

Molecular Cytogenetics Group



Juan C. Cigudosa

Group Leader

Juan C. Cigudosa, 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 he 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 Center (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 September 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. Juan's scientific contributions have been recognised through the 1998 Award to the Best Young Investigator in Human Genetics from the *Asociación Española de Genética Humana* (AEGH) and the Plate of Honour from the *Asociación Española de Científicos* (AEC) in 2002. This year, 2010, he has been elected as a Member of the Board of Directors of the European Cytogeneticists Association (ECA).

Summary

In almost all cases, 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. An oncogene may therefore be transferred to a genomic region resulting in its ectopic expression, a new chimaeric gene may be formed after the rearrangement displaying new or altered activities, and a tumour suppressor gene may be disrupted, its expression abrogated. All these events play a major role in oncogenesis and have in the past provided the rationale for molecular therapies. Our Group's main interests surround the discovery of new fusion genes, the study of the biological and genomic effects of genetic aberrations in the tumour cell behaviour and the translation of these findings into putative applications in the clinic.

Strategic Goals

- Design of human cellular models with inducible chromosome translocations to study their genetic role in oncogenesis
- Characterise molecular cytogenetic and epigenetic markers such as chromosome translocations and copy number variations in solid tumours and leukaemia
- Provide molecular cytogenetic technology such as spectral human and mouse karyotypes and customised designed FISH probes



Staff scientists: Sara Álvarez and Sandra Rodríguez. **Graduate students:** Francesco Acquadro, Bibiana I. da Silva (until March), Ana del Río, Alba Maiques, Juliane Menezes and Jaroslaw K. Sochacki. **Technicians:** M. Carmen Carralero, Almudena Gil (since March), Miguel A. Grillo, M. Carmen Martín and Gloria Soler (until April).

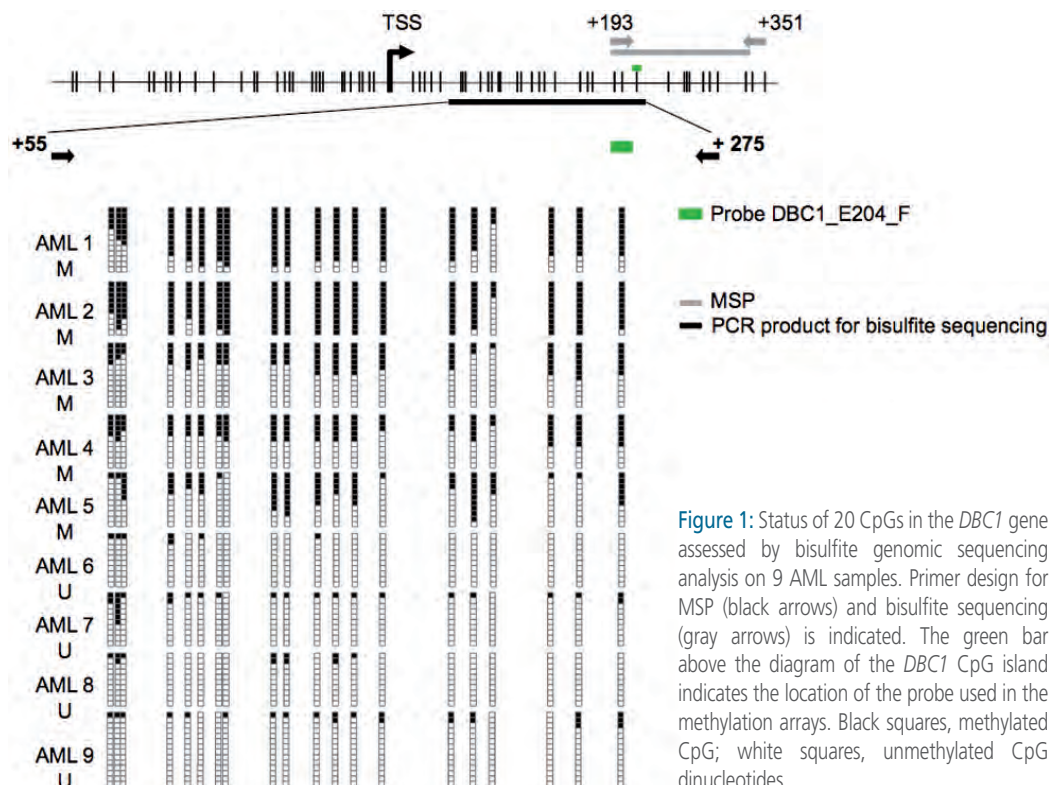
Highlights

Epigenomics and chromosome translocations in acute myeloid leukemia (AML)

After an exhaustive genome-wide array CGH analysis of *de novo* AML cases we have accumulated increasing evidence to suggest 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 biologic and clinical heterogeneity found in AML.

We carried out high-throughput methylation profiling on 116 *de novo* AML cases and validated key biomarkers in an independent cohort of 244 AML cases. Methylation signatures were found to be associated with the presence of a specific cytogenetic status. In cases with normal karyotype, the aberrant methylation of the *DBC1* promoter was validated as



a predictor of disease-free and overall survival (Figure 1). In addition, DBC1 expression was significantly silenced in the aberrantly methylated samples, thus, warranting more functional studies with this molecular marker.

Patients with chromosome rearrangements showed distinct methylation signatures. We also work with a model system of human progenitor hematopoietic stem cells expressing AML1/ETO, CBF β /MYH11 or MLL/AF9 fusion proteins in a collaborative project with J.C. Mulloy from the Division of Experimental Haematology, Cincinnati Children's Hospital Medical Center (USA). Using this model, we established the role of fusion proteins in the epigenetic profiles. These data were analysed and compared with patient samples carrying the same rearrangements.

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

Several 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 translocations may be present in colon, bladder and pancreatic cancer. We are also adopting several genomic approaches to gain insight into a possible cause of the chromosome aneuploidy affecting multiple myeloma cells: array-CGH and microRNA expression profiling. Finally, we are designing human stem cell-based models of cell lines that are genetically and chromosomally stable (Figure 2) and that 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), that fuses 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.

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 2010 we carried out over 2,000 assays including high resolution karyotyping of leukaemia

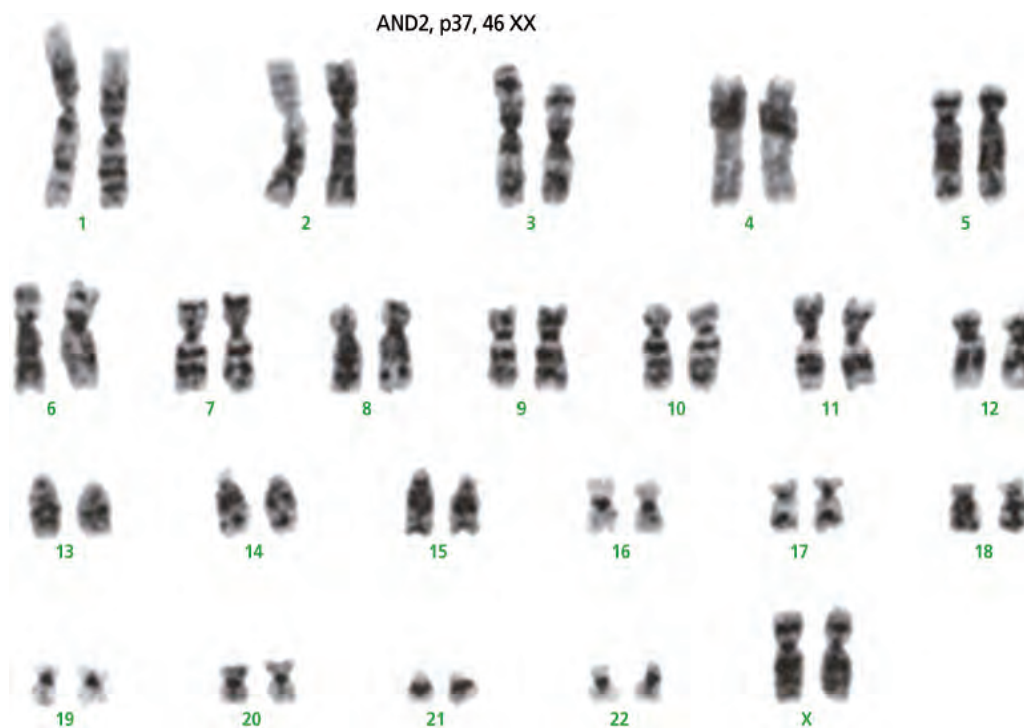
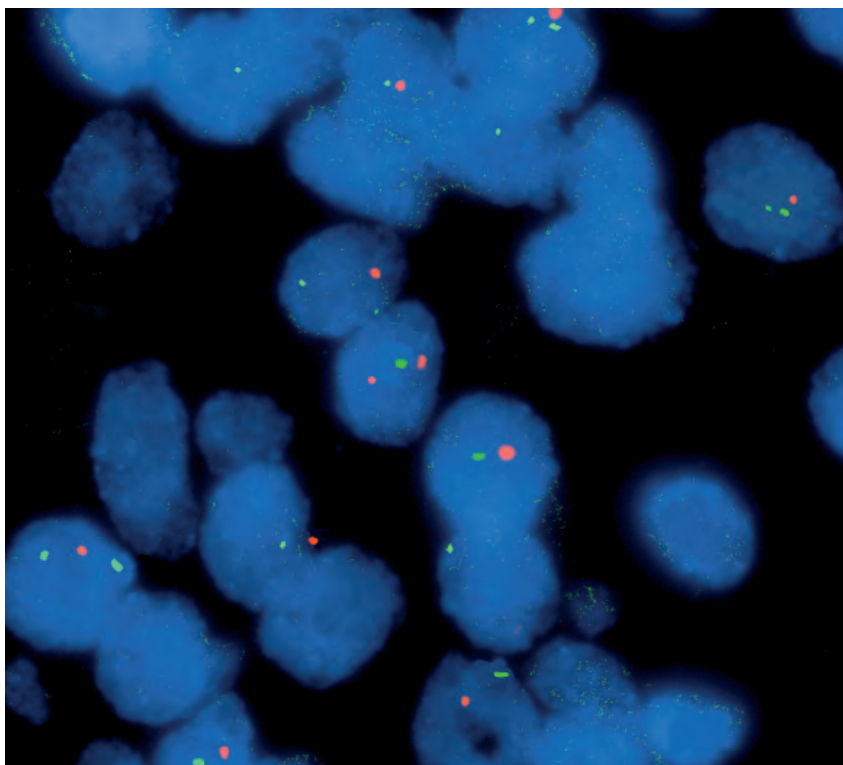


Figure 2: G-banded karyotype of a human embryonic stem cell line. This particular cell line, after 37 cell culture passages, still displays a normal female karyotype, 46, XX.



and other tumour samples, design of FISH probes (Figure 3), 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 for Molecular Cytogenetics, we are participating in several clinical assays, collaborative networks, and quantity performance studies – both at the national and European level.

Figure 3: Validation of a new FISH probe on a paraffin-embedded tissue. The image depicts a section of a colon cancer tissue sample hybridised with: a FISH probe for the *ETV6* gene (red signal) and a FISH probe the centromeric regions of chromosome 12 (green signal, serving as a control for aneuploidy). Blue circles correspond to the nuclei. Red and green signals can be identified inside the nuclei, showing the number of copies of the gene of interest versus the number of copies of the control chromosome.

Publications

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Awards and Recognition

Member, Board of Directors, European Cytogeneticists Association (2010-2014)