MOLECULAR CYTOGENETICS UNIT

Sandra Rodríguez-Perales
Unit Head

Staff Scientist
Raúl Torres

Graduate Students
Maria Cruz Casado (since May), Pilar Puig

Technicians
M. Carmen Martín, Marta

OVERVIEW

Recurrent chromosomal rearrangements — changes in the structure of native chromosomes — are very common and well-known hallmarks of cancer. A better understanding of these cancer-causing mechanisms will lead to novel therapeutic regimens to fight cancer. The research activity of the Molecular Cytogenetics and Genome Editing Unit focuses on increasing the knowledge about the role of chromosomal rearrangements in cancer development and progression and discovering new therapeutic targets. With the combined use of CRISPR genome editing and cytogenetic technologies, we are creating models that recapitulate chromosomal and genetic cancer alterations. The goal of the Unit is to provide CNIO and external researchers with the latest technologies used in the fields of molecular cytogenetics and genome editing. The Unit is continuously implementing and developing new technologies in those fields.

"We apply genome engineering approaches to reproduce and eliminate chromosome rearrangements and gene alterations. We also provide access to the latest cytogenetic and CRISPR technologies."

We also participate in collaborative projects with clinical and basic science investigators across the CNIO and other institutions.

Martínez-Lage (until December) (TS)*, Francisco José Moya (TS) *(PEJ) **

*Titulado Superior (Advanced Degree)
**Plan de Empleo Joven (Youth Employment Plan)

Students in Practice
Alejandro Alonso (since September) (Maastricht University, The Netherlands), Alejandro Nieto (since November) (*Universidad Autónoma de Madrid*, Spain), Paula M. Ojeda (February-June) (*Universidad de Alcalá de Henares*, Spain), Beatriz Olalla (March-August) (*Universidad Politécnica de Valencia*, Spain)

RESEARCH HIGHLIGHTS

A new protocol to quantify the alterations (copy number changes) in cancer samples. Chromosomal instability (CIN), a type of genomic instability, favours changes in chromosome number and structure and is associated with the progression and initiation of multiple diseases, including cancer. Therefore, CIN identification and analysis represent a helpful tool for cancer diagnosis and treatment. In 2021, we reported an optimised molecular cytogenetic protocol to detect CIN in formalin-fixed, paraffin-embedded mouse and human tissues, using fluorescent *in situ* hybridization to visualise and quantify chromosomal alterations such as amplifications, deletions, and translocations.

Technological and translational activities. Our Unit offers rapid, precise, and affordable technologies to analyse cancer cells at the chromosome level and to functionally interrogate the cancer genome. We provide state-of-the-art molecular cytogenetic and genome editing services. The Unit focuses on making available a complete repertoire of gene editing tools for cellular and genetic manipulation and an array of delivery vehicles, offering a flexible, modular platform for precision genome manipulation. The Unit provides molecular cytogenetics technologies for human and mouse chromosomes analysis, including conventional karyotyping, FISH, SKY and CGH array. In 2021, we carried out over 2,500 assays for experimental and clinically oriented projects.

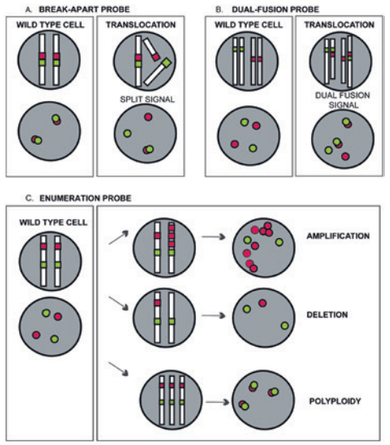

FIGURE Schematic representation of chromosomal abnormalities detected by fluorescence in situ hybridization (FISH). Schematic representation at genomic level of wild-type and rearranged FISH signal patterns detected by: (**A**) a break-apart probe and (**B**) a dual-fusion probe. (**C**) Schematic representation of amplification, deletion and polyploidy FISH signalling patterns detected by an enumeration FISH probe. Amplification shows multiple red signals, deletion shows loss of a red signal, and polyploidy where the probe signal shows multiple sets of chromosomes, observed by 3 pairs of fusion signals.

PUBLICATIONS

- Álvarez EG *et al.* (incl. Torres-Ruiz R, Rodriguez-Perales S) (2021). Aberrant integration of Hepatitis B virus DNA promotes major restructuring of human hepatocellular carcinoma genome architecture. *Nat Commun* 12, 6910.
- García-Silva S *et al.* (incl. Torres-Ruiz R, Rodriguez-Perales S, Peinado H) (2021). Melanoma-derived small extracellular vesicles induce lymphangiogenesis and metastasis through an NGFR-dependent mechanism. *Nat Cancer* 2, 1387-1405.
- Tejedor JR *et al.* (incl. Torres-Ruiz R) (2021). Integrative methylome-transcriptome analysis unravels cancer cell vulnerabilities in infant MLL-rearranged B cell acute lymphoblastic leukemia. *J Clin Invest* 131, e138833.
- Zanetti SR *et al.* (incl. Torres-Ruiz R) (2021). A novel and efficient tandem CD19- and CD22-directed CAR for B cell ALL. *Mol Ther*. PMID: 34478871.
- Castro-Sánchez P *et al.* (incl. Rodriguez-Perales S, Torres-Ruiz R) (2021). Fast diffusion sustains plasma membrane accumulation of phosphatase of regenerating liver-1. *Front Cell Dev Biol* 8, 585842.
- Monteagudo M *et al.* (incl. Torres-Ruiz R, Rodriguez-Perales S, Robledo M) (2021). Analysis of telomere maintenance related genes reveals *NOP10* as a new metastatic-risk marker in pheochromocytoma/paraganglioma. *Cancers (Basel)* 13, 4758.
- Baquero JM *et al.* (incl. Torres-Ruiz R, Rodriguez-Perales S, Benítez J, Osorio A) (2021). Small molecule inhibitor of OGG1 blocks oxidative DNA damage repair at telomeres and potentiates methotrexate anticancer effects. *Sci Rep* 11, 3490.
- Manso R, Rodríguez-Perales S, Torres-Ruiz R, Santonja C, Rodríguez-Pinilla SM (2021). PD-L1 expression in peripheral T-cell lymphomas is not related to either *PD-L1* gene amplification or rearrangements. *Leuk Lymphoma* 62, 1648-1656.
- Torres-Ruiz R, Grazioso TP, Brandt M, Martinez-Lage M, Rodriguez-Perales S, Djouder N (2021). Detection of chromosome instability by interphase FISH in mouse and human tissues. *STAR Protoc* 2, 100631.
- Calvete O *et al.* (incl. Torres-Ruiz R, Rodriguez-Perales S, Benítez J) (2021). Alterations in SLC4A2, SLC26A7 and SLC26A9 drive acid-base imbalance in gastric neuroendocrine tumors and uncover a novel mechanism for a co-occurring polyautoimmune scenario. *Cells 10*, 3500.

PATENTS

- Malats Riera N, Bork P, Kartal E, Molina Montes E, Rodríguez S, Estudillo L, Real FX, Schmidt TSB, Zeller G, Wirbel J, Maistrenko OM (2021). Faecal Microbiota Signature for Pancreatic Cancer. *EP21382876.7*.
- Rodriguez Perales S, Torres Ruiz R, Martínez-Lage M (2018). Gene Editing Based Cancer Treatment. *WO 2020/079243. National phase entry* (2021).

AWARDS AND RECOGNITION

- 1st prize "Paper of the Year 2020-2021" contest in the gene therapy category for the article: *In vivo* CRISPR / Cas9 targeting of fusion oncogenes for selective elimination of cancer cells (*Nature Communications*, 2021), bestowed by The Spanish Society for Gene and Cell Therapy (*SETGyC*).
- 2021 Molecular Cloud Infinite Possibilities Teams Award, MolecularCloud™ Distinguished Research Awards for Genome Editing 2021.