# BIOGRAPHICAL SKETCH

NAME: Garcia Bermudez, Javier

POSITION TITLE: Assistant Professor, Children's Research Institute at University of Texas Southwestern Medical Center.

EDUCATION/TRAINING

| INSTITUTION AND LOCATION | DEGREE *(if applicable)* | START DATE MM/YYYY | END DATE *(or expected end date)* MM/YYYY | FIELD OF STUDY |
|---|---|---|---|---|
| Universidad Autonoma de Madrid (Spain) | Bachelor's degree | 09/2005 | 07/2010 | Biochemistry |
| Universidad Autonoma de Madrid (Spain) | Master's degree | 09/2010 | 07/2011 | Molecular and Cellular Biology |
| Center of Molecular Biology Severo Ochoa, (Spain) | PhD | 09/2011 | 12/2015 | Biochemistry and molecular biology |
| The Rockefeller University, New York (USA) | Postdoc | 04/2016 | 11/2021 | Cancer metabolism |

## A. PERSONAL STATEMENT

Metabolism plays a critical role in tumors by allowing them to resist stresses imposed by the microenvironment and fulfil the demands of excessive cell proliferation. The precise metabolic pathways through which cancer cells adapt to their environment and whether these pathways can be exploited therapeutically remain unknown. Tumors experience oxidative stress during all stages of cancer progression due to the generation of oxygen-derived reactive oxygen species (ROS), molecules that react with cellular macromolecules and compromise their function. One major deleterious effect of ROS accumulation is lipid peroxidation, which damages cellular membranes and, when uncontrolled, leads to a non-apoptotic and iron-dependent form of cell death, ferroptosis. Susceptibility to lipid peroxidation has emerged as a major opportunity for new cancer therapies. Indeed, growing evidence suggests that lipid peroxides accumulate during radiation therapy, and that mechanisms to suppress ferroptosis result in radioresistance and metastasis. I believe that identifying novel ferroptosis-regulating genes will help us develop ways to improve therapeutic outcomes in cancer. In our laboratory we have developed three methodologies that, applied to these questions, will aid in unveiling novel oxidative dependencies of tumors: i) forward CRISPR-Cas9 genetic screens to find in an unbiased manner candidate genes that are essential under certain stresses; ii) a collection of DNA-barcode cancer cell lines from different origin that can be used in a proliferation competition assay to assess dependency on different nutrients; and iii) a metabolomic platform allowing metabolite analysis of whole-cell or of immunocaptured organelles such as mitochondria. I hypothesize that cancer cells contain other, as-yet uncharacterized mechanisms to suppress lipid peroxidation. My laboratory will identify these antioxidant pathways and test whether disrupting them increases the efficacy of radiation therapy and inhibits metastasis.

**B. POSITIONS AND HONORS**

**Academic Appointments:**

2011-2015 Ph.D. Student, Laboratory of Jose M. Cuezva - CBMSO/Autonomous University of Madrid, Spain.
2016-2021 Postdoctoral Fellow, Laboratory of Kivanc Birsoy – The Rockefeller University, New York, NY, USA.
2021 Assistant Professor in Children's Research Institute and Assistant Professor in Pediatrics, UT Southwestern Medical Center, Dallas, TX.

## Honors and Awards:

2010 Fellowship for Graduate Studies, Graduate Division
2011 Fellowship Formacion de Personal Investigador (FPI-MICINN)
2013 EMBO Travel Award High-Throughput RNAi and Data Analysis Course
2015 Honor mention (cum laude) in PhD Thesis defense
2016 EMBO Long Term Postdoctoral Fellowship
2016 Pilot Project Award from The Rockefeller University Center for Clinical and Translational Science
2017 Pilot Project Award from Center for Basic and Translational Research on Disorders of the Digestive System
2018 Anderson Cancer Center Postdoctoral Fellowship
2019 Robertson Proof of Concept Award from Robertson Therapeutic Development Fund
2019 Leukemia and Lymphoma Society Special Fellow Award
2020 NIH K99/R00: Pathway to Transition Award
2021 CPRIT Scholar in Cancer Research

**C. CONTRIBUTIONS TO SCIENCE**

1. **Phosphorylation of the ATPase Inhibitory Factor 1 by PKA regulates bioenergetics and stem cell differentiation.**

During my PhD I described a novel regulatory mechanism of IF1 activity: the phosphorylation in serine 39 of human IF1 by mitochondrial protein kinase A (PKA). Phosphorylation of IF1 abolishes its capacity to bind the ATP synthase and hence to exert its biological activity on the enzyme. Only dephosphorylated IF1 binds and inhibits both the hydrolase and synthase activities of the enzyme, being able of demonstrating how ATP synthesis was affected by the binding of the inhibitor, thought for a long time as a unidirectional inhibitor of only the hydrolitic activity of the complex. Additionally, we demonstrated the role of dephosphorylated IF1 in human stem cells. Active IF1 is a short-lived protein whose degradation depends on the activity of mitochondrial serine proteases, being this degradation process a key step in the differentiation of human mesenchymal stem cells (hMSCs) into osteocytes by switching from a glycolytic phenotype to a higher dependency on oxidative phosphorylation.

**a)** **García-Bermúdez J**, Sánchez-Aragó M, Soldevilla B, Del Arco A, Nuevo-Tapioles C and Cuezva JM. (2015) *PKA phosphorylates the ATPase Inhibitory Factor 1 and inactivates its capacity to bind and inhibit the mitochondrial H+-ATP synthase.* **Cell Reports**. 12: 1-13.

**b)** Sánchez-Aragó M, **García-Bermúdez J**, Martínez-Reyes I and Cuezva JM. (2013) *Degradation of IF1 controls energy metabolism during osteogenic differentiation of hMSC.* **EMBO Reports**. 14(7):638-44.

2. **Aspartate is a limiting metabolite for cancer cell proliferation under hypoxia and in tumors.**

Oxygen levels are generally lower in tumors than in normal tissues. As oxygen is a major electron acceptor for many essential metabolic pathways, tumor hypoxia often impairs cancer cell proliferation. The specific

metabolites that are limiting for proliferation under hypoxia and in tumors, however, are not well defined. To address this, we performed a proliferation assay on a collection of cancer cell lines using inhibitors of the mitochondrial electron transport chain (ETC), a major metabolic pathway that requires molecular oxygen to function. Sensitivity to ETC inhibition varies amongst cancer cell lines, and a metabolomics analysis identified aspartate availability as a major determinant of proliferation under ETC inhibition, consistent with the essential role of ETC in aspartate synthesis. Cancer cell lines most resistant to pharmacologic inhibition of ETC maintain aspartate levels by importing aspartate through a glutamate/aspartate transporter, SLC1A3. Genetic or pharmacologic inhibition of SLC1A3 sensitizes cancer cells to ETC inhibitors. Aspartate levels also decrease at oxygen levels expected to inhibit mitochondrial respiration, and increasing its import by SLC1A3 provides a proliferative advantage to cancer cells under hypoxic conditions in vitro and in tumor xenografts. Finally, aspartate levels in primary human tumors negatively correlate with the expression of hypoxia markers, suggesting that tumor hypoxia is sufficient to inhibit ETC and aspartate synthesis in human tumors. We therefore conclude that aspartate is a limiting metabolite for cancer cell proliferation under hypoxia and in some tumors and its availability could be targeted for cancer therapy.

**a)** **Garcia-Bermudez J,** La K, Baudrier L, Zhu XG, Sviderskiy VO, Papagiannakopoulos T, Snuderl M, Lewis C, Possemato R and Birsoy K. (2018) *Aspartate is a limiting metabolite for cancer cell proliferation under hypoxia and in tumors.* **Nature Cell Biology** (Jul;20(7):775-781).

**b)** **Garcia-Bermudez J***, Prasad S*, Baudrier L, Badgley MA, Liu Y, La K, Soula M, Williams RT, Yamaguchi N, Hwang RF, Taylor LJ, De Stanchina E, Rostandy B, Alwaseem H, Molina H, Bar-Sagi D and Birsoy K. (2021) *Adaptive stimulation of macropinocytosis overcomes aspartate limitation in cancer cells under hypoxia.* **bioRxiv.** *co-1st authors.

3. **Disruption of cholesterol metabolism in cancer opens therapeutic opportunities**

Cholesterol is essential for cells to grow and proliferate. Normal mammalian cells meet their need for cholesterol through its uptake or de novo synthesis. We have found that, due to loss of expression of a cholesterol synthesis gene, *SQLE*, some lymphomas are unable to de novo synthesize cholesterol, enabling them extremely sensitive to depletion of cholesterol from the media. Importantly, disruption of cholesterol uptake by knocking out low-density lipoprotein receptor (LDLR) resulted in a strong impairment of tumor growth in lymphoma cell lines and patient-derived xenografts. Dependency on cholesterol synthesis can vary depending on the cancer type, as we have seen in gliomas treated with the compound MI-2, a menin inhibitor that showed promising results for the treatment of this cancer type. Treatment with MI-2 directly inhibited another cholesterol synthesis enzyme, *LS*, depleted intracellular cholesterol levels and triggered cell death. The reasons underlying why cancer cells rely on synthesis or uptake of cholesterol remain poorly understood.

**a)** **Garcia-Bermudez J**, Baudrier L, Bayraktar E, Shen, Y, La K, Guarecuco R, Yucel B, Fiore S, Tavora B, Freikman E, Lewis C, Min W, Inghirami G, La K, Sabatini DM and Birsoy K. (2019) *Squalene accumulation in cholesterol auxotrophic lymphomas prevents oxidative cell death.* **Nature**. 2019 Mar;567(7746):118-122.

**b)** Phillips RE, Yang Y, Smith RC, Thompson BM, Yamasakie T, Soto-Feliciano YM, Funato K, Liang Y, **Garcia-Bermudez J**, Wang X, Garcia BA, Yamasaki K, McDonald JG, Birsoy K, Tabar V, and Allis CD. (2019) *Target identification reveals lanosterol synthase as a vulnerability in glioma.* **PNAS.** 2019 Apr 16;116(16):7957-7962.

4. **Understanding the role of antioxidant pathways in cancer.**

The high metabolic rates required for proliferation and the activation of oncogenic signaling pathways within tumors result in the generation of high levels of reactive oxygen species (ROS) and oxidative stress. One major effect of ROS is the damage of cellular structures including peroxidation of membrane lipids. Excessive accumulation of these lipid peroxides compromises the normal function of cellular membranes and leads to a non-apoptotic form of cell death, ferroptosis. During my postdoc, I asked how cancer cells adapt to oxidative stress in the tumor environment. By using forward genetics and transcriptomics I found two metabolites that

enable resistance to lipid peroxidation stress in different cancer types. Among these, anaplastic large cell lymphomas (ALCLs) accumulate high levels of squalene, a cholesterol synthesis intermediate which alters the cellular lipid profile and renders them resistant to lipid peroxidation and ferroptosis. Inhibiting squalene synthesis alone impaired proliferation under conditions of oxidative stress *in vitro* and in tumor xenografts. Analogous to this study, a CRISPR-screen in cancer cells upon induction of lipid peroxidation identified a subset of leukemias that require tetrahydrobiopterin (BH4) biosynthesis for proliferation under oxidative stress. Our work revealed that BH4 acts as a potent endogenous antioxidant to quench reactive oxygen species within lipid membranes. These studies show that different cancer types rely on the accumulation of two antioxidant metabolites to cope with the oxidative stress of the tumor microenvironment.

**a)** **Garcia-Bermudez J**, Baudrier L, Bayraktar E, Shen, Y, La K, Guarecuco R, Yucel B, Fiore S, Tavora B, Freikman E, Lewis C, Min W, Inghirami G, La K, Sabatini DM and Birsoy K. (2019) *Squalene accumulation in cholesterol auxotrophic lymphomas prevents oxidative cell death.* **Nature**. 2019 Mar;567(7746):118-122.

**b)** Soula M, Weber R, Zilka O, Alwaseem H, La K, Yen F, Molina H, Pratt D*, **Garcia-Bermudez J*** and Birsoy K*. (2020). *Metabolic determinants of cancer cell sensitivity to canonical ferroptosis inducers*. **Nature Chemical Biology** 16:1351-1360. PMID: 32778843. ***co-corresponding author**

**Complete List of Published Work:**

https://www.ncbi.nlm.nih.gov/myncbi/javier.garcia%20bermudez.1/bibliography/public/