter, were tested in primary human T cells activated via CD3/CD28. Results showed that GFP expression peaked at 8 hours post-activation, decreased by 24 hours, increased again until 72 hours, and subsequently declined. While these promoters achieved a fivefold increase in GFP expression upon activation, they also exhibited high basal activity in the absence of activation, limiting their precision. To address this, a second generation of the most promising promoter was developed. Enhancer sequences were removed, and repressor sequences from genes involved in CAR-T cell activation, identified through RNA sequencing, were added. This modification aims to reduce basal expression while maintaining inducibility, potentially improving the safety and efficacy of CAR-T therapy.

Celia Jiménez, Rosa M. Montes, Cristina Requena, Laura Rodríguez, Jose Manuel Sánchez, Gonzalo Zarco, Faustino Palomo, María del Carmen Noguera, Pedro Real, Verónica Ramos

Centro Pfizer-Universidad de Granada-Junta de Andalucía de Genómica e Investigación Oncológica

Carcinogenesis model in human stem cells: The effect of NUP98-KDM5A on embryonic hematopoiesis

During embryonic development, hematopoiesis is an essential process tightly regulated by various signalling pathways and transcription factors. The disruption of such mechanisms can produce an accumulation of undifferentiated and non-functional blood cells, which leads to leukemia. In pediatric cases, driver mutations often occur during embryonic development, frequently resulting from chromosomal translocations that produce oncogenic fusion proteins. These proteins typically involve dysfunctional genes related to cell differentiation or proliferation.

One example is the NUP98-KDM5A (NK5A) fusion oncoprotein, which is found exclusively in pediatric acute myeloid leukemia cases with poor prognosis. Developing new treatments and therapies to improve patient outcome relies on understanding the disease and its underlying mechanisms. In this project, we used a human embryonic stem cell line engineered to express NK5A under induction, serving as a model for leukemogenesis.

This model was used to study the impact of NUP98-KDM5A on various cell populations during the differentiation from hESCs to early hematopoietic progenitors. We found that NK5A causes a block in differentiation and alters the expression of genes associated with cell differentiation and proliferation, particularly in hematoendothelial cells and early hematopoietic progenitors. These findings enhance our understanding of the cellular origins of this leukemia subtype and the mechanisms driving the disease.

Cerón-Hernández, J ^{1,2*}; García, A ^{1,3*}; Denningoff, V ^{1,4}; Molina, MP¹; Martínez-Navajas, G ^{1,2}; Aznar-Peralta, I ¹; Simón-Sáez, I ⁵; Giovannetti, E ^{6,7}; Javier Lopez-Hidalgo ⁸; Fresno, C ^{9*}; Real, P ^{1,2,3#}; Serrano, MJ ^{1,3,10#}

* These authors equally contribute to this work.

1. Centro Pfizer-Universidad de Granada-Junta de Andalucía de Genómica e Investigación Oncológica (GENYO), Granada, Spain. 2. University of Granada, Department of Biochemistry and Molecular I, Granada, Spain 3. Instituto de Investigación Biosanitaria ibs. GRANADA, Granada, Spain 4. Molecular Clinical Lab, University of Buenos Aires (UBA) - National Council for Scientific and Technical Research (CONICET), Buenos Aires, Argentina. 5. Mayo Clinic, Rochester, Minnesota, USA 6. Department of Medical Oncology, Cancer Center Amsterdam, Amsterdam UMC, VU University, Amsterdam, The Netherlands. 7. Cancer Pharmacology Lab, Fondazione Pisana Per La Scienza, San Giuliano, Pisa, Italy. 8. Unit of Pathological Anatomy. University Hospital San Cecilio. 18016, Granada, Spain. 9. Health Sciences Research Center (CICSA), Anáhuac University México, México. 10. Integral Oncology Division, Virgen de las Nieves University Hospital, Av. Dr. Olóriz 16, 18012, Granada, Spain.

Platelet Direct Interactions Drive Cisplatin Resistance in Non-Small Cell Lung Cancer

Background: Platelets are pivotal in the progression, spread, and metastasis of non-small cell lung cancer (NSCLC). Recent studies indicate that a complex, bidirectional interaction exists between tumor cells and platelets, characterized by the exchange of biomolecules. This research aimed to explore the influence of platelet-tumor interactions on the development of cisplatin resistance in NSCLC. Methods: A series of functional assays were performed using cocultures of NSCLC cell lines and platelets, followed by treatment with cisplatin. Subsequently, transcriptomic profiling was conducted on these cells, along with functional enrichment analysis, to identify the molecular pathways involved in platelet-mediated chemoresistance.

Results: NSCLC cells cocultured with platelets and treated with cisplatin showed a marked decrease in apoptosis and an increase in proliferation compared to control groups. Tumor cells exposed to platelets exhibited enhanced clonogenic potential. Transcriptomic analysis revealed significant alterations in gene expression, particularly the upregulation of genes associated with the PI3K-AKT, NF-kappa B, and apoptosis-related pathways, indicating their role in fostering a chemoresistant phenotype. Conclusions: This study offers new insights into the behavioral and transcriptomic changes induced by

platelet interactions in the context of cisplatin treatment. It elucidates the mechanisms underlying platelet-mediated chemoresistance and positions platelets as potential targets for therapeutic intervention.

Charisios Triantafyllou ¹, Julius Lindblom ², Lorenzo Beretta ³, PRECISESADS Clinical Consortium ¹, Ioannis Parodis ^{2,4}, Marta Alarcón-Riquelme ^{1,5}, Guillermo Barturen Briñas ¹

1. Department of Medical Genomics, GENyO, Center for Genomics and Oncological Research Pfizer/University of Granada, Granada, Spain 2. Division of Rheumatology, Department of Medicine Solna, Karolinska Institutet and Karolinska University Hospital, Stockholm, Sweden 3. Scleroderma Unit, Referral Center for Systemic Autoimmune Diseases, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico di Milano, Milan, Italy 4. Department of Rheumatology, Faculty of Medicine and Health, Örebro University, Örebro, Sweden 5. Institute for Environmental Medicine, Karolinska Institutet, Stockholm, Sweden.

TRANSCRIPTOMIC ANALYSIS REVEALS A SYNERGY OF HYDROXYCHLOROQUINE AND GLUCOCORTICOIDS IN MODULATING B CELL-RELATED IMMUNE PROCESSES

Background/Purpose: The combination of antimalarial drugs (e.g., hydroxychloroquine) and corticosteroids (e.g., prednisone) is a common treatment for autoimmune diseases, due to their anti-inflammatory effects. While believed to have synergistic benefits, it remains unclear whether this synergy exists at the molecular level. The aim of this study is to investigate whether molecular synergy exists between hydroxychloroquine and corticosteroids, to identify which drug drives the amplified therapeutic effect, and to underline specific gene sets that are involved in this interaction. *Methods:* We analyzed bulk RNA sequencing data from 582 samples from the PRECISESADS project. The patients were diagnosed with systemic lupus erythematosus (SLE), primary Sjögren's disease (pSjD), undifferentiated connective tissue disease (UCTD), and mixed connective tissue disease (MCTD), and were grouped into four categories based on treatment status: no current exposure to HCQ or GC or any immunosuppressive treatment (off-treatment; n = 244), on HCQ (n = 198), on GC (n = 45), or on HCQ and GC combined (n = 95). We performed differential gene expression (DGE) analysis across treatment groups (HCQ, GC, and HCQ+GC) with the off-treatment group of patients serving as the comparator. Meta-analysis and functional analysis followed.

Results: Combination of HCQ and GC generated a synergistic molecular response, with a higher number of differentially expressed genes and greater effect size compared to the individual treatments. Functional analysis pointed to numerous immune-related pathways. Notably, B cell-related gene proliferation and activity modules were significantly suppressed in the group of patients on combination treatment.

Conclusions: The combination of HCQ and GC results in a synergistic molecular response. Functional analysis revealed significant involvement of immune-related pathways, with a notable suppression of B cell-related gene modules. Overall, these findings suggest a synergy at the molecular level when HCQ and GC are administered concurrently in combined regimens, enhancing the modulation of key immune processes.

Diaz-Ruano AB ¹, Gomez-Jimenez E ¹, Marchal JA ^{1,2,3}, Picon-Ruiz M ^{1,2,3}

1. Biopathology and Medicine Regenerative Institute (IBIMER), University of Granada, Spain 2. Biopathology and Regenerative Medicine Institute (IBIMER), Centre for Biomedical Research (CIBM), University of Granada, Granada, Spain; Biosanitary Research Institute of Granada (ibs.GRANADA), University Hospitals of Granada-University of Granada, Granada, Spain; 3. Department of Human Anatomy and Embryology, Faculty of Medicine, University of Granada, Granada, Spain; Excellence Research Unit "Modeling Nature" (MNat), University of Granada, Granada, Spain.

Increased intracellular levels of estrone by HSD17B14 overexpression induces inflammation, CSC enrichment and migration in ER + breast cancer cells

Breast cancer is the most frequently diagnosed malignancy and the leading cause of cancer-related mortality among women worldwide. Estrogen receptor-positive (ER+) breast cancer represents the most common subtype, largely influenced by estrogens and their biosynthetic pathways. Among the enzymes involved, the 17 β -hydroxysteroid dehydrogenase (HSD17B) family plays a pivotal role, although its precise contributions to breast cancer progression remain unclear. This study investigates the impact of HSD17B14 overexpression on inflammation, epithelial-mesenchymal transition (EMT), and cancer stem cell (CSC) properties in ER+ breast cancer cells. Using MCF7 cells, we achieved successful transfection to overexpress HSD17B14, reaching expression levels approximately 200 times higher than control cells. Overexpression resulted in a marked increase in the expression of pro-inflammatory cytokines, suggesting a pro-tumorigenic role of HSD17B14 in the tumor microenvironment. Furthermore, migration capacity was significantly enhanced, accompanied by upregulation of key EMT markers, such as Vimentin, ZEB1, TWIST, ZO-1, and DCLN1. These changes indicate that HSD17B14 contributes to cellular plasticity and metastatic potential. In addition to promoting inflammation and EMT, HSD17B14 overexpression enriched the CSC population. Functional assays, including mammosphere formation and wound healing tests, demonstrated an increase in self-renewal capacity and cellular migration. These findings highlight the multifaceted role of HSD17B14 in driving inflammation, CSC enrichment, and metastatic behavior in ER+ breast cancer cells.

This research provides novel insights into the molecular mechanisms linking HSD17B14 with breast cancer progression, emphasizing its role in promoting inflammation and aggressive tumor phenotypes. Targeting HSD17B14 or its associated signaling pathways could represent a promising therapeutic approach for managing ER+ breast cancer, particularly in cases driven by inflammation and EMT. These findings pave the way for further studies to explore HSD17B14 as a potential biomarker and therapeutic target in breast cancer treatment strategies.

Diaz-Ruano AB ¹, Martinez-Alarcon N ¹, Gomez-Jimenez E ¹, García Martínez MA ², Preda O ², Ramirez-Tortosa C ², Marchal JA ^{1,3,4}, Picon-Ruiz M ^{1,3,4}

1. Biopathology and Medicine Regenerative Institute (IBIMER), University of Granada, Spain 2. Department of Pathological Anatomy, University Hospital San Cecilio, Granada, Spain 3. Biopathology and Regenerative Medicine Institute (IBIMER), Centre for Biomedical Research (CIBM), University of Granada, Granada, Spain; Biosanitary Research Institute of Granada (ibs.GRANADA), University Hospitals of Granada-University of Granada, Granada, Spain; 4. Department of Human Anatomy and Embryology, Faculty of Medicine, University of Granada, Granada, Spain; Excellence Research Unit "Modeling Nature" (MNat), University of Granada, Granada, Spain.

Estradiol and estrone have different biological functions to induce NF- κ B-driven inflammation, EMT and stemness in ER+ cancer cells

This study explores the distinct biological roles of estradiol (E2) and estrone (E1) in regulating NF- κ B-driven inflammation, epithelial-mesenchymal transition (EMT), and stemness in estrogen receptor-positive (ER+) cancer cells. NF- κ B activation, a key process in inflammation and tumorigenesis, is analyzed in the context of obesity, a significant risk factor for breast cancer. E2, the dominant estrogen in premenopausal women, demonstrates a protective effect against NF- κ B activation and inflammation. Conversely, E1, the predominant estrogen in postmenopausal women, collaborates with TNF- α to promote NF- κ B activation, exacerbating inflammation, tumorigenesis, EMT, and cancer stem cell characteristics.

The study employed HeLa pER α cells to assess the impact of prolonged exposure to TNF- α , either alone or in combination with E1 or E2. Biomarker analysis revealed that E1 and E2 exert opposing influences on inflammation, EMT regulation, and stemness. These findings underscore the distinct biological functions of these estrogens in obesity-driven breast cancer mechanisms, highlighting the pro-tumorigenic role of E1 in postmenopausal women. Significantly, this research provides a foundation for developing new therapeutic approaches. By targeting E1 or its biosynthesis, clinicians could design treatments to reduce the risk of ER+ breast cancer, especially in obese postmenopausal women. Furthermore, the study supports the potential of E1-reduced hormone replacement therapies as preventive measures to lower breast cancer incidence in this vulnerable population.

The findings advance the understanding of estrogen-specific roles in cancer biology and offer actionable insights for improving treatment outcomes for ER+ breast cancer patients. By identifying E1 as a key driver of inflammation and tumorigenesis, this research opens avenues for more precise and effective therapies against breast cancer.

Fabián Vergara Rubio ^{1,2,3}; **Bernardino Alcázar Navarrete** ^{3,4}; **Francisco Gabriel Ortega** ^{1,2,4}; **Teresa Valero** ^{1,2,3}

1. Pfizer University of Granada Andalusian Genomics and Oncology Research Center (GENYO), Liquid Biopsy and Cancer Interception Group, PTS Granada. 18016 Granada, Spain. 2. Instituto de Investigación Biosanitaria ibs.GRANADA, Granada, Spain. 3. University of Granada, Campus de Cartuja s/n, 18071, Granada, Spain. 4. Pneumology Unit, Hospital Universitario Virgen de las Nieves, Granada, Spain.

A Novel Approach for Assessing T Cell-Mediated Immunity by Using Extracellular Vesicles

Introduction: Cellular immunity mediated by T cells plays a crucial role in infectious, autoimmune, oncological, and degenerative processes. However, its analysis is not applied due the complexity of conventional methods (ELISPOT, FLUOROSPOT, or Flow-Cytometry kits), which require fresh blood, cell culture, specialized equipment, and highly trained personnel.

Objectives: This study aims to assess the feasibility of utilizing the specific binding of a peptide to its T cell receptor for the selective isolation of extracellular vesicles (EVs) to evaluate specific cellular responses.

Methodology: Plasma samples were collected from three distinct cohorts: 1: Plasma from 5 donors obtained in 2019. 2: Plasma from 5 donors less than a month after recovering from the disease (COVID-19). 3: Plasma from 5 donors vaccinated against COVID-19. Magnetic beads were functionalized with a pool of peptides from the spike protein subunit 2 of the virus. EV-capture was performed by direct incubation of EVs isolated from plasma and functionalized beads. EV-quantification was carried out using a colorimetric ELISA method, with CD81 as the quantified protein. Specific binding of EVs to the antigenic peptide was confirmed through TEM and competitive tests. Finally, results were compared across the three cohorts.

Results: The outcomes demonstrated successful functionalization of magnetic beads, with specific binding of EVs to functionalized beads confirmed through SEM and competitive tests. The quantification of captured EVs was significantly higher in samples from patients who had been in contact with the virus.

Conclusions: This innovative approach to assess T cell-mediated immunity could enhance speed, efficiency, and versatility compared to conventional methods. Its simplicity and versatility make it a valuable tool for investigating specific immune responses in diverse clinical contexts.

Fernando Montenegro-Elvira^{1,2}, Sergio Castro-González^{1,2}, Pedro P. Medina^{1,2}

1. University of Granada, Department of Biochemistry and Molecular Biology I, Granada, Spain; 2. Centre for Genomics and Oncological Research (GENYO), Granada, Spain

SWI/SNF as a therapeutic target in B-Acute Lymphoblastic Leukemia

The mammalian SWI/SNF complex, a chromatin remodeler composed of 10-15 subunits, includes SMARCA2 or SMARCA4 as its catalytic core. Alterations in SWI/SNF subunits are common in hematological malignancies and, depending on the disease context, these subunits can also contribute to tumor maintenance or exhibit oncogenic properties. Recent advances in dual SMARCA2/SMARCA4 inhibitors, including classic inhibitors and PROTACs, have underscored the potential of targeting the SWI/SNF complex as a therapeutic strategy in cancer. Promising results have been reported in acute myeloid leukemia (AML) and T-cell acute lymphoblastic leukemia (T-ALL), but the potential impact of SWI/SNF inhibition in B-cell acute lymphoblastic leukemia (B-ALL) remains unexplored. In this study, we evaluated the effects of a dual SMARCA2/SMARCA4 ATPase inhibitor (BRM014) and a SMARCA2/SMARCA4 PROTAC degrader (ACBI1) on a panel of leukemia cell lines. Our findings demonstrate that B-ALL cells are sensitive to SWI/SNF inhibition, with sensitivity comparable to that observed in AML and T-ALL. Mechanistic investigations revealed that SWI/SNF inhibition in B-ALL cells leads to reduced proliferation, G1 cell cycle arrest, and increased apoptosis. These results highlight the therapeutic potential of SWI/SNF complex inhibitors in B-ALL, paving the way for their application in this hematologic malignancy.

Gonzalo Gómez Hernández ¹, Daniel Toro ¹, Georgina Galicia ¹, María Botía ¹, María Morell ¹ and Marta E. Alarcón-Riquelme ¹

1. Genetics and Genomics of Immune-Mediated Diseases, GENYO, Granada, 18016, Spain

Bank1 modulates the differentiation and molecular profile of key B cell populations in autoimmunity

This study aimed at defining the role of the B cell adaptor protein BANK1 in the appearance of age-associated B cells (ABCs) in 2 SLE mouse models (TLR7.tg6 and imiquimod-induced mice), crossed with Bank1^{-/-} mice. The absence of Bank1 led to a significant reduction in ABC levels, also affecting other B cell populations. To gain deeper insights into their differentiation pathway and the effect of Bank1 on B cell populations, a single-cell transcriptome assay was performed. In the TLR7.tg6 model, we identified 10 clusters within B cells, including an ABC-specific cluster that was decreased in Bank1-deficient mice. In its absence, ABCs exhibited an antiinflammatory gene expression profile, while being proinflammatory in Bank1-sufficient lupus-prone mice. Trajectory analyses revealed that ABCs originated from marginal zone and memory-like B cells, ultimately acquiring transcriptional characteristics associated with atypical memory cells and long-lived plasma cells. Also, Bank1 deficiency normalized the presence of naive B cells, which were nearly absent in lupus-prone mice. Interestingly, Bank1 deficiency significantly reduced a distinct cluster containing IFN-responsive genes. These findings underscore the critical role of Bank1 in ABC development, affecting early B cell stages toward ABC differentiation, and the presence of IFN-stimulated gene-containing B cells, both populations determinant for autoimmunity.

Inés Aznar-Peralta ¹, Amparo Roa-Colomo ², M. Pilar Molina-Vallejo ¹, M. José Serrano ^{1,3}

1. Liquid Biopsy and Cancer Interception Group, GENYO Centre for Genomics and Oncological Research, Pfizer - University of Granada - Andalusian Regional Government, Granada, Spain 2.

Gastroenterology and Hepatology Department, San Cecilio University Hospital, Granada, Spain 3. Molecular Pathology Lab. Intercenter Anatomical Pathology Unit, San Cecilio and Virgen de las Nieves University Hospital, Granada, Spain

Circulating free DNA Profile in Cirrhosis and Hepatocellular Carcinoma: Diagnostic and Prognostic Value

Background and aims:

Hepatocellular carcinoma (HCC) remains a substantial global health burden, with early detection being a critical factor for patient outcomes. Despite established screening protocols for high-risk populations, particularly those with liver cirrhosis (LC), current detection methods lack sensitivity, leading to delayed diagnosis and limited treatment options. This study aimed to evaluate the potential of circulating cell-free DNA (cfDNA) analysis as a novel approach for HCC detection and prognosis.

Approach and results:

We analyzed cfDNA concentration, fragments patterns, and methylation status in plasma samples from 39 patients with HCC and 46 patients with LC. The concentration of cfDNA was significantly higher in HCC than in LC patients and was associated with advanced HCC stage, lymphovascular invasion, and several liver function markers. Fragments of cfDNA were longer in patients with HCC than in those with LC. A multivariate model (CSAC) incorporating cfDNA characteristics, alpha-fetoprotein (AFP), and C-reactive protein (CRP) levels was developed. The CSAC model demonstrated high diagnostic performance (AUC 0.949) for differentiating HCC from LC, outperforming individual markers. Moreover, a higher CSAC index was correlated with shorter overall survival (OS) in the HCC cohort. In patients with advanced-stage HCC, cfDNA concentration was the only independent prognostic factor for OS.

Conclusions:

The integration of cfDNA analysis with established clinical markers in the CSAC model demonstrates significant potential as a powerful tool for the early detection of HCC in patients with LC. This approach could significantly enhance screening accuracy and consequently improve patient outcomes. Further validation in larger, multi-center studies is warranted to confirm these findings and optimize the model for clinical implementation.

Iria Alonso-Alves ^{1,2}, Belén Rubio-Ruiz ^{1,2}

1. Department of Medicinal & Organic Chemistry, Faculty of Pharmacy, University of Granada, Campus Cartuja s/n, 18071, Granada, Spain, 2. GENYO, Centre for Genomics and Oncological Research, Pfizer/University of Granada/Andalusian Regional Government, PTS Granada, Avda. Ilustración 114, 18016, Granada, Spain.

Fluorescent isoquinolium-based derivatives as ChoK inhibitors: design, synthesis, antiproliferative effects and functionalization into Pd-loaded nanospheres.

One of the most promising approaches to address cancer heterogeneity is combination therapy, which is based on the use of simultaneous strategies to achieve a synergistic effect. Among the different possibilities to be explored, the use of photothermal therapy together with cancer chemotherapy is attracting considerable attention due to the advantages of combining physical and biochemical targeting.

Choline kinase (ChoK) catalyzes the first step of phosphatidylcholine biosynthesis by converting choline to phosphocholine. In cancer, overexpression of ChoK can drive abnormal lipid metabolism, making it a validated drug target for cancer therapy. Although numerous ChoK inhibitors have been developed, the synthesis of fluorescent ChoK inhibitors to track their uptake and distribution remains largely unexplored.

Our research is focused on the development of a Pd-functionalized nanosystem capable of specifically delivering a fluorescent ChoK inhibitor into cancer cells and mediating photothermal ablation under near-infrared irradiation.

Firstly, we have performed the synthesis and biological evaluation of a library of isoquinolinium-based fluorescent ChoK inhibitors featuring electron-donating and electron-withdrawing groups at different positions on the isoquinolinium ring, as well as alkyl or aryl chains attached to the nitrogen of the heterocycle. All the synthesised molecules include a ketone functional group for their subsequent conjugation into Pd-functionalised polymeric nanospheres through a pH-sensitive hydrazone bond. Preliminary results demonstrate a high fluorescence capability of the structures at different wavelengths and a moderate antiproliferative activity against ChoK-overexpressed cancer cell lines.

Iván Fernández Rengel ^{1,2,3}, Mencía Espinosa Martínez ^{1,2,3}, María Alcázar Fabra ^{1,2,3}, David Landeira ^{1,2,3}

1. Centre for Genomics and Oncological Research (GENYO), Avenue de la Ilustración 114, 18016 Granada, Spain. 2. Department of Biochemistry and Molecular Biology II, Faculty of Pharmacy, University of Granada, Granada, Spain. 3. Instituto de Investigación Biosanitaria ibs.GRANADA, Hospital Virgen de las Nieves, Granada, Spain

Epigenetic silencing of transposable elements in mouse embryonic stem cells

Embryonic stem cells are characterized by a relatively relaxed chromatin state, which is regulated by several cellular mechanisms. This less-restricted chromatin configuration enables stem cells to activate distinct sets of genes in response to differentiation signals during early embryonic development. Transposable elements (TEs) take advantage of this chromatin state to induce their expression, which is crucial at specific stages of early embryonic development. However, the widespread activation of TEs can be detrimental to the cell, leading to replication stress, cellular senescence, or promoting diseases such as cancer. Several epigenetic mechanisms exist to silence the expression of these elements. In this study, we have demonstrated for the first time the involvement of specific epigenetic mechanisms in the silencing of TEs in embryonic stem cells.

L. Algeciras-Jiménez^{1*}, M. Ortiz-Bueno^{1*}, V. Ronco-Díaz¹, P. Heredia¹, A. Millan^{2,3}, A. Ballesteros^{2,3}, K. Pavlovic^{1,2}, J. Rädler⁴, S. El Andaloussi⁴, MD. Carmona^{2,3}, E. Attila⁵, D. Gupta^{4#}, IC. Herrera^{3#} and K. Benabdellah^{1#}

*,#: Contributed equally

1. Department of Genomic Medicine, Pfizer-University of Granada-Andalusian Regional Government Centre for Genomics and Oncological Research (GENYO), Granada, Spain; 2. Maimonides Institute of Biomedical Research in Cordoba (IMIBIC), Cell Therapy, 14004, Córdoba, Spain; 3. Reina Sofía University Hospital, Córdoba, Spain.; 4. Department of Laboratory Medicine, Karolinska Institute, Stockholm, Sweden.; 5. Fred Hutchinson Cancer Research Centre, Clinical Research Division, Fairview Ave N, Seattle, WA 98109, USA.

Enhancing CAR-T Therapy Through Engineered EVs Targetting AML-Specific Antigens

Myeloid neoplasms, including myelodysplastic syndromes (MDS), myeloproliferative neoplasms (MPN), and acute myeloid leukaemia (AML), are clonal diseases of hematopoietic stem cells (HSCs), with AML being the most common acuteleukaemia in adults and characterized by aggressive behaviour and low overall survival rates. Despite advances in immunotherapy for haematological malignancies, CAR-T cell therapies in AML have shown limited success compared to lymphoid neoplasms due to several challenges: lack of leukaemia-specific antigens, which increases off-tumour toxicity; the immunosuppressive tumour microenvironment characterized by factors like adenosine that inhibit CAR-T cellfunction; and manufacturing difficulties, as AML cells can hinder T cell production and patients often have compromised T cell quality due to prior chemotherapy. To address the limitations associated with patient cell features and standardize a more cost-effective CAR-T production, we propose an alternative combinatorial therapeutic strategy. Our approach involves utilizing our gene editing expertise to produce allogenic off-the-shelf anti-CLL1 CAR-T cells combined with extracellular vesicles (EVs) as carriers for an immunosuppressive gene-silencing shRNA payload. The core of our

project focuses on precision targeting using CRISPR/Cas9 to simultaneously eliminate the PD-1 and TRACgenes in CAR-T cells, which target the C-type lectin-like molecule-1 (CLL-1). This strategy aims to avoid triggering harmful and potentially toxic reaction: graft-versus-host (GvH). The resulting off-the-shelf anti-CLL1 CAR-T cells will be codelivered with EVs targeting CD123, loaded with shRNA against CD73, an enzyme associated with adenosine production and immunosuppression. In this presentation, we outline the strategy we have followed to generate EVs with affinity toward CD123-positive leukemic cells.

Lea Talpašová ¹, Katarína Bhide ¹, Jakub Víglaský ¹, Jana Jozefiaková ¹, Monika Slaviková ³, Amod Kulkarni ^{1,2}, Ján Čurlík ⁴, Katarína Kucková ¹, Tomáš Maľarik ¹, José Antonio García Salcedo ⁵, Mangesh Bhide ^{1,2}

1. Laboratory of Biomedical Microbiology and Immunology, The University of Veterinary Medicine and Pharmacy, Košice, Slovakia 2. Institute of Neuroimmunology, Slovak Academy of Sciences, Bratislava, Slovakia 3. Biomedical Research Center, Institute of Virology, Slovak Academy of Sciences, Bratislava, Slovakia 4. Department of Breeding and Diseases of Game, Fish and Bees, Ecology and Cynology, The University of Veterinary Medicine and Pharmacy, Košice, Slovakia 5. GENyO. Pfizer-University of Granada-Junta de Andalucía Center for Genomics and Oncological Research

Development of CDR3-Fc fusion proteins against SARS-CoV-2 brain infections

Drug delivery across the blood-brain barrier (BBB) is difficult and challenging. Conventional antibodies are ineffective due to their size (150 kDa), which limits their biodistribution in the central nervous system (CNS). Nanobodies derived from the Camelid variable domain of heavy chain only antibodies offer a promising alternative thanks to their small size (15 kDa). Above all, their complementarity-determining region 3 (CDR3; ~5 kDa) plays a dominant role in binding nanobodies to antigens. The goal was to produce CDR3 capable of binding the SARS-CoV-2, fused with the Fc domain of human IgG1 (to increase stability) and angiopep-2 (to increase translocation of the fusion protein across the BBB). Llama glama was immunized with recombinant protein S of SARS-CoV-2, blood was collected, RNA was isolated and reverse transcribed to cDNA. To create a CDR3-E. coli library, CDR3 was amplified with degenerated primers, cloned into pSEX81-Fc-angiopep-2 phagemid, and transformed into E. coli XL1-Blue. Library was superinfected with M13 K07ΔpIII Hyper Phage and two rounds of panning on inactivated SARS-CoV-2 were performed. Gene coding CDR3-Fc was amplified from eluted phages, cloned into Pichia pastoris and soluble proteins were produced. A total of 96 clones were screened for their ability to bind protein S and 15 were shortlisted for ability to neutralize pseudovirus (Delta variant). DNA from four clones neutralizing ≥80 % was sequenced and electroporated into Pichia pastoris and proteins were produced. These proteins were tested for cytotoxicity, neurotoxicity and binding affinity to inactivated SARS-CoV-2 (Omicron variant). Clones 34, 43 and 2B3 with non cytotoxic / neurotoxic effect at concentration of 1 μg showed affinity to SARS-CoV-2 (A450nm > 1). These clones are being studied for their EC50 against live virus and ability to cross the BBB in vitro. Research was funded from APVV-22-0084, VEGA1/0381/23, VEGA1/0348/22, EURONANOMED2021-105.

Lucía Chica-Redecillas ^{1,2}, Juan Miguel Guerrero-González ², Sergio Cuenca López ¹, Elena Arance ², Fernando Marín-Benesiu ^{1,2}, Carmen María Molares-Álvarez ^{1,2}, María Jesús Álvarez-Cubero ^{1,2,3}, Luis Javier Martínez-González ^{1,2}

1. Faculty of Medicine - University of Granada, Department of Biochemistry and Molecular Biology III and Immunology, Faculty of Medicine, Granada, Spain.; 2. Centre for Genomics and Oncological Research: Pfizer, University of Granada, Andalusian Regional Government (GENYO), Granada, Spain.; 3. Biosanitary Research Institute (ibs. GRANADA), University of Granada, Spain

Potential Biomarkers in Familial Prostate Cancer Revealed by miRNA Analysis

"Background/Objectives. About 5-10% of prostate cancer (PC) cases have a hereditary component. Men with a family history face a twofold increased risk, with a tendency for more aggressive cancer. Current guidelines for early diagnosis and patient stratification are inadequate. Present study aims to identify potential miRNA biomarkers for the detection and stratification of high-risk familial PC.

Methods. We analysed miRNA expression in two PC multicase families. We used the edgeR package to study the transcriptome and only considered results with FDR<0.05 as significant. To ensure the validity of the results, we compared them with The Cancer Genome Atlas (TCGA) database.

Results. Eight miRNAs significantly expressed in hereditary PC patients were identified and validated by TCGA: hsa-miR-205-5p, hsa-miR-197-3p, hsa-miR-144-5p, hsa-miR-423-3p, hsa-miR-101-3p, hsa-miR-501-5p, hsa-miR-671-3p and hsa-miR-744-5p. Enrichment analysis revealed the involvement of target genes of identified miRNAs (APP, SOX9, ZEB2, NLK, FOXO3, LRP1, LRRK2, SRC, SMAD3 and TGFB1) in crucial pathways related to cancer development, highlighting the Wnt pathway. Construction of miRNA-mRNA network also revealed a high interconnection among identified miRNAs with genes involved in the Wnt pathway.

Conclusion. miRNAs have the potential to be significant cancer biomarkers. Specifically, hsa-miR-205-5p, hsa-miR-101-3p and hsa-miR-144-5p play important roles in crucial pathways in PC. Our study underscores the importance of the Wnt pathway in the development of hereditary PC. These findings are consistent with our previous research on this disease.

M Cortijo-Gutiérrez ¹, N Maldonado-Pérez ¹, M Tristán-Manzano ², K Pavlovic-Pavlovic ¹, P Justicia-Lirio ², Manel Juan ⁶, María Castella ⁶, RO Bak ⁸, IC Herrera ^{3,4}, K Benabdellah ^{1#}, F Martín ^{1,5,7#}

1. Department of Genomic Medicine, Pfizer-University of Granada-Andalusian Regional Government Centre for Genomics and Oncological Research (GENYO), Granada, 18016, Spain; 2. LentiStem Biotech. Pfizer-University of Granada-Andalusian Regional Government Centre for Genomics and Oncological Research (GENYO), Granada, 18016, Spain; 3. GC1 4Cell Therapy, IMIBIC. University of Cordoba, Reina Sofia University Hospital, Cordoba, 14004, Spain.; 4. Hematology Unit, Reina Sofia Hospital, Córdoba, 14004, Spain.; 5. Departamento de Bioquímica y Biología Molecular III e Inmunología, Facultad de Medicina, Universidad de Granada, Av. de la Investigación 11, 18007 Granada, Spain; 6. Hospital Clinic, Barcelona, 08036, Spain.; 7. Instituto de Investigación Biosanitario de Granada (ibs.GRANADA). Granada 19400, Spain; 8. Department of Biomedicine - Forskning og uddannelse, Skou-bygningen, 8000 Aarhus C, Denmark

Enhancing CAR-T Cell Therapy Through PD-1 deletion and Controlled IL-15 Expression

Adoptive Cell Therapy (ACT) with Chimeric Antigen Receptor (CAR) T cells holds promise for treating refractory leukemia and lymphoma, but faces challenges such as toxicity, tumor microenvironment inactivation, and poor cell persistence. This study develops a gene editing strategy to overcome inhibitory signals from Programmed Death-Ligand 1 (PD-L1) in tumors. The approach involved deleting PD-1 in CAR-T cells, using CRISPR/Cas9 to target the PDCD1 gene. This achieved efficient PD-1 depletion (80%) without major T cell phenotype alterations. However, PD-1 knockout (KO) CAR-T cells exhibited decreased metabolic fitness, lower proliferative capacity, and increased exhaustion markers. Despite this, they showed improved memory phenotype, persistence, and cytotoxicity against PD-L1+ tumor cells. To enhance these cells' therapeutic potential, controlled IL-15 expression was introduced using a CRISPR/Cas9-Homology-Directed Repair (HDR) system, targeting the PD-1 promoter. The AAV6 delivery method proved effective for IL-15 integration in primary T cells. The IL-15-expressing pdTRUCKIL-15 cells exhibited improved proliferation, enhanced metabolic features, and greater cytotoxicity compared to CAR-T PD-1 KO and wild-type cells. In conclusion, PD-1 deletion enhances CAR-T activity against PD-L1+ tumors but reduces metabolic fitness. However, IL-15 expression under the PD-1 promoter rescues these deficiencies, offering a promising strategy to improve CAR-T cell persistence and efficacy against PD-L1+ cancers. This platform can potentially be applied to other PD-L1+ tumors.

M Tristán-Manzano^{1*#}, PL Justicia-Lirio^{1*}, N Maldonado-Pérez², C Barbero-Jiménez¹, M Cortijo-Gutiérrez², K Pavlovic^{2,3}, C Panisello, A Hinckley-Boned², FJ Molina-Estévez², P Muñoz², C Griñán-Lisón⁴, S Marchal-Navarro⁴, J Muñoz-Ballester⁶, P Gonzalez-Sierra⁶, C Herrera³, JR Rodríguez-Madoz⁷, F Prósper⁷, C Bueno⁵, P Menendez⁵, JA Marchal⁴, F Martin^{2 8#}

#Share senior authorship *Share first authorship.

1. LentiStem Biotech (Granada, Spain) 2. GENyO- Centro de Genómica e Investigación Oncológica: Pfizer, University of Granada, Junta de Andalucía (Granada, Spain); 3. Maimonides Institute of Biomedical Research in Cordoba (IMIBIC) 4. Department of Human Anatomy and Embryology, University of Granada (Spain); 5. Josep Carreras Leukemia Research Institute; 6. Virgen de las Nieves University Hospital Granada; 7. Hemato-Oncology Program Adoptive Cell Therapy, CIMA Universidad Clínica de Navarra (Spain); 8. Department of Biochemistry, Molecular Biology and Immunology III, University of Granada (Spain).

Transactivator-free Doxycycline-inducible TRUCKs releasing IL-18 for enhancing the antitumoral potential

Chimeric Antigen Receptor (CAR) T cell therapy has revolutionized type B cancer treatment, while efficacy remains limited in various lymphomas and solid tumors. Reinforcing conventional CAR-T cells to release cytokines can improve their efficacy but also increase safety concerns. Several strategies have been developed to regulate their secretion using minimal promoters that are controlled by chimeric proteins harboring transactivators. However, these chimeric proteins can disrupt the normal physiology of T cells which makes them not ideal for the generation of Advanced Therapy Medicinal Products (ATMPs). Here, we present the first transactivator-free anti-CD19 CAR-T cells able to control IL-18 expression (iTRUCK19.18) under ultra-low doses of doxycycline (Dox) and without altering cellular fitness. Interestingly, IL-18 secretion requires of T cell activation in addition to Dox, allowing the external regulation of CAR-T cell potency. This effect was translated into an increased CAR-T cell antitumor activity against aggressive CD19+ hematologic and solid tumor models such as pancreatic cancer (PDAC) and Ewing Sarcoma (ES). In a clinically relevant context, we have generated patient-derived iTRUCK19.18 cells capable of eradicating primary B cells tumors in a doxycycline-dependent manner. Furthermore, IL-18-releasing CAR-T cells polarized pro-tumoral macrophages (M2) towards an M1-antitumoral phenotype in the presence of tumoral PDAC cells, suggesting the potential for modulating the tumor microenvironment. In this line, we have further explored that behavior in 3D multicellular models where iTRUCKS19.18 (+Dox) increased tumour killing in ES-3D spheroids containing M2 macrophages. In addition, spheroids with M1 and M2 macrophages decrease the mean expression of CD14, FRb, CD163 and CD209 markers that are overexpressed in M2-like TAMs when treated with iTRUCKS19.18 (+Dox). In summary, we showed that our platform can generate exogenously controlled CAR-T cells with enhanced antitumor potency and in the absence of transactivators.

M. C. Ortega-Liebana,^{1,2,3} T. J. Widmann¹, J. J. Diaz-Mochon^{1,2,3} and R. M. Sanchez-Martin^{1,2,3}

1. GENYO, Centre for Genomics and Oncological Research, Pfizer/University of Granada/Andalusian Regional Government, PTS Granada, Avda. Ilustración 114, Granada 18016, Spain. 2. Department of Medicinal & Organic Chemistry and Excellence Research Unit of "Chemistry applied to Biomedicine and the Environment", Faculty of Pharmacy, University of Granada, Campus de Cartuja s/n, Granada 18071, Spain. 3. Instituto de Investigación Biosanitaria ibs.GRANADA, Granada, Spain.

Smart Nanosystems Targeting B-Cell for Precise Cancer Therapy

Although significant progress has been made in the development of cancer nanomedicine co-formulated with drugs, the challenges of generating a nanosystem which allows therapeutics to efficiently and selectively reach tumoral areas remain to be overcome. Our project aims to develop an unprecedented 'track and treat' nanosystems armed with navigational ability to achieve antigen-specific targeting and controlled therapeutic agent delivery capabilities to treat B cell lymphoma. Specifically, the light-controlled thermosensitive polymer-functionalised gold(Au)-based nanodevices we present have been designed to (1) track and tag tumours by recognising a target antigen using the single-chain variable fragment (scFv) mimics present on chimeric antigen receptor (CAR) T cells, and (2) release therapeutic agents in situ via NIR-controlled photothermal therapy (PTT). The first nanocarrier have been successfully synthesized and characterized, and we assessed their selectivity towards target cancer cells vs. healthy cells, as well as their anticancer drug release and PTT capabilities upon NIR irradiation. The internalisation of the nanocarriers and the combined effect of the release of an anticancer drug and PTT in the target cells were assessed in detail by viability studies, confocal fluorescence microscopy and CyTOF mass cytometry. Finally, we tested the efficacy of the nanocarrier in a zebrafish xenograft model for the treatment of lymphoma cancer. Overall, this study advances drug delivery approaches toward clinical applicability and opens a promising avenue to design reasonable efficient NIR-activated nanomedicines.

María Alcázar-Fabra ^{1,2,3}, Efres Belmonte Reche ^{1,2,3}, Mencía Espinosa-Martínez ^{1,2,3}, David Landeira ^{1,2,3}

1. GENYO. Centre for Genomics and Oncological Research Granada (Spain); 2. Department of Medicinal & Organic Chemistry and Excellence Research Unit of "Chemistry applied to Bio-medicine and the Environment", University of Granada,(Spain); 3. Instituto de Investigación Biosanitaria ibs.GRANADA (Spain); 4. CRISPNA Bio S.L (Spain); 5. Centro Nacional de Investigaciones Oncológicas (CNIO), Madrid (Spain); 6. Centro de Investigación Energéticas Medioambientales y Tecnológicas (CIEMAT), Madrid (Spain); 7. Department of Biochemistry and Immunology III, University of Granada (Spain).

Mitotic bookmarking by Polycomb proteins in stem cells

Understanding the molecular mechanism that facilitates the inheritance of gene expression programs and cell identity across mitotic cell generations is a key transversal question in biomedicine. Polycomb repressor complexes (PRCs) are hallmark epigenetic regulators that repress developmental genes by catalysing histone post-translational modifications and participating in the 3D organization of chromatin within the nucleus. Importantly, they have been proposed to endorse cells with the epigenetic memory required to restore gene expression programs on daughter cells after mitosis. However, the molecular mechanisms by which Polycomb regulation is reinstated upon DNA synthesis and inherited through mitosis remain largely unknown. Here, we have analysed the distribution of Polycomb proteins on mitotic chromosomes in nocodazole-treated mouse embryonic stem cells (mESCs), by using fluorescence microscopy, biochemical fractionation and calibrated chromatin immunoprecipitation followed by sequencing (ChIP-seq).

Maria J Tello-Lopez ^{1,2*}, Victoria Sanchez-Martin ^{1,3,4,5*}, Dusan Ruzic ^{6*}, Andrea Ortiz Morales ¹, Javier Murciano-Calles ⁷, Miguel Soriano ^{1,2}, Katarina Nikolic ^{6#}, Jose Antonio Garcia-Salcedo ^{1,3,4#}

* These authors equally contribute to this work.

1. GENYO. Centre for Genomics and Oncological Research: Pfizer, University of Granada, Andalusian Regional Government, PTS Granada-Avenida de la Ilustración, 18016 Granada, Spain. 2. Center for Intensive Mediterranean Agrosystems and Agri-Food Biotechnology (CIAIMBITAL), University of Almería, 04001 Almería, Spain. 3. Servicio de Microbiología, Hospital Universitario Virgen de las Nieves, Granada, Spain. 4. Instituto de Investigación Biosanitaria ibs.GRANADA, Granada, Spain. 5. Department of Biochemistry, Molecular Biology III and Immunology, University of Granada, Granada,

18016, Spain. 6. Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Belgrade, Belgrade, 11221, Serbia. 7. Department of Physical Chemistry, Unit of Excellence for Chemistry Applied to Biomedicine and the Environment, and Institute of Biotechnology, University of Granada, Granada, 18071, Spain

The histone deacetylase inhibitor Scriptaid targets G-quadruplexes

Scriptaid is a chemical compound with antitumoral effects due to its role as a histone deacetylase inhibitor. Despite sharing part of the chemical structure with other ligands of G-quadruplexes (G4s), the interaction of Scriptaid with G4s has not been explored before. We synthesized Scriptaid and screened its cytotoxic activity in cellular models of colorectal cancer (CRC). We extensively evaluated its biological activity by cell cycle, immunofluorescence, qRT-PCR and Western Blot experiments. To identify the G4 targets of Scriptaid, we conducted a panel of binding assays. Here, we show that Scriptaid induced cytotoxicity, cell cycle arrest and nucleolar stress in CRC cells. Moreover, Scriptaid impaired RNA polymerase I (Pol I) transcription, stabilized G4s and caused DNA damage. Finally, we disclose that these effects were attributable to the binding of Scriptaid to G4s in ribosomal DNA. In conclusion, our work reveals that a primary impact of Scriptaid on human cells is the interaction with G4s.

Marina Vargas-Fernández ^{1, 2}, Jordi Martorell-Marugán ^{2, 3}, Pedro Carmona-Sáez ^{1, 2}

1. Department of Statistics and Operations Research, University of Granada, Spain. 2. GENYO, Centre for Genomics and Oncological Research: Pfizer / University of Granada / Andalusian Regional Government. PTS Granada - Avenida de la Ilustración, 114 - 18016, Granada, Spain. 3. Fundación para la Investigación Biosanitaria de Andalucía Oriental (FIBAO)

Parkinson's Disease Symptoms Network Analysis using Longitudinal Linear Mixed Models from Electronic Health Records

Parkinson's Disease (PD) is a neurodegenerative movement disorder characterized by motor and nonmotor symptoms, which worsens over time. It also presents an extensive prodromal phase long before main symptoms are developed. Understanding clinical manifestations, risk factors and comorbidities during this time is fundamental in order to prevent and delay first symptomology, to perform early diagnoses and support medical decision. Due to heterogeneity present in this pathology, association between clinical variables and response to treatment classification remain one of the main challenges to overcome. Currently used network analysis methods often impose strict criteria of structure and functionality poorly adapted to the reality of data. We aim to develop a methodology that would facilitate the analysis of interactions between clinical variables, overcoming the usability and functionality limitations of existing approaches. Our method, called LMNET, is based on network analysis using linear mixed models. LMNET builds iterative models where each variable is successively represented as a response and the others as independent predictors, allowing to capture the hierarchical and specific influence of subgroups or longitudinal patterns. The result is a network in which variables are positively connected when their values are correlated and negatively connected when the influence is inverse.

Marín-Benesiu F ^{1,2}, Arenas-Rodríguez V ^{1,2}, Cuenca-López S ¹, Porrás-Quesada PM ^{1,2}, Tamayo-Gómez A ⁴, Vázquez-Alonso F. ⁴, Álvarez-Cubero MJ ^{1,2,3}, Martínez-González LJ ^{1,2}.

1. GENYO, Centre for Genomics and Oncological Research: Pfizer, University of Granada, Andalusian Regional Government, Parque Tecnológico de la Salud, Granada, Spain. 2. Department of Biochemistry, Molecular Biology III and Immunology, Faculty of Medicine, University of Granada, Parque Tecnológico de la Salud, Granada, Spain. 3. Ibs Granada, Biosanitary Research Institute of

Granada, Granada, Spain. 4. Department of Urology, Virgen de las Nieves University Hospital, Granada, Spain.

SINGLE CELL DIFFERENTIAL ABUNDANCE ANALYSIS OF PROSTATE CANCER CELLS REVEALS TUMORAL-SPECIFIC GENE MARKERS IN FUSION-GUIDED BIOPSY SAMPLES

Prostate cancer, the second most common malignancy worldwide, is characterized by a heterogeneous tumor microenvironment that complicates treatment. This study leverages single-cell RNA sequencing (scRNA-seq) to identify tumor subtypes in fusion biopsy samples, advancing precision medicine. From 31 prostate tissue samples—12 tumor cell-enriched (T) and 19 non tumor cell-enriched (N)—cancer cells and cancer-associated fibroblasts (CAFs) were identified. Cancer cells accounted for 13.02% in T samples and 9.25% in N samples, while CAFs were 5.32% and 7.33%, respectively. Reclustering revealed five cancer cell subtypes, with Clusters 0 and 3 enriched in N samples, and Cluster 1 dominant in T samples. Differential abundance (DA) analysis identified nine DA neighborhoods, mostly enriched in T samples, with key marker genes including SLC9A9, SCHLAP1, PTPRG, PLA2G2A, and ERG. Subcluster analysis highlighted unique gene expression patterns, such as overexpression of SCHLAP1, FHIT, and CRISP3 in Cluster 1, and KLK2, HPN, and PCA3 in Cluster 3. These findings provide critical insights into tumor heterogeneity, offering potential biomarkers and therapeutic targets to improve prostate cancer management.

Marta García Cerezo¹, **Francisco Gabriel Ortega Sánchez**^{1,2}, **Teresa Valero**^{1,2,3}, **Laura Rosa Fernández Castro**¹, **Fabián Vergara Rubio**¹, **Juan Sainz**^{1,2,4}, **Antonio Jesús Láinez Ramos-Bossini**^{1,5,6}

1. Instituto de Investigación Biosanitaria ibs.GRANADA, Granada, Spain. 2. Genomic Oncology Area, GENYO, Centre for Genomics and Oncological Research: Pfizer/University of Granada/Andalusian Regional Government, PTS, Granada, Spain 3. Department of Medicinal & Organic Chemistry and Excellence Research Unit of “Chemistry applied to Biomedicine and the Environment”, Faculty of Pharmacy, University of Granada, Campus de Cartuja s/n, 18071 Granada, Spain 4. Department of Biochemistry and Molecular Biology I, Faculty of Sciences, University of Granada 5.Servicio de Radiodiagnóstico. Hospital Universitario Virgen de las Nieves, España 6Departamento de Anatomía y Embriología Humana. Facultad de Medicina, Universidad de Granada, España

Proteomic signature in extracellular vesicles for colorectal cancer diagnosis

Colorectal cancer (CRC) is the third most common cancer worldwide and the fourth leading cause of cancer-related deaths. Its treatment involves a multidisciplinary approach, including surgery, radiotherapy, and chemotherapy. The choice of treatment depends on factors like cancer stage, the patient's health, and personal preferences. Surgery is the primary curative treatment for non-metastatic CRC, but its success is closely linked to the quality of the surgical intervention, preoperative staging, and treatment decisions.

Extracellular vesicles (EVs), heterogeneous lipid bilayer structures released by almost all cell types into biological fluids, play a crucial role in CRC. EVs transfer nucleic acids, proteins, and lipids to target cells, influencing the physiological functions of recipient cells. In CRC, EVs contribute to processes such as epithelial-mesenchymal transition (EMT), extracellular matrix degradation, premetastatic niche formation, and angiogenesis, all of which facilitate metastasis. This study aims to investigate EVs as potential diagnostic biomarkers for CRC.

To isolate small extracellular vesicles (sEVs) from blood plasma, a combination of size-exclusion chromatography (SEC) and filtration was used. Plasma samples were processed and concentrated, followed by separation using a qEV size chromatography column. EVs were then concentrated by ultracentrifugation and lysed for protein analysis. Protein lysates were analyzed using reverse-phase protein arrays (RPPA) at MD Anderson Cancer Center.

A total of 39 patients participated, including 15 controls and 24 cancer patients. Differential expression analysis identified 18 proteins with significant changes, with G6PD-R-V and NF2-RC showing the most impact in distinguishing cancer patients. Statistical analyses, including LIMMA, k-Nearest Neighbor, Random Forest, and Logistic Regression with LASSO, were performed to refine the protein selection. Validation was carried out using cross-validation and ROC curves.

These findings suggest that EVs and their protein content could serve as promising diagnostic biomarkers for CRC.

Mehmet Serdar Koca ¹, Katrien L.A. Quintelier ^{2,3}, Lucía Rodríguez-Doña ¹, Adrián Barreno ⁴, Axel Schulz ⁴, Sonia Gavasso ^{5,6}, Patrice Hemon ⁷, Divi Cornec ⁷, Sofie Van Gassen ^{2,3}, Yvan Saeys ^{2,3}, Marta E Alarcón-Riquelme ^{1,8}, Concepción Marañón ¹, Paulina Rybakowska ¹

1Pfizer-University of Granada-Junta de Andalucía Centre for Genomics and Oncological Research (GENYO), Granada, Spain. 2Department of Applied Mathematics, Computer Science and Statistics, Ghent University, Ghent, Belgium. 3Data Mining and Modeling for Biomedicine Group, VIB Center for Inflammation Research, Ghent, Belgium.. 4Mass Cytometry Lab, German Rheumatism Research Center (DRFZ), A Leibniz Institute, Berlin, Germany. 5Department of Clinical Medicine, University of Bergen, 5009 Bergen, Norway. 6 Department of Neurology, NeuroSys-Med, Haukeland University Hospital, 5021 Bergen, Norway. 7B Lymphocytes, Autoimmunity and Immunotherapies, UMR1227, Immunology Department, Augustin Morvan Hospital, Brest, France. 8 Institute for Environmental Medicine, Karolinska Institute, Stockholm, Sweden.

CyTOF harmonization for multicenter immune monitoring studies

Background: 3TR is a multicenter consortium aiming to identify common molecular signatures across 7 chronic diseases. Among various OMICs technologies, cytomics data will be obtained and whole blood samples will be analyzed using mass cytometry. Due to high numbers of individuals (>1000) and limited CyTOF throughput, various instruments need to be involved. Objectives: Optimize samples acquisition protocol and verify if multiple CyTOF instruments can be used. Methods: To select acquisition solution, identical samples were acquired using CAS, CASPLUS, and water. FLOWSOM clustering and PCA were used to compare different settings. To study the spillover matrices stability, compensation beads were stained with 12-plex panel and acquired the following day or kept frozen for longer time. To test multiple CyTOF instruments, whole blood from 3 donors was barcoded, stained with 22-plex panel, aliquoted and frozen. Next, aliquots were acquired using 4 HELIOS and 1 CyTOF XT instrument. Coefficient of variation (CVs) was calculated for cell frequencies and median signal intensities (MSI). Results: Increasing background issues were detected using CAS. Water has the best signal to noise ratio, however, introduce artifact during clustering. CASPLUS presented the highest sample stability. Spillover compensation was consistent during 3 different experiments (3 months). After bead normalization, batch effects were reduced; however, center-based differences in some markers persisted, alongside high CVs in cell frequency and MSI. Following batch normalization using a reference sample, improved parameters were observed, particularly in reducing center-based clustering accumulation. Conclusions: Multicenter CyTOF experiments are feasible, however special protocol for sample acquisition and additional normalization steps are needed to align the centers. IMI, PRECISESADS, (GA#115565), 3TR, (GA#831434), EFPIA, SIGNATURE (GA#101072891)

P Heredia ^{1,6}, K Pavlovic ^{1,2}, L Algeciras ¹, V Ronco ¹, MD Carmona ², IC Herrera ^{2,7}, JA Marchal ^{3,4,5,6#} and K Benabdellah ^{1#}

1. Department of Genomic Medicine, Pfizer-University of Granada-Andalusian Regional Government Centre for Genomics and Oncological Research (GENYO), Granada, Spain; 2. Maimonides Institute of Biomedical Research in Cordoba (IMIBIC), Cell Therapy, 14004, Cordoba, Spain; 3. Instituto de

Investigación Biosanitaria IBS.GRANADA, University Hospitals of Granada – University of Granada, 18016 Granada, Spain; 4. Excellence Research Unit “Modeling Nature” (MNat), University of Granada, 18016 Granada, Spain; 5. Biopathology and Regenerative Medicine Institute (IBIMER), Center for Biomedical Research (CIBM), University of Granada, 18016 Granada, Spain; 6. Department of Human Anatomy and Embryology, Faculty of Medicine, University of Granada, Biosanitary Research Institute of Granada (IBS.GRANADA), Granada, Spain; 7. Department of Hematology, Reina Sofía University Hospital, Córdoba, Spain

Optimizing exosome production from CAR-T cells for cancer immunotherapy

Chimeric antigen receptor (CAR) T cell therapy has revolutionized immunotherapy, offering a promising approach for treating various conditions. However, significant challenges remain, including cytokine release syndrome and neuroinflammation, as well as complications like tumor lysis syndrome and graft-versus-host disease. Moreover, current CAR-T cells are not efficient for the treatment of solid tumors due to antigenic diversity, immune escape within the tumor microenvironment, and the challenge of achieving sufficient proliferation and infiltration. Exosomes derived from CAR-T cells (EXO-CAR-T) can offer a complementary therapy due to the unique properties of extracellular vesicles (EVs). These EVs, including exosomes, are capable of mediating intercellular communication and carrying a repertoire of therapeutic molecules. By using EXO-CAR-T, we hypothesize that we can mitigate some of the limitations and side effects of traditional CAR-T cell therapy. Exosome-based therapies offer biocompatibility, low toxicity, high permeability, and stability in biological fluids, with the ability to cross biological barriers and be engineered for targeted drug delivery. However, the large-scale preparation of exosomes from CAR-T cells is expensive, and the safety and immunogenicity of engineered exosomes need careful consideration. Our research focuses on several key areas: 1. Exosome Biogenesis and Production: Through gene editing techniques, we aimed to improve the production yield of exosomes from CAR-T cells. Our preliminary data showed an increase in exosome output. 2. Safety and Immunogenicity: To address concerns regarding the safety and immunogenicity of engineered exosomes, we analyzed the effects of eliminating HLA-I expression to produce allogeneic EXO-CAR-T. Our preliminary findings suggest that exosomes derived from CAR-T cells represent a promising complementary therapy for cancer treatment, particularly for solid tumors. By enhancing exosome production and engineering them to evade immune detection, we can potentially overcome many of the limitations associated with traditional CAR-T cell therapy.

Patricia Concha-Moral ^{1,2}, **José M. Ruiz-Jiménez** ^{1,2}, **Ana M. Matia-Gonzalez** ^{1,3,4} and **Pedro P. Medina** ^{1,3,4#}

1. Pfizer-University of Granada-Junta de Andalucía Centre for Genomics and Oncological Research (GENYO). Granada, Spain 2. Andalusian Public Foundation for Biomedical Research in Eastern Andalusia - Alejandro Otero (FIBAO). Granada, Spain 3. Department of Biochemistry and Molecular Biology I, University of Granada. Granada, Spain 4. Institute for Biomedical Research IBS. Granada, University Hospital Complex of Granada/ University of Granada. Granada, Spain

NFAT5::VPS4A AS A NOVEL FUSION GENE IN LUNG CANCER

"Fusion genes play an important role in biomedicine, acting as diagnostic markers and therapeutic targets. In non-small cell lung cancer (NSCLC), numerous fusions have been identified, including those formed by genes such as ALK, ROS1, NTRK, and RET35. The main objective of this study is to identify and characterize new fusion genes present in lung cancer.

The methodology employed genetic dependency databases derived from CRISPR and RNA interference studies. Validation was performed through amplification of the implicated genes and study of chimeric proteins. We characterized phenotypic effects through overexpression models, cell viability assays, and clonogenicity. We studied the localization using immunofluorescence.

This study identifies the NFAT5::VPS4A fusion in an NSCLC cell line, confirming the expression of its chimeric protein and its oncogenic role. Localization analyses revealed changes in protein distribution, which could help understand its function in cancer cells.

In summary, this work identifies the presence of a new fusion gene called NFAT5::VPS4A in lung cancer, which could serve as a diagnostic marker or therapeutic target. These findings expand our understanding of NSCLC genetics and open new opportunities for personalized diagnostics and treatments."

Pilar González-Marchante ^{1,2*}, Ana Ariza-Cosano ^{1,2*#}, Ana Gázquez-Gutiérrez ^{1,2}, Ana Colomer-Boronat ^{1,2}, Álvaro Méndez-Maguilla ¹, Eva Álvarez-Barrero ¹, Guillermo Peris ^{1,3}, Francisco J Sánchez-Luque ⁴, José Luis García-Pérez ¹, Miguel Ángel Moreno-Mateos ⁵, Sara R. Heras ^{1,2#}

* These authors equally contribute to this work.

1. GENYO, Centre for Genomics and Oncological Research: Pfizer/University of Granada/Andalusian Regional Government. PTS Granada, Av. de la Ilustración, 114, 18016, Granada, Spain; 2. Dept. of Biochemistry and Molecular Biology II, Faculty of Pharmacy, University of Granada, Campus Universitario de Cartuja, 18071, Granada, Spain; 3. Dept. of Computer Languages and Systems, Universitat Jaume I – Castellón de la Plana, 12071, Spain; 4. Institute of Parasitology and Biomedicine "López-Neyra" (IPBLN), CSIC, P.T. Ciencias de la Salud, Av. del Conocimiento s/n, Armilla, 18100 Granada; 5. Andalusian Center for Developmental Biology (CABD). Pablo de Olavide University, CSIC, Junta de Andalucía, Ctra. Utrera Km.1, 41013 Seville, Spain.

The endogenous retrovirus ERV1-3 plays an essential role during morphogenesis in zebrafish

Endogenous retroviruses (ERVs) have shaped the genomes of vertebrates, but the extent of their influence on evolution, functionality and disease remains to be elucidated. Although most ERVs have lost the ability to retrotranspose, their transcription is tightly regulated during pluripotency where they play critical roles. However, their involvement in later development during morphogenesis remains unknown.

Here we focus on the ERV1-3 subfamily, a member of the zebrafish ERV1 superfamily that shows specific expression in the paraxial mesoderm and somites. We show that this expression is primarily driven by three recently inserted full-length copies that contain only the pol gene and harbour a unique combination of binding sites for specific mesodermal transcription factors, including tbx16, a Nodal-induced transcription factor. Indeed, we demonstrate that ERV1-3 expression is mediated by the Nodal signalling pathway, an essential pathway for early embryonic development. Furthermore, depletion of ERV1-3 RNA using CRISPR-RfxCas13d resulted in early and late developmental phenotypes that strikingly resemble Nodal mutants, like cyclopia, open neural tubes and somite defects. Importantly, Nodal inhibition and ERV1-3 depletion phenotypes were partially rescued overexpressing a predicted transcript derived from ERV1-3 potentially encoding an RNase H domain.

Taken together, our findings demonstrate that a relatively young endogenous retrovirus has been co-opted for developmental roles within one of the most evolutionarily conserved signalling pathways.

Samuel Pérez-Fernández^{1 2}, Juan A. Villatoro-García^{1 3}, Sara Bandrés-Ciga⁴, Pedro Carmona-Sáez^{1 3}, Jordi Martorell-Marugán¹

1. Centro Pfizer-Universidad de Granada-Junta de Andalucía de Genómica e Investigación Oncológica (GENYO), 2. Fundación para la Investigación Biosanitaria de Andalucía Oriental (FIBAO), 3. Departamento de Estadística de la Universidad de Granada, 4. Centre for Alzheimer's and Related Dementias (CARD), National Institute of Aging, National Institutes of Health, Bethesda, MD, United States

Gene expression profiling for Parkinson's Disease subtyping over time

Parkinson's disease (PD) is a neurodegenerative disease with a large genetic and clinical heterogeneity. Our hypothesis is that patients with PD can be stratified into molecular subtypes with different pathological mechanisms, on which precision medicine can be applied. We have access to the Accelerating Medicines Partnership program for Parkinson's disease (AMP-PD), a project that integrates different cohorts, including 8461 transcriptomics samples and clinical information. Patient stratification is performed with the Monte Carlo reference-based consensus clustering (M3C) method: This method is based on the Consensus Clustering algorithm applying a Monte Carlo hypothesis testing framework to obtain the optimal number of patient groups. The analysis to find gene modules is performed with the Weighted Correlation Network Analysis (WGCNA) method, a well-known analysis based on Pearson correlation. Applying these methodologies we have found that PD patients are optimally stratified into 5 molecular clusters at the start of the study. These groups are replicated over different timepoints, although many individuals change their assigned subgroup. Future work includes functional characterization of the gene modules, clinical assesment of the patient subtypes and integration with other omics data.

Soledad Romero-Tamudo ^{1,2}, M. Dora Carrión ¹, Jose Manuel Espejo-Román ^{1,2}, Meriem Chayah-Ghaddab ^{1,2}, Ana Conejo-García ¹, Olga Cruz-López ¹

1. Department of Medicinal and Organic Chemistry and Excellence Research Unit of Chemistry Applied to Biomedicine and the Environment, Faculty of Pharmacy, Campus Cartuja s/n, 18071, University of Granada, Granada, Spain, 2. GENYO, Centre for Genomics and Oncological Research, Pfizer/University of Granada/Andalusian Regional Government, PTS Granada, Avda. Ilustración 114, 18016 Granada, Spain 3. Biosanitary Institute of Granada (ibs.GRANADA), SAS-University of Granada, Avenida de Madrid, 15, 18012, Granada, Spain

N-ARYL THIQ SULFONYL ESTERS AS CD44-HA INTERACTION INHIBITORS: DESIGN, SYNTHESIS AND BIOLOGICAL STUDIES.

Cluster of differentiation 44 (CD44) through its interaction with hyaluronic acid (HA) is associated with cancer and angiogenesis [1]. Crystal structure and mutagenesis studies of murine and human CD44 have identified critical residues involved in the CD44-HA binding domain (HABD). Through biophysical binding assays, fragment screening and crystallographic analysis, an inducible pocket adjacent to the HA binding groove was discovered suggesting a potential binding site for small molecules and the tetrahydroisoquinoline (THIQ) pharmacophore was identified as a promising lead for optimization [2]. Nevertheless, these THIQ derivatives were shown to bind to CD44-HABD with low millimolar affinity. Before our recent reports on a N-alkyl and N-aryl THIQ derivatives [3][4] etoposide was the only antitumor compound found to inhibit the binding of CD44 to HA, disrupting key functions that drive malignancy [5]. Based on our previous results [4], here we present the design, synthesis and biological evaluation of a new series of N-aryl THIQ sulfonyl esters with antiproliferative activity against the CD44+ breast cancer cell line MDA-MB-231. The two most active compounds, SRT7 and SRT8, have EC50 values in the low micromolar range. Inhibition of the HA-CD44 interaction is proposed as one of the mechanisms by which they exert their antiproliferative activity, since the reduced antiproliferative activity was observed either in cells which CD44 was blocked or in CD44- cell lines.

Tomáš Maľarik ¹, Jana Jozefiaková ¹, Katarína Bhide ¹, Amod Kulkarni ^{1,2}, Ján Čurlík ⁴, Katarína Kucková ¹, Jakub Viglaský ¹, Lea Talpašová ¹, José Antonio Garcíá Salcedo ³, Mangesh Bhide ^{1,2}

1. Laboratory of Biomedical Microbiology and Immunology, University of Veterinary Medicine and Pharmacy in Košice, Slovakia; 2. Institute of Neuroimmunology, Slovak Academy of Sciences,

Unravelling the therapeutic potential, of CDR3 of nanobodies against TBEV and WNV

Nanobodies (Nb) are getting popular in therapy and diagnosis due to the enhancement of solubilization, resistance against proteases, changes in temperature and pH. The variable region of nanobodies (VHH) consists of 4 frameworks (FR 1 – 4) and complementarity determining regions (CDR1 – 3). The main epitope binder, CDR3, is longer in VHH than in classical VH in IgG and more diverse. We still lack effective therapeutics, against brain-related infections. The aim was to produce CDR3 only molecule that can bind TBEV and WNV and neutralize them. Treatment of CNS infection caused by both viruses is complicated due to impermeable blood brain barrier to most of the drugs. Both viruses adhere to the cells via DIII domain of envelope glycoprotein. Thus, to produce DIII blocking CDR3, we immunized Lama glama with recombinant DIII of TBEV and WNV, collected blood of the immunized animal, isolated RNA, reverse transcribed to cDNA, amplified CDR3, and cloned into pSEX81 phagemid to create CDR3 E. coli XL-1Blue library. The CDR3 library was superinfected with Hyperphage M13 KO7DpIII to pack CDR3-phage library. The library was then exposed to 3 rounds of panning on inactivated TBEV and WNV. ssDNA eluted from panned phages (3rd round) was subjected to PCR amplification and cloning into pQE-30-UA-mCherry-GFP plasmid and 48 CDR3 proteins fused with GFP were produced in E. coli Shuffle. The purity, toxicity and binding affinity to rDIII and viruses were tested, in which 11 CDR3-GFP clones showed interaction with DIII of TBEV and 7 with DIII of WNV. Five clones showed considerable binding with inactivated TBEV (strain Hypr) and 5 with WNV (strain Goshawk). Two shortlisted candidates, CDR3TB3 and CDR3TB5 effectively neutralized TBEV virus, while CDR3WB1 and CDR3WB4 neutralized pseudoviral particles of WNV when 1 ug of CDR3 was used. Research funded from APVV-22-0084, VEGA1/0381/23, VEGA1/0348/22, EURONANOMED2021-105.

Torres-García A. ^{1,2,3}, Álvarez-González B. ², Pozo-Agundo A. ^{2,3}, Ceballos-Ramírez J. ⁴, Gutiérrez-Tejero F. ⁴, Arrabal-Martín M. ⁴, Morón-Romero R. ⁴. Cabeza-Barrera J. ⁴, Martínez-González LJ. ^{1,2}, Álvarez-Cubero MJ. ^{1,2,3}

1. Universidad de Granada, Departamento de Bioquímica y Biología Molecular III e Inmunología, Faculty of Medicine, Granada, Spain. 2. Centro de Genómica e Investigación Oncológica: Pfizer-Universidad de Granada-Junta de Andalucía (GENYO), Granada, Spain. 3. Instituto de Investigación Biosanitaria (ibs.GRANADA), Universidad de Granada, Spain. 4. Hospital Universitario Clínico San Cecilio, Granada, Spain.

PHARMACOGENETICS OF HSD3B1: PREDICTING OUTCOMES IN ANDROGEN DEPRIVATION THERAPY FOR PROSTATE CANCER PATIENTS

Introduction and objectives: Prostate cancer (PC) is the most prevalent neoplasm among men in Spain. Androgen deprivation therapy (ADT) is a key treatment, which aims to maintain testosterone levels at castration to inhibit tumour growth. However, in many patients, this treatment fails, resulting in castration-resistant prostate cancer (CRPC). The aim of this study is to analyse the influence of genetic variants in the genes HSD3B1 (rs1047303), SRD5A2 (rs523349), CYP17A1 (rs2486758) and CYP19A1 (rs4775936) on the response to ADT and disease progression. Materials and methods: The rs1047303, rs523349, rs2486758 and rs4775936 variants were genotyped in 151 patients using Taqman Genotyping technology in QuantStudio™ 12K-Flex from Applied Biosystems. Clinical variables were extracted from the medical records of patients in the Andalusian Health System. Integration of experimental and clinical data was performed using SPSS Statistics software.

Results: A recessive model for HSD3B1 (rs1047303) was established, showing that those patients carrying two recessive alleles (C/C) presented a higher therapeutic failure ($\chi^2=0.022$, $p=0.027$, $OR=2.932$, $95\%CI=1.132-7.593$) as well as a higher predisposition to develop CRPC ($\chi^2=0.004$, $p=0.008$, $OR=4.349$, $95\%CI=1.470-12.865$) compared to heterozygous and wild-type patients. We also compared

patients who had developed CRPC in less than 3 years of ADT with patients who had treatment efficacy greater than 5 years and observed that those patients with a homozygous recessive (C/C) genotype showed a higher risk of rapid progression ($\chi^2=0.013$, $p=0.019$, $OR=4.219$, $95\%CI=1.271-13.975$). Therefore, those patients carrying two alleles of rs1047303 have a worse prognosis due to experiencing greater therapeutic failure.

Conclusions: The rs1047303 polymorphism of the HSD3B1 gene is a good predictive biomarker of response to ADT as well as disease progression in patients with PC.

Viviana Ramírez ^{1,2}, Patricia González-Palacios ¹, Silvia Martínez-Diz ³, Luis Javier Martínez-González ^{2,4}, María Jesús Álvarez-Cubero ^{2,4}, Ana Rivas ¹.

1. Department of Nutrition and Food Science, Faculty of Pharmacy, University of Granada, 18071 Granada, Spain. 2. GENYO Centre for Genomics and Oncological Research, Pfizer/University of Granada/Andalusian Regional Government PTS Granada—Avenida de la Ilustración, 114, 18016 Granada, Spain. 3. Preventive Medicine and Public Health Service, Hospital Universitario Clínico San Cecilio, Granada, Spain. 4. Department of Biochemistry and Molecular Biology III, Faculty of Medicine, University of Granada, 18012 Granada, Spain.

Genetic Polymorphisms and Dietary Exposure to Bisphenols: Role of their Interaction in Children's Cognitive Functioning

Current evidence highlights the importance of the genetic component in neurodevelopmental disorders (NDDs), such as attention-deficit hyperactivity (ADHD), autism spectrum disorder (ASD) and intellectual disability (ID), due to their elevated heritability. Cognitive dysfunction in general is a common condition of NDDs. Likewise, endocrine disrupting chemicals (EDCs), including bisphenols, have been termed neuroendocrine disruptors given their ability to reach the brain and affect neurodevelopmental processes. Herein, the main goal was to evaluate the role of several genetic variants (SNPs) in cognitive impairment in children exposed to bisphenols through the diet. A total of 102 children aged 6-12 years having cognitive function assessed by WISC-V, dietary consumption and genotyping data were included. Ten SNPs in genes involved in brain development, synaptic plasticity, and neurotransmission (BDNF, NTRK2, HTR2A, MTHFR, OXTR, SLC6A2, and SNAP25) were genotyped by microarray technology and commercial probes. Bisphenols (BPA plus BPS) in food items were determined via UHPLC-MS/MS. In genetic association analysis, BDNF rs11030101-T and SNAP25 rs363039-A allele carriers scored better on the fluid reasoning domain, except for those inheriting the BDNF rs6265-A allele, who had lower scores. Secondly, relevant SNP-bisphenol interactions existed in verbal comprehension (NTRK2 rs10868235 ($p\text{-int}=0.043$)), working memory (HTR2A rs7997012 ($p\text{-int}=0.002$)), MTHFR rs1801133 ($p\text{-int}=0.026$), and OXTR rs53576 ($p\text{-int}=0.030$)) and fluid reasoning (SLC6A2 rs998424 ($p\text{-int}=0.004$)). These results demonstrate for the first time that studying the synergistic or additive effects of genetic and environmental components on cognitive function in vulnerable populations may help to better understand the multifactorial, complex and polygenetic aetiology of NDDs.

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Caracuel Peramos, Rita

Colomer Boronat, Ana

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Premiados – Comunicación póster

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