ACUTE LEUKAEMIA IN CHIMERIC MICE BY A *bcr-ABL^{p190}* FUSION GENE MADE BY HOMOLOGOUS RECOMBINATION

Andrés CASTELLANOS[1], Belén PINTADO[2], Eduardo WERUAGA[3], Rosario AREVALO[3], Antonio LOPEZ[4], Alberto ORFAO[4] and Isidro SANCHEZ-GARCIA[1]

[1]Departamento Proliferación-Diferenciación, Instituto Microbiología Bioquímica, CSIC/Universidad de Salamanca, Edificio Departamental, 37007-Salamanca; [2]Area de Reproducción Animal Centro de Investigación y Tecnología , Ctra de la Coruña Km 5.9, 28040-Madrid; [3]Departamento Biología Celular y Patología y [4]Servicio de Citometría, Universidad de Salamanca.

Fusion proteins are commonly the result of chromosomal translocations in leukaemias and solid tumours in which chromosomal breaks occur within genes on each chromosome. In assessing the relevance of such proteins in tumorigenesis, in vivo experimental models are needed that facilitate the study of tumor development and progression and overt tumor outgrowth. Transgenesis is an important method that allows various stages of tumour development to be studied. However, it has the difficulty of choosing a promoter to engineer expression in the appropriate cell type in vivo. An homologous recombination strategy could therefore be utilized to mimic the consequence of chromosomal translocations by fusion of coding sequences into an endogenous gene.Thus, in-frame fusion of sequences with the endogenous gene in embryonal stem (ES) cells would create a fusion gene that utilizes the endogenous promoter to control expression, as would occur following chromosomal translocation.

A well-characterized example of chomosomal abnormality involves the rearrangements of the *BCR* and *ABL* genes in Philadelphia chromosome-positive chronic myelogenous leukaemia and acute lymphocytic leukaemia. Depending on the precise breakpoint within the *BCR* gene, fusion proteins of 210 kDa (p210) or 190 kDa (p190) are produced which are associated with chronic and acute leukaemias, respectively.

We have used homologous recombination in ES cells to create an in-frame fusion of *ABL* with exon 1 of mouse *bcr* in order to produce a *bcr-ABLp190* chimaeric gene. Mutant ES cells were introduced into blastocysts, which were used to produce chimeric mice. These chimeric mice develop hematological tumors which were only acute lymphoid leukaemias. *bcr-ABLp190* chimaeric mice began to show signs of distress from 4 months of age characterized by indolent habit, slow movement, and ruffled hair. Postmortem examination showed consistent evidence of hematological disease, which was characterized as acute leukemia due to hypercellularity, and blasts accounted for more than 30% of bone marrow cells. While a high proportion of *bcr-ABLp190* mice developed acute leukaemia, no disease appeared in control group at 12 months. This does not appear simply to reflect the lack of ES cell contribution in these mice, since the level of chimerism, evaluated by coat color, was as high as 90% in some mice. The leukaemic proliferation was clonal by analysis of immunoglobulin gene rearrangement in spleen DNA.

When in the *bcr-ABLp190* ES cells the normal *bcr* allele was disrupted by homologous recombination and the cells injected to produce chimeric mice, these mice also develop acute lymphoid leukamias. These data show that *bcr-ABLp190* fusion protein does not require the endogenous *bcr* gene product to induce tumour development.

Our results provide proof that *bcr-ABLp190* fusion gene is crucial for Philadelphia chromosome-positive acute lymphocytic leukaemia.

References

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