MOLECULAR CYTOGENETICS AND GENOME EDITING UNIT

Juan C. Cigudosa
Unit Head

Staff Scientist
Sandra Rodríguez

OVERVIEW

Chromosomal translocations are very common events involved in the development of several cancers, especially in sarcomas and haematological malignancies. The research activity of the Molecular Cytogenetics and Genome Editing Unit covers the main topics related to cancer cytogenetics and genome engineering: from classical cytogenetics techniques to new genome engineering tools, including the CRISPR-Cas9 system. We are focusing on the implementation and development of new technologies to enhance knowledge about the biology of tumours and to discover new potential therapeutic targets. With the combined use of CRISPR-Cas9 genome editing and cellular technologies, we are creating *in vitro* models that recapitulate chromosomal and genetic cancer alterations. Members of the Unit also participate in collaborative projects with clinical and basic science investigators across the CNIO and other institutes.

"By way of different molecular approaches, we generate human cancer cell models carrying tumour-associated chromosomal translocations in order to study their functional contribution to oncogenesis."

Post-Doctoral Fellow
Raúl Torres

Technicians
Angelo Bertini (until April) (TS)*, M. Carmen Carralero, M. Carmen Martín, Marta Martínez-Lage (since June) (TS)*, Francisco J. Moya (TS)*

**Titulado Superior* (Advanced Degree)

RESEARCH HIGHLIGHTS

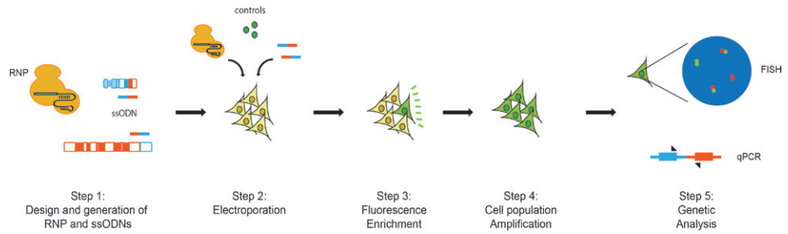

Figure Targeted chromosomal translocations workflow using CRISPR ribonucleoprotein (RNP), GFP molecule controls and single-stranded oligodeoxynucleotides (ssODNs) transfection approach.

Optimising CRISPR-Cas9 to model cancer aberrations in primary cells

In vitro modelling of complex tumour-associated chromosome translocations at native loci is feasible with CRISPR. However, the generation of translocations must be optimised, especially for mimicking events in human primary cells. We have optimised our CRISPR protocol to efficiently obtain those cells, thereby enabling the rescue of translocation+ populations of human primary cells, including induced pluripotent stem (iPS) cells and mesenchymal stem cells (MSCs). These models can surely help us to understand the molecular mechanisms underlying the initiation of human cancers, and can also be used for high-throughput drug screening, toxicological testing and biomarker identification.

From the patient's chromosome translocations to their functional effects

We have worked on the oncogenic role of the translocation t(8;21)(q22;q22)/*RUNX1-RUNX1T1*, which occurs in 4% of acute myeloid leukaemia patients. We deciphered a new function for the activation of MAPK8, observed in t(8;21)+ cells, which is responsible for the stabilisation of SP1. Our data show the essential role of SP1 in t(8;21)+ cell maintenance through the regulation of key genes, such as *CDKN1A*. These results provide new evidence for the inclusion of pharmacological approaches leading to degradation of SP1 in the treatment of these patients.

Technological and translational activities

We provide state-of-the-art molecular cytogenetics and genome editing services. The Unit makes available various techniques to the CNIO Research Groups; these techniques provide more sensitive and accurate tools to analyse cancer cells, such as RNA-FISH, chromosome stability studies based on a combined array CGH-FISH approach, or the use of CRISPR libraries to perform high-throughput functional analysis. For gene editing experiments, we have set up a specific FISH analysis to detect genomic integration sites of small constructs including LV particles. In 2016, we carried out over 1,000 assays for experimental and clinically-oriented projects. ■

PUBLICATIONS

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- Torres-Ruiz R, Rodriguez-Perales S (2016). CRISPR-Cas9 technology: applications and human disease modelling. *Brief Funct Genomics*. PMID: 27345434