

Molecular Cytogenetics Group

Summary

In almost all instances, chromosomes in human cancer cells show structural and/or numerical rearrangements. These types of mutations target genes and other non-coding genomic elements which become aberrantly expressed as a consequence of the rearrangement. Fusion genes arising from chromosomal translocations play a major role in oncogenesis and have in the past offered the rationale for molecular therapies. Our Group's main focus is the discovery of new fusion genes and the study of the biological and genomic effects of genetic aberrations in tumour cell behaviour.

Strategic Goals

- Characterise molecular cytogenetic markers such as chromosome translocations and copy number variations (CNV) in solid tumours and leukaemia
- Design of human cellular models with inducible chromosome translocations to study their genetic role in oncogenesis
- Provide CNIO researchers with state-of-the-art molecular cytogenetic technology such as spectral human and mouse karyotypes (SKY), fluorescence *in situ* hybridisation (FISH) diagnosis and customised designed FISH probes, and array-based CGH

Juan C. Cigudosa Group Leader

Juan C. Cigudosa was born in San Adrian, Navarra, Spain, in 1964, graduated in Biological Sciences and obtained his PhD from the *Universidad de Navarra* in 1991, for which received the 1992 First Class Honours Award.

He carried out his postdoctoral studies (1993 – 1997) at the Paterson Institute for Cancer Research, Manchester, UK, and at the Genetic Service of the Memorial Sloan Kettering Cancer Centre (MSKCC), New York (USA), during which time, he contributed to the clinical cytogenetic management of patients focusing on the study of genomic alterations occurring in lymphoma and myeloma.

He returned to Spain in 1998 to work as a Genetic Consultant at the *Hospital Universitario de La Laguna*, Tenerife. He joined the CNIO in the year 2000 where he has been responsible for providing extremely specialised, cutting edge technologies in molecular cytogenetics such as spectral karyotyping (SKY), fluorescence *in situ* hybridisation (FISH) for diagnosis and research, and array-based comparative genomic hybridisation (aCGH) for the analysis of genomic aberrations.

His research has focused on the role of chromosome aberrations in multiple myeloma, acute myeloid leukaemia and childhood sarcomas. His scientific contributions have been recognised through the 1998 Award to the Best Young Investigator in Human Genetics of the *Asociación Española de Genética Humana* (AEGH) and the Plate of Honour of the *Asociación Española de Científicos* (AEC) in 2002.





Staff scientists: Sara Álvarez, Sandra Rodríguez. **Graduate students:** Francesco Acquadro, Bibiana I. Ferreira, Ana del Rio, Alba Maiques, Juliane Menezes (since November), Jaroslaw K. Sochacki. **Technicians:** M. Carmen Carralero, Miguel A. Grillo, M. Carmen Martín, Gloria Soler.

Highlights

Acute myeloid leukaemia (AML)

After an exhaustive genome-wide array CGH analysis of *de novo* AML cases, we have accumulated increasing evidence suggesting that, in addition to genetic aberrations, therapeutically reversible epigenetic events also play a critical role in the pathogenesis of human leukaemia. To reveal the nature and characteristics of this role, we employ high-throughput

methylation profiling to systematically explore the epigenomic variation underlying the biological and clinical heterogeneity found in AML.

In addition, we work with a model system of human progenitor hematopoietic stem cells (HSC) expressing AML1/ETO, CBF β /MYH11 or MLL/AF9 fusion proteins in a collaborative project with J.C. Mulloy, the Division of Experimental

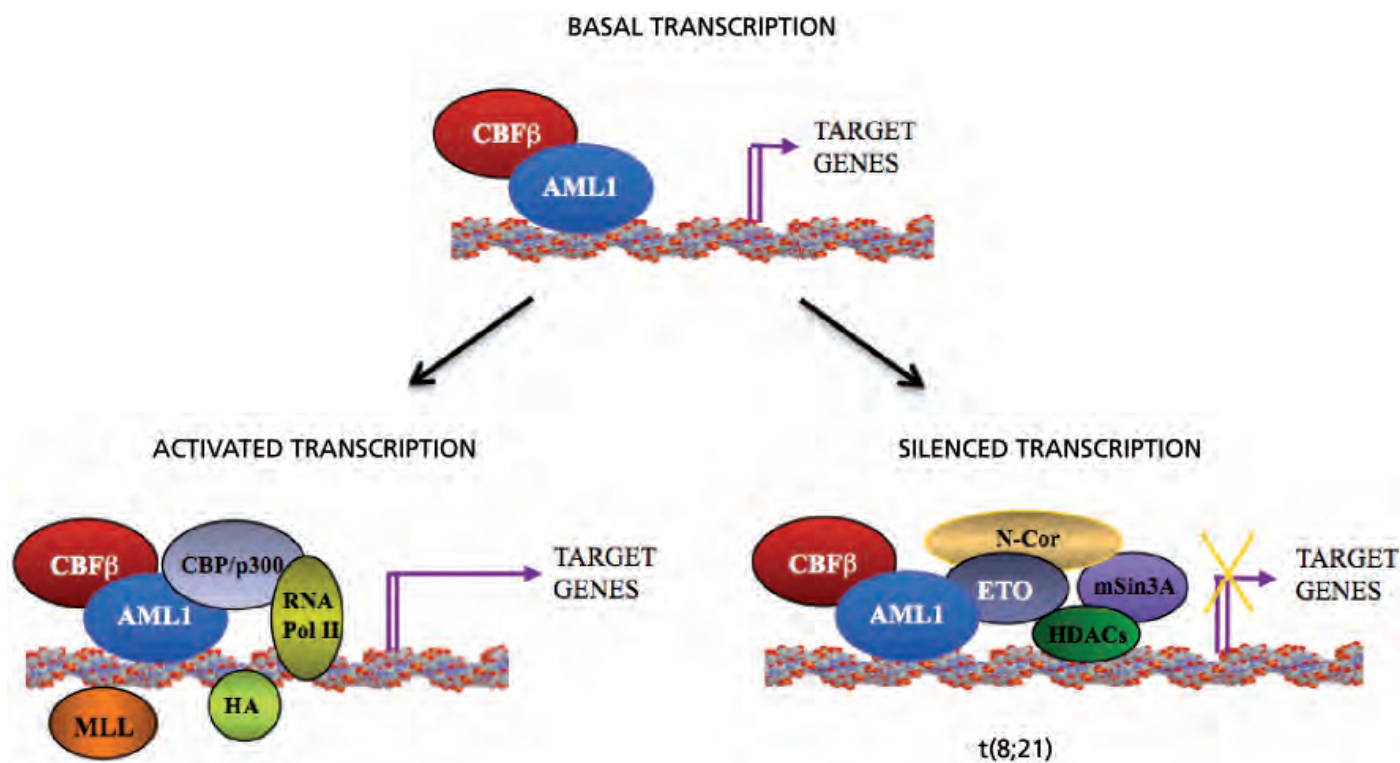


Figure 1: Schematic representation of transcriptional regulatory conformations of the AML1 gene. To be fully operational, the CBF transcriptional complex requires other proteins (CBP/300, MLL, RNA PolII). Upon a reciprocal translocation, t(8;21), the fused gene AML1/ETO silences transcription of target genes by recruiting some co-repressor molecules with histone deacetylase activity.

Haematology, Cincinnati Children's Hospital Medical Centre (USA). We observed that AML samples are correctly separated from bone marrow (BM) controls and do segregate into two main categories. While one of them shows a rather plane profile (similar to the one observed in the control BM), the other presents dramatic changes with an aberrant methylation signature. A significant difference on the distribution of AML cases based on their cytogenetic characterisation is observed among the two methylation categories.

Taking advantage of our model using hematopoietic stem cells stably transfected with the fusion proteins, we found that the epigenetic signature of MLL leukaemia may also be observed in human progenitor cells fully transformed

in vitro by this single oncogenic event. However, HSC expressing the AML1/ETO and CBF/MYH11 fusion proteins (Figure 1), which do not show a full transformed phenotype, do not recapitulate the methylation signature observed in the AML primary cases.

These results suggest that a dramatic change in the epigenetic profile of a myeloid cell is observed in the presence of single genetic abnormalities such as t(8;21), t(15;17) or MLL rearrangements. We are also investigating alternative fusion gene transcripts (Figure 2) that may act as secondary genetic events and be required for a full leukaemic transformation.

Multiple myeloma

In the context of whole genome analysis, we – and others – have reported the occurrence of mutations in genes causing the inactivation of TRAF3, TRAF2, API2 or API1 genes. By array CGH analysis, we have identified the presence of a homozygous deletion covering the API2 gene locus in 2 cases out of 26, as well as in an additional case with a hemizygous deletion (around 10% of the cases). To further validate this result, we are conducting a FISH (with a customised probe) and alternative molecular analyses, to investigate the status of the API2 gene in a new cohort of MM patients. Preliminary data suggest that the deletion of this gene is a frequent event and, subsequently, a deeper evaluation of the role of API2 in multiple myeloma is warranted.

Generation of biological models and tools to study the role of chromosome translocations in cancer

Two ongoing projects deal with chromosome translocations. Firstly, we are carrying out a multidisciplinary evaluation of the role of reciprocal chromosome translocations and gene fusions in the pathogenesis of epithelial tumours. By *in silico* analysis we have found that some chromosome translocation may be present in colon and pancreatic cancer. We have produced a list of candidate genes that

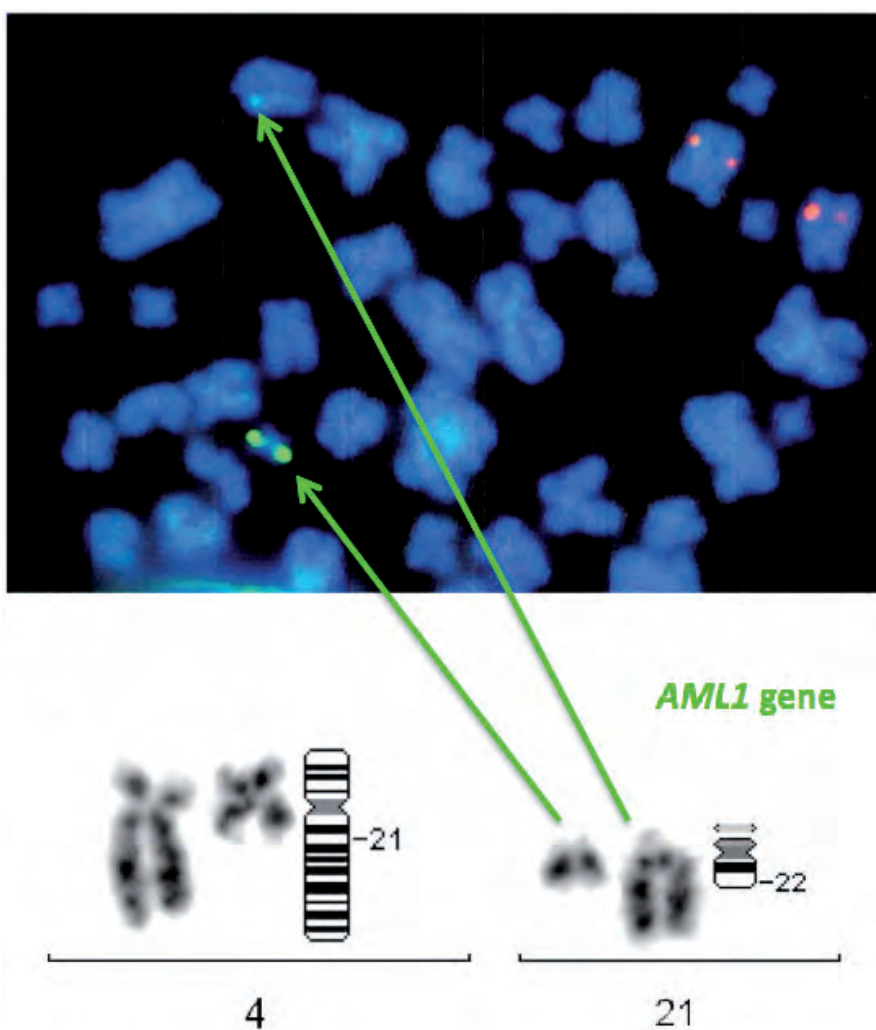


Figure 2: A new fusion partner for the AML1 gene. A new translocation has been found in a *de novo* AML case of that involves chromosomes 4 and 21 (left panel) and results in the rearrangement of the AML1 gene (labelled in green in the FISH plate shown in the right panel).

may be involved in these translocations and are currently conducting *in vitro* assays to further validate these findings. Secondly, we are engaged in the design of human stem cell-based cellular models that, upon chemical exposures, can be genetically engineered to develop a previously designed cancer-related chromosome translocation. We have started to model translocations such as the t(8:21)(q21,q12); fusing the AML1 and ETO genes and the t(7;11)(p15;p15); that fuses the NUP98 and HOXA9 genes. Both translocations occur in stem cells involved in the myeloid differentiation pathway and give rise to different types of AML (Figure 2).

Molecular cytogenetics service

Our group provides a state-of-the-art molecular cytogenetic service to both CNIO researchers and the clinical and research community externally. In 2009 we carried out over 2,000 assays

including high resolution karyotyping of leukaemia and other tumour samples, design of FISH probes (Figure 2), spectral karyotyping (SKY) of human and mouse tumours and cell lines, aneuploidy analysis for mouse models, and aCGH for experimental and clinically-oriented projects.

As a reference laboratory in Molecular Cytogenetics, we are participating in several clinical assays, collaborative networks, and quantity performance studies – both at the national and European level where our expertise is required.

Publications

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