

Chromosomal Abnormalities, Cancer and Mouse Models: The Critical Role of Translocation-Associated Genes in Human Cancer

I. Sánchez-García*

Departamento de Diferenciación y Proliferación Celular, Instituto de Microbiología Bioquímica, CSIC/ Universidad de Salamanca, Edificio Departamental, Avda del Campo Charro, s/n, 37.007-Salamanca, Spain



Abstract: Cancer results from subversion of the processes that control the normal growth, location and mortality of cells. This loss of normal control mechanisms arises from the acquisition of mutations in three broad categories of genes: proto-oncogenes, tumor suppressor genes and DNA repair enzymes. Proto-oncogene activation may occur by mutation, gene amplification or DNA rearrangement. Chromosomal translocations entail the generation of gene fusions in both haematopoietic and solid mesenchymal tumors. Despite the successful identification of these specific and consistent genetic events, the nature of the intimate association between the gene fusion and the resulting phenotype is pending to understand. The application of transgenic methods to the study of these cancer-associated gene fusions have provided insights into their *in vivo* functions and suggested mechanisms by which lineage selection may be achieved. Herein these studies are reviewed to illustrate how manipulation of their loci in the mouse have contributed to current understanding in unique and unexpected ways.

It has been realized for many years that cancer has a genetic component and at the level of the cell it can be said to be a genetic disease. In 1914, Boveri suggested that an aberration in the genome might be responsible for the origin of cancer. This was subsequently supported by the evidence that cancer, or the risk of cancer, could be inherited; that mutagens could cause tumors in both animals and humans; and that tumors are monoclonal in origin, that is, the cells of a tumor all show the genetic characteristics of the original transformed cell. It is only in recent years that the involvement of specific genes has been demonstrated at the molecular level. Cancer cells contain many alterations with accumulate as tumors develop. Over the last 20 years, considerable information has been gathered on regulation of cell growth and proliferation leading to the identification of the proto-oncogenes and the tumor suppressor genes.

WHAT IS CANCER?

In normal cell growth there is a finely controlled balance between growth-promoting and growth-restraining signals such that proliferation occurs only when required. The balance is tilted when increased cell numbers are required, for example

during wound healing and during normal tissue turn-over. Differentiation of cells during this process occurs in an ordered manner and proliferation ceases when no longer required. In malignant tumor cells this process is disrupted, continued cell proliferation occurs and loss of differentiation is found. In addition the normal process of programmed cell death may no longer operate. Cancer arise from a single cell which has undergone mutation. Mutations in genes such as those described below give the cell increased growth advantages compared to others and allows them to escape normal controls on proliferation.

IDENTIFICATION OF ONCOGENES

Oncogenes were first directly identified in viruses capable of inducing tumors in animals and/or of transforming cells *in vitro*. There are presently nearly 200 known oncogenes that, under certain conditions, can contribute to the release of cells from normal controls of proliferation, death, migration and adhesion to cause neoplastic transformation. It is probable that the majority of genes that possess oncogenic potential have now been identified and this figure of approximately 200 proto-oncogenes from about 60.000 functional human genes thereby sets an upper limit to the number of points at which the biochemical pathways controlling normal cell growth might be subverted by oncoproteins. Fortunately, the actual number of general mechanisms is probably much smaller, as

*Address correspondence to this author at the Departamento de Diferenciación y Proliferación Celular, Instituto de Microbiología Bioquímica, CSIC/ Universidad de Salamanca, Edificio Departamental, Avda del Campo Charro, s/n 37.007- Salamanca, Spain; Tel: +34-923-238403; Fax: +34-923-224876; E-mail: isg@gugu.usal.es

oncoproteins fall into groups of similar activity and the members of each group are presumed to act at corresponding points in signalling pathways (Fig. 1). In the main, oncogene activation is the result of somatic events rather than hereditary genetic causes transmitted by mutation in the germline. It is, in other words, a consequence of evolution (mutation and selection) within the body of one animal.

diseases, many investigators have endeavored to define genetic alterations that are specific for a given cancer category. Though such cancer type-specific alterations have generally not been found in the common epithelial malignancies, there has been considerable success in studies of mesenchymal tumors. A series of gene fusions resulting from chromosomal translocations have been identified in the leukemias, lymphomas and sarcomas (for a review see references [1] and [2]). The

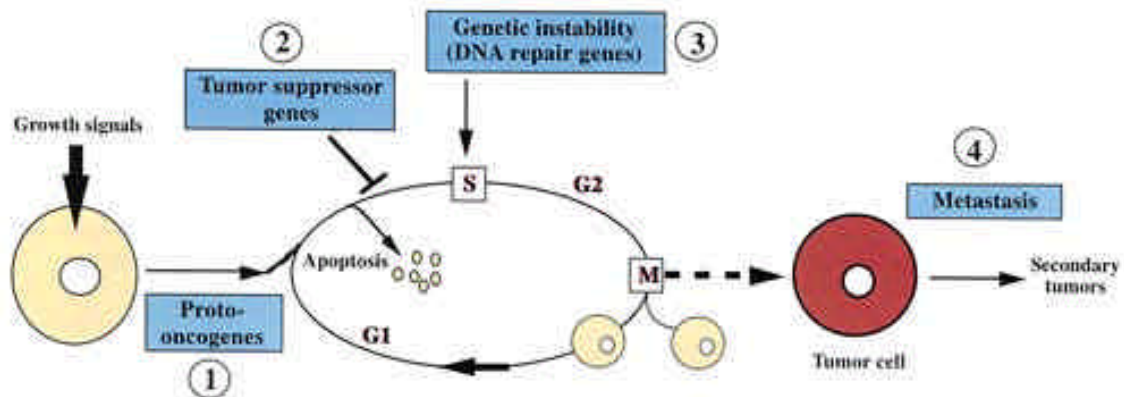


Fig. (1). Genetic signals in cancer development. Three broad functional categories may be distinguished within which mutations may arise: (1) and (2) pathways driving cell proliferation and controlling cell cycle progression and apoptosis, (3) in the promotion of genetic instability through mutations in DNA repair genes, and (4) effects associated with metastasis.

ONCOGENE ACTIVATION BY CHROMOSOMAL TRANSLOCATIONS IN HUMANS

The fact that human cancers do not generally appear to be caused by retrovirally activated oncogenes raises the question of how proto-oncogenes become activated in human cancers. In normal cells proto-oncogene activation may occur by mutation, DNA rearrangement or gene amplification (Fig. 2). Chromosomal translocations entail the generation of gene fusions in both haematopoietic and solid mesenchymal tumors (for a review see references [1] and [2]). Despite the successful identification of these specific and consistent genetic events, the nature of the intimate association between the gene fusion and the resulting phenotype is pending to understand. The application of transgenic methods to the study of these cancer-associated gene fusions have provided insights into their *in vivo* functions and suggested mechanisms by which lineage selection may be achieved. Herein, these studies are reviewed to illustrate how manipulation of their loci in the mouse have contributed to current understanding in unique and unexpected ways.

Following the common belief that different categories of cancer represent biologically distinct

translocations juxtapose portions of two cellular genes to generate chimeric gene products and/or alter regulation of gene expression, thereby providing a putative oncogenic stimulus (Fig. 2).

From a biological perspective, the successful identification of these specific and consistent genetic events now demands that we explain the nature of the intimate association between the gene fusions, the phenotype with which they are associated and the target cell from which the cancers arise. Due to the absence of a direct link between a cell carrying the cytogenetic abnormality and a test of whether this cell has the capacity to maintain the disease *in vivo*, two different hypothesis try to explain the link between the gene fusion and the resulting phenotype. One hypothesis suggests that many cell types in the stem/progenitor hierarchy are susceptible to transformation [3, 4]. The gene fusion event would alter the normal development program, resulting in the expansion of abnormal cells that are blocked at a particular stage of differentiation. The degree of commitment of the target cell influences the characteristics of the resulting tumor cells. The second hypothesis suggests that mutations responsible for transformation and progression occur in primitive cells only [5]. According to this, the phenotype results from the ability of these primitive target stem

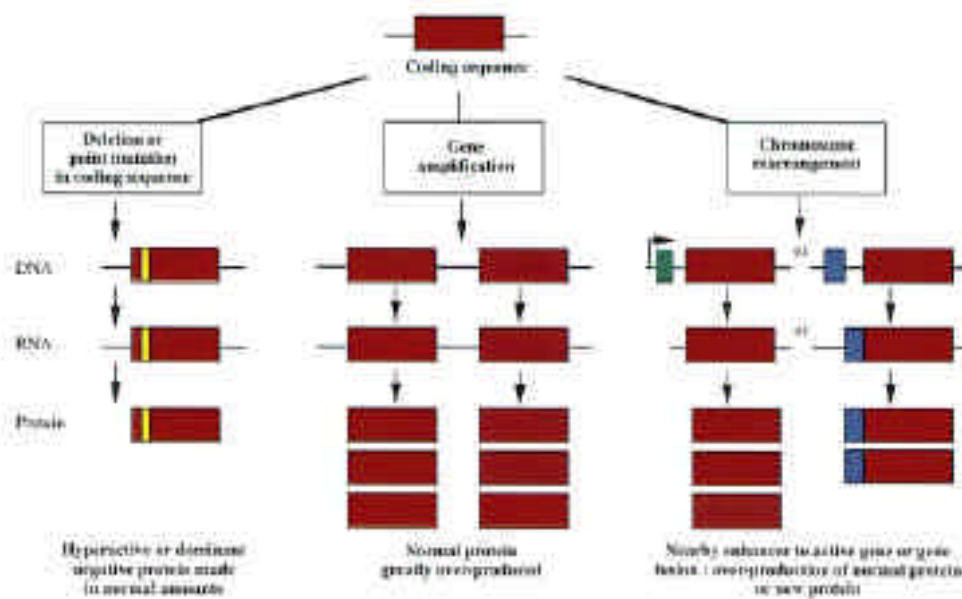


Fig. (2). Schematic representation of mechanisms of oncogene activation.

cells to differentiate, depending on the influence of the specific gene fusion. This article will examine the data from the knock-in mouse model systems that investigate the link between the oncogenic gene fusions and the resulting phenotype in human cancer.

PROTO-ONCOGENES AND LINEAGE SPECIFICITY

According to the pattern of expression, the proto-oncogenes can be divided into two different categories. The former category includes proto-oncogenes such as members of the RAS family and tumor-suppressor genes such as p53. The associated gene products generally have a widespread pattern of expression and function within fundamental signal-transduction pathways in the mammalian cell. Such genes are often altered in a variety of cancers because their gene products are part of a general lineage-independent mechanism that regulates transit through the cell cycle. In contrast, despite the apparent diversity of gene rearranged by tumor type-specific fusion event, one of the two gene products involved in each fusion often functions in lineage-specific developmental pathways. Examples include ABL and CHOP that are rearranged in leukemias [6, 7] and sarcomas [8, 9], respectively. Such gene products have a more restricted pattern of expression or function, and contribute to the control of growth, differentiation and survival within a restricted set of cell types. Support for their lineage-specific role comes from the finding of specific developmental defects in mice

with inactivating mutations of these genes [10-12]. This suggests that these gene fusions may only exert their oncogenic influence in certain cellular environments through a lineage-dependent mechanism, in spite of the gene fusion expression as a result of chromosomal rearrangement is usually regulated by endogenous elements from the 5' partners which have a widespread pattern of expression, such as BCR, FUS/TLS, MLL, SIL [1, 2].

INFLUENCE OF LINEAGE IN CELL MODELS OF FUSION GENES

The possibility that these gene fusions may only exert their oncogenic influence in certain cellular environments through a lineage-dependent mechanism indicates that caution must be exercised when interpreting the phenotypic effect of these gene fusions in heterologous environments. In particular, the activity of these gene fusions in model cell-culture systems may not be appropriate indicators of their actual function in the specific tumors in which the fusion specifically occurs. For example, several of these fusions are not transforming in NIH-3T3 mouse fibroblasts [1]. For a variety of these gene fusions, more relevant cell-culture systems have been developed in which the effect of the fusion on growth, differentiation and survival can be examined in cell types related to the specific associated lineages [13-16].

For the gene fusions that generate chimeric transcription factors, the influence of cellular

environment has been further examined by monitoring changes in expression of downstream genes. Genes whose expression was induced in NIH-3T3 cells by the E2A-PBX1 fusion [17], which is typically found in pre-B cell acute lymphoblastic leukemias, were not expressed in E2A-PBX1 immortalized mouse myeloblasts, nor in human pre-B cell leukemia lines. These findings indicate that transcriptional function of these chimeric proteins is cell-type specific. This intimate relationship between gene fusions and cell lineage must be considered in the design of future cell culture models to explore the role of these gene fusions. As additional lineage markers are identified by developmental and cell biologists, these markers must be examined in the tumors to precisely define the characteristics of the tumor lineage. In turn, these lineage characteristics must be incorporated into the selection of such variables as cell lines and retroviral constructs to introduce the gene fusion into smaller cellular subsets. Then, functional endpoints must be compared among multiple lineages in these cell-culture models to dissect how the effect of the fusion product is influenced by the different cell types.

TRANSGENIC MOUSE MODELS OF GENE FUSIONS

Further issues of lineage specificity have been investigated in transgenic mouse models in which these fusions are introduced into the germline (Table 1). In these transgenic experiments, the phenotype is highly influenced by the choice of an attached expression cassette that regulates when and where the transgene is expressed. Furthermore, unlike the translocations that occur sporadically in single cells during prenatal or postnatal development, the transgenic fusion genes will be expressed in all developing and/or adult cells in which the expression cassette is active. The difficulties with this approach are illustrated by initial attempts to express the BCR-ABL fusion under control of the BCR promoter [18]. The absence of live transgenic animals in these experiments was attributed to transgene expression in various developing lineages that resulted in pleiotropic lethal effects. However, several transgenic models of leukaemia-associated fusions have been described. In transgenic mice expressing BCR-ABL from the metallothionein-1 promoter, the lineage specificity of the BCR-ABL fusion has only been partly recapitulated [19]. Despite the activity of the promoter in a wide range of tissues, transgenic mice expressing BCR-ABL from this promoter specifically developed lymphoid or myeloid

leukemias. This finding indicates that BCR-ABL may preferentially exert oncogenic effects in the hematopoietic compartment.

Transgenic models resembling acute promyelocytic leukemia were successfully generated by directing PML-RARA expression to the early myeloid lineage [20, 21]. However, expression of the PML-RARA from a promoter which is active in myelomonocytic cells but not myeloid progenitor resulted in impaired myelopoiesis without leukemogenesis [22], thus, emphasizing that the effect of this fusion is dependent on the stage of haematopoietic development.

In general, the transgenic mouse models have failed to reproduce the intimate relationship between the gene fusions and the resulting phenotype in human cancer. The reason for this lack of success could be related to the tandem-repeat nature of the transgene insertion (often 5-50 copies), which appears to contribute to a phenomenon akin to variegation [23]. The many examples of variegation, co-suppression and related phenomena emphasize the conclusion that mechanisms exist by which different copies of a DNA sequence can intercommunicate in some real sense such as, jointly, their activity state can differ substantially from that of a single copy acting alone. There is a route by which these problems can be avoided: single-copy gene insertions do not appear to variegate. This argues for the use of targeted integration of single transgene constructs by homologous recombination in embryonic stem (ES) cells.

KNOCK-IN MOUSE MODELS OF GENE FUSIONS

The genetic changes underlying human cancer are somatic in nature; therefore, creating germline mutations is not always suitable to produce proper animal models in order to understand the intimate relationship between gene fusions and resulting phenotypes in human cancer. A newly developed tool has opened up many ways to circumvent these problems. This approach employs homologous recombination to generate fusion genes at the endogenous mouse locus in embryonic stem cells followed by blastocysts injections to create chimeric mice and provides not only unprecedented opportunities to dissect the function of chromosomal-associated genes, but also the proper means to query their role in disease processes (Table 2).

Table 1. Transgenic Mouse Models of Genes Activated/Fused in Chromosomal Translocations

Translocation	Tumour type	Construct	Mouse strain	Phenotype
t(8;14)q24;q32)	BL, B-ALL	MTV/c-myc	C57BL/6J	Mammary adenocarcinomas
		E μ lg-c-myc	C57BL/6	Pre-B cell lymphoblastic lymphomas
		E μ -c-myc	C57BL/6	Lymphoma. Leukemia
		E κ -SV40-c-myc	C57BL/6	Lymphoma (35%)
		LTR-c-myc	C57BL/6	Lymphoma (7%)
		SV-c-myc	C57BL/6	Lymphosarcoma. Fibrosarcoma (14%)
		MT-c-myc	C57BL/6	Normal
		GATA1 ^{Pr} -c-MYC	FVB/N	Erythroleukemia
t(14;18)(q32;q21)	FL	bc12-Ig	C57BL/6	Follicular lym ^{Pr} oliferation
		bc12-E μ	SWR/J x SLJ/J	Polyclonal expansion of B-cells
		WAP-bc12-SV40pA	C57BL/6	Increased MMTV-myc-induced tumours
		H2K ^b pro-BCL2-MoMuLV-LTR	C57BL/6	Normal. Increased radioresistance
		E μ -bc12	BJF1	Enhanced B and T cells survival
		E μ -bc12	SCID	Promotes B-lymphoid development
		Ick ^{Pr} -bc1-2	C57BL/6	Apoptosis delayed in T-cells
		Ick ^{Pr} -bc1-2	B6 x C3H	Peripheral T-cell lymphoma
t(1;14)(p32;q11)	T-ALL	CD2 ^{enh} -SR ^{Pr} -tall	----	Normal
		CD2-TAL1	----	Normal
		Ick ^{Pr} -TAL1-hGHpA	C57BL/6	T-ALL
		^{Pr} oxIck ^{Pr} -tall-hGHpA	FVB/N	T-ALL
t(11;14)(p15;q11)	T-ALL	Ick ^{Pr} -Ttg1	C57BL/6	T-ALL
		TCRbenh-RBTN1 ^{Pr} -RBTN1	CBA x C57/B	T-ALL
		Ins ^{Pr} -RBTN1-SV40pA	CBA x C57/B	Normal
t(11;14)(p15;q11)	T-ALL	CD2 ^{Pr} -rbtn2-CD2 ^{enh}	CBA x C57/B	T-ALL
t(10;14)(q24;q11)	T-ALL	IgH μ -HOX11 Ick-HOX11	----- -----	B-cell lymphomas Not viable
Inv(14)& t(14;14)(q11;q32)	T-PLL, T-CLL	Lck ^{Pr} -TCL1-hGHpA	B6C3	T-cell leukemias (long latency)

(Table-1). contd....

Translocation	Tumour type	Construct	Mouse strain	Phenotype
t(14;X)(q32;q28)	T-PLL	CD2-p13MTCP1	-----	T-PLL-like syndrome
t(11;14)(q13;q32)	B-CLL	E μ -SR -CyclinD1-pA-SV40tag	C57BL/6J x SJL/J	Normal
		E μ -VHpr-CyclinD1-globinA	C57BL/6 x C3H	Normal
		MMTV-CyclinD1-SV40pA	-----	Mammary adenocarcinomas
t(14;19)(q32;q13)	B-CLL	E μ -VHpr-BCL3- globinA	C3HHeCrMTV	Lympho ^{Pf} oliferative disorders
t(5;14)(q31;q32)	pre-B-ALL	GFAF-IL3	-----	Motor disfunction
		CMV-II3	FVB/n	Autoimmune disorder
t(1;19)(q23;p13)	pre-B-ALL	E μ -VH-E2A-PBX1a-SV40pA	FVB/N	T-lymphoblastic lymphoma
t(9;22)(q34;q11)	CML, B-ALL	E μ -VH-bcr-v-abl-SV40pA	C57BL/6/J Wehi x SJL/J Wehi	Pre-B or T-lymphoma (25%)
		MPSV-LTR-bcr-v-abl-SV40pA	C57BL/6/J Wehi x SJL/J Wehi	Pre-B or T-lymphoma (25%)
		E μ -gag-v-abl	BSF1	Plasmacytomas
	B-ALL	MT-p190bcr-ab1	C57BL/6 x CBA	Myeloid or B-lymphoid AL
	B-ALL	BCRpr-p210bcr-abl	-----	Embryonic lethal
	CML	MTpr-p210BCR-ABL	C57BL x CBA	B- and T-cell leukemias
	CML	MTpr-p210bcr-abl-SV40PA	C57BL/6 x DBA/2	T-cell leukenias
	CML	Tec-p210bcr-abl-SV40pA	C57BL/6 x DBA/2	ALL (founder). CML-like (F1)
t(15;17)(q21;q21-22)	ANLL (M3, APL)	CD11bpr-PML-RARA	-----	Myelopoiesis impairment
		hCGpr-PML-RARA	C57BL/6 x C3H/He	AML(30%, long latency)
		hMRP8-PML-RARA	FVB/N	APL (low frequency, long latency)
		hCGpr(+5' flank)-PML-RARA-Catb	-----	APL (low frequency, long latency)
		Actinpr-PML-RARA	-----	Embryonic lethal
		MT-PML-RARA	C57BL/6 x SJL/J	Hepatocellular carcinoma
t(1;7)(q34;q34)	T-ALL	lckpr-lck(exons1-12)-hGHpA	DBA2 x C57BL/6J	Thymic tumours
inv(16)(p13;q22)	ANLL	hMRP8pr-PEBP2b-MYH11	FVB/N	Neutrophil impairment

Abbreviations used: Ch: chimeric; Het: heterozygous; BL: Burkitt's lymphoma; FL: follicular lymphoma; AML: acute myelogenous leukemia; ALL: acute lymphoblastic leukemia; PLL: ^{Pf}olymphocytic leukemia; CLL: chronic lymphocytic leukemia; CML: chronic myelogenous leukemia; ANLL: acute nonlymphoblastic leukemia; pA:polyA; tk: tyrosine kinase; pr:promoter; enh: enhancer.

Table 2. Knock-in Mouse Models of Genes Fused in Chromosomal Translocations

Translocation	Tumour type	Construct	Mouse strain	Phenotype
t(9;11)(p22;q23)	AML	HSVtk-Mll(exon8)-AF9pA-MC1neopA	C57BL/6	AML (Ch)
t(9;22)q34;q11)	ALL	MC1neopA-ber(exon1)-ABL-pA	C57BL/6	ALL (Ch). Lethal(Het)
t(8;21)(q22;q22)	AML	AML1(exon5)-ETO-Neo-HSV-tk	C57BL/6	Normal(Ch). Embryonic lethal(Het)
		AML1(exon4)-ETO-Neo-Difteria tox. A	C57BL/6	Normal(Ch). Embryonic lethal(Het)
inv(16)(p13;q22)	AML(M4E ₀)	PGKtk-Cbfb(exon5)-MYH11-PGKNeo	C57BL/6	Normal(Ch). Embryonic lethal(Het)

Abbreviations used: Ch: chimeric; Het: heterozygous; BL: Burkitt's lymphoma; AML: acute myelogenous leukemia; pA: polyA; tk: tyrosine kinase; pr: promoter; enh: enhancer

In contrast to the transgenic mice models described above, only a single copy of the fusion gene is generated by inserting the 3' end of the fusion cDNA into the endogenous locus of the 5' partner, and in this position, fusion gene expression is regulated by endogenous elements from the 5' partner (Fig. 3). Making chimeras between wild-type blastocysts and mutant ES cells provides one of these easiest ways to attain a nondirected restriction for genome alteration (a genome

alteration that occurs in a restricted but random manner, so that a limited number of cells in the organism undergo the genomic alteration, but they can be any cells in the organism). Normal ES cells can contribute to any cells in the embryo proper as well as the amnion and yolk sac mesoderm. Thus, if the mutant ES cells have a biased contribution in the embryo or animal, it can be very informative about the nature of the defect caused by the cancer gene. Chimera studies have been also useful in

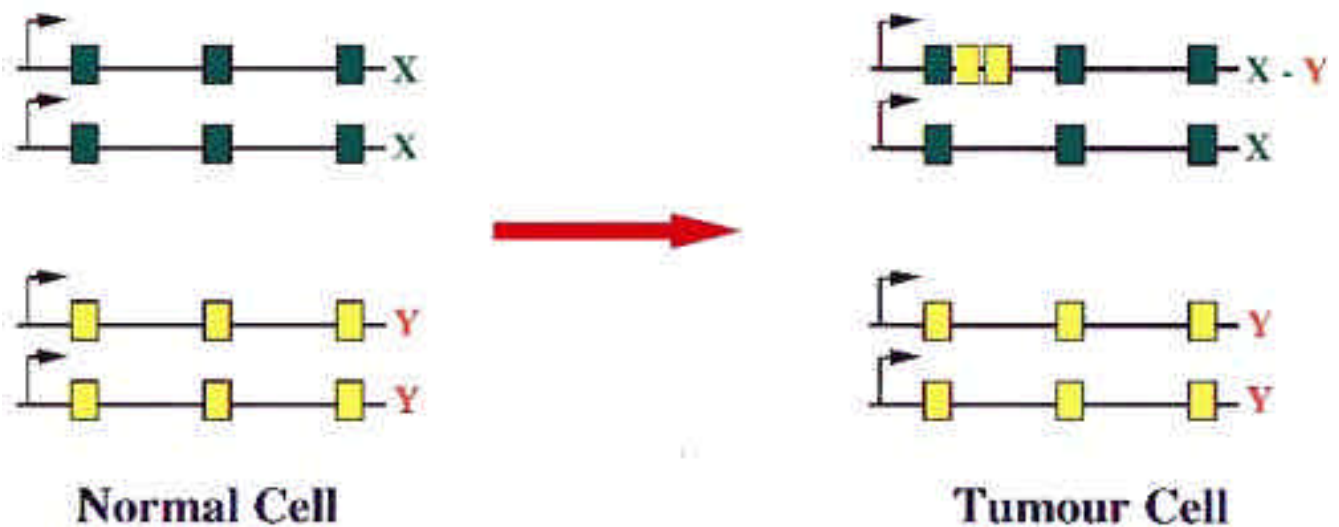


Fig. (3). Knock-in mouse models of gene fusions. The fusion gene is generated by homologous recombination at the endogenous mouse locus in ES cells followed by blastocyst injection. In contrast to the classical transgenic mouse models, only a single copy of the fusion gene is generated by inserting the 3' end of the fusion cDNA (gene Y) into the endogenous locus of the 5' partner (gene X), and in this position, fusion gene expression is regulated by the endogenous elements from the 5' partner.

answering the question of whether the mutation is cell autonomous or non autonomous in nature.

A chimera approach was used to investigate the biological role of BCR-ABL^{P190} and MLL-AF9 oncogenes [24, 25]. Both studies demonstrated oncogenicity and lineage specificity in the chimeric mice. Despite the activity of the BCR and MLL endogenous promoters in a variety of lineages, only leukemias developed in this mice. These leukemias recapitulated specifically fusion genes are associated in humans [24, 25]. Thus, these findings suggest that BCR-ABL^{P190} and MLL-AF9 activity is cell autonomous and that it likely acts in stem or multipotential progenitor cells. Moreover, this knock-in approach has also been used to show the influence of the gene product from the remaining intact allele on the oncogenic activity of the chimeric protein [24].

Similar studies were carried out with the AML1-ETO and CFBF-MYH11 fusions associated with myeloid leukemia [26-28]. To determine the biological role of the AML1-ETO and CFBF-MYH11 fusions, a conventional gene knock-in was first carried out (Fig. 3). When the AML1-ETO and CFBF-MYH11 fusion genes were transmitted through the germline, the resulting heterozygotes demonstrated severely impaired haematopoiesis and died in midgestation. The similarity of these phenotypes with those of homozygous Aml-1 and Cbfb mutants suggest that the fusion products exert a dominant-negative effect to block haematopoiesis. Although this block may contribute to leukemogenesis when it sporadically occurs in a single myeloid progenitor cell, it seems most compatible with embryonic viability when it occurs throughout the hematopoietic progenitor populations. This finding clarifies that the oncogenicity of some of these fusions is restricted to the setting of sporadic acquired and not inherited germline events. To check whether this was a cell autonomous defect, the modified ES cells were used to make chimeric animals. These modified ES cells were excluded from contributing to the hematopoietic lineages and leukemia did not develop in the chimeric animals [26-28]. Together, these studies demonstrated not only their requirement for hematopoiesis, but also that their activity is cell autonomous and that it likely acts in stem or multipotential progenitor cells as well.

Overall these studies, with the knock-in mouse models suggest, that leukemia-initiating genetic event might regularly occur at the stem cell level, irrespective of the phenotypic makeup of the bulk population of leukemic blasts. An explanation could

be that the gene fusion itself determines the differentiation program of the affected cell clone, which contrasts with the opinion that the leukemia phenotype is a reflection of the level of the hematopoietic hierarchy at which the genetic defect occurs. Thus, data coming from the knock-in mouse models support the hypothesis that the leukemogenic event most often occurs in the primitive cell [5] and that the nature of this genetic defect itself determines the differentiation program of the leukemic clone. These results favor the idea that hematopoietic lineage determination is driven intrinsically (as described in stochastic models) [29] rather than extrinsically (through external influences), though it still is pending to understand how a common progenitor cell become one type rather than another. This question cannot be answered with the current knock-in mouse models of gene fusions, as in the knock-in mice generated by conventional methods [24-28] the genetic change exists in all the cells through their life span. After establishment, there is no way to modulate the presence of these mutations. An appropriate model of these diseases would be one in which only a few cells or a single stem cell undergoes the genetic alteration, rather than the majority of cells in a tissue.

FUTURE PROSPECTS: CONDITIONAL KNOCK-IN MOUSE MODELS

Germline mutations do not model many genetic human diseases including cancer and even preclude analysis of a post-natal phenotype when the mutation is embryonic lethal, as we pointed out previously. Therefore, new models are needed to specifically address the relationship between the target cell and the expression and function of the fusion genes. A solution could be to restrict the genome alteration, either by limiting the type and/or number of cells that carry it, or by introducing a silent genetic alteration that can be activated in a spatial- or temporal-specific manner. One way to achieve a model would be to use an inducible and lineage specific recombinase. The Cre recombinase of the P1 bacteriophage and the FLP recombinase of yeast have been the choice for experiments in mammalian systems because they require only a specific, short (34-bp) consensus recognition site (loxP and FRT sites, respectively) to catalyze recombination. If two different sites are placed in the same orientation in trans, recombination excision will result in creation of specific chromosomal rearrangements [30, 31]. Therefore, the general strategy for conditional knock-in will be to place two recognition sites for a site-specific recombinase

within the appropriate introns in such a way that the genes can function normally after this alteration. Thus, completely normal mice carrying this altered allele in homozygous form can be established. If a transgene expressing the recombinase under the control of a tissue/cell type-specific promoter is introduced into this homozygous animal, it will rearrange both genes in the lineage of specificity, rendering the fusion gene functional. These models would provide an imitation of the stochastic event that probably occurs in initiation of human cancer and a new framework for viewing the cellular and molecular mechanisms that underlie the heterogeneity seen in human cancer; particularly the impact that fusion gene expression has on the development program of normal stem cells. These studies should determine whether the common mesodermal origin of the haematopoietic and mesenchymal lineages provides a common link for the frequent occurrence of these translocations in sarcomas and hematopoietic tumors. By exploring these questions of lineage specificity in the new mouse models, these studies will contribute to understand the fundamental diversity of the different diseases that are unified under the common heading of cancer.

CONCLUSIONS AND FUTURE PROSPECTS

There have been remarkable advances in our understanding of the molecular biology of cancer that provides new selective tumor destruction mechanisms. The molecular characterization of the tumor-specific chromosomal abnormalities has led to the identification of genes (mainly novel transcription factors) involved in the development of cancer. It is intriguing how these molecular changes are integrated into the development program of the tumor-specific target cells, either by exploiting the constitutive activity of recombinases and rearranging genes or by commonly gene fusion. The fact that most of these chromosomal abnormality-associated proteins share a common structural composition suggests that there may exist a common pathway leading to tumorigenesis. The viral oncoproteins are continuing to point the way to important intracellular pathways involved in growth regulation. Deregulation of cell cycle control is becoming increasingly important [1, 2], and most cancer cells lack the Rb G1 checkpoint through mutation of one of the elements in the cyclin D/Cdk4/p16/Rb axis. We can anticipate that the perturbation of such networks will be a fundamental cause of cancer.

An emerging common theme is that alteration of these genes disrupts the normal development of

tumor-specific target cells by uncoupling of the apoptotic and proliferative regulatory signals. Loss of this coupling by translocation-associated genes diminishes tumor cell death by apoptosis and results in malignant hyperproliferation. Diminished tumor cell apoptosis could contribute both to tumor progression by enhancing the survival of cells sustaining DNA damage and to determine the responsiveness of tumor to commonly employed modalities of anti-neoplastic therapy. When the chromosomal abnormality product also blocks a differentiation program, an acute malignancy will appear. If the tumor-associated protein only alters proliferation control without blocking differentiation, a chronic malignancy will develop, being required to block a specific differentiation program to achieve a full malignant phenotype.

Nevertheless, these advances provide new molecular tools for diagnosis and patient management during treatment and hold some promise for more selective nontoxic therapy in the future, because the chimeric molecules as a result of chromosomal abnormalities are ideal therapeutic targets since they are unique to the disease, allowing the design of new and specific anti-tumor proteins [14, 32, 33], and the presence of the fusion protein is necessary for the persistence of tumor [13, 14]. Moreover, these strategies allow specifically the introduction of therapeutic molecules into the target cell [14, 33]. However, there are some vital pieces of the acute malignancy puzzle missing. In particular, we are ignorant of the larger picture of the natural history of these acute malignancies, which includes issues such as the role of genetic and environmental factors, the disruption of differentiation program in the tumor-specific target cells and the timing of critical molecular events in relation to initiation, progression, and latency of disease. It is our task to not only identify these mechanisms, but also to determine their relative importance for each stage and type of cancer. Our hope, then, is to translate that knowledge into clinical applications.

ACKNOWLEDGEMENTS

I apologize to all those whose work has not been directly referenced due to space constraints. Research in the author's laboratory is supported by European Commission (BMH4-CT96-0375), DGCYT (UE96-0041, PB96-0816 and 1FD97-0360), Fundación Científica of the AECC, FIS (99/0935), and NIH grant (1 R01 CA79955-01).

REFERENCES

- [1] Sánchez-García, I. (1997). *Annu. Rev. Genetics.*, **31**, 429-453.
- [2] Cobaleda, C.; Pérez-Losada, J. and Sánchez-García, I. (1998). *BioEssays*, **20**, 922-930.
- [3] Cline, M.J. (1994). *N. Engl. J. Med.*, **330**, 328-336.
- [4] Barr, F.G. (1998). *Nature Genet.*, **19**, 121-124.
- [5] McCulloch, E. (1983). *Blood*, **62**, 1-13.
- [6] Chan, L.C. *et al.* (1987). *Nature*, **325**, 635-637.
- [7] de Klein, A.; *et al.* (1981). *Nature*, **300**, 765-767.
- [8] Crozat, A.; Aman, P.; Mandahl, N.; and Ron, D. (1993). *Nature*, **363**, 640-644.
- [9] Rabbitts, T.H.; Forster, A.; Larson, R.; and Nathan, P. (1993). *Nature Genet.*, **4**, 175-180.
- [10] Tybulewicz, V.L.J.; Crawford, C.E.; Jackson, P.K.; Bronson, R.T.; and Mulligan, R.C. (1991). *Cell*, **65**, 1153-1163.
- [11] Schwartzberg, P.L.; *et al.* (1991). *Cell*, **65**, 1165-1175.
- [12] Zinszner, H.; *et al.* (1998). *Genes & Dev.*, **12**, 982-995.
- [13] Sánchez-García, I. and Grütz, G. (1995). *Proc. Natl. Acad. Sci. U.S.A.*, **92**, 5287-5291.
- [14] García Hernández, B.; and Sánchez-García, I. (1996). *Molecular Medicine*, **2**, 125-133.
- [15] Grignani, F. *et al.* (1993). *Cell*, **74**, 423-431.
- [16] Kuroda, M. *et al.* (1997). *Am. J. Pathol.*, **151**, 735-744.
- [17] Fu, X.; and Kamps, M.P. (1997). *Mol. Cell. Biol.*, **17**, 1503-1512.
- [18] Heisterkamp, N.; Jenster, G.; Kioussis, D.; Pattengale, P.K.; and Groffen, J. (1991). *Transgenic Res.*, **1**, 45-53.
- [19] Voncken, J.W.; *et al.* (1995). *Blood*, **86**, 4603-4611.
- [20] Grisolano, J.L.; Wesselschmidt, R.L.; Pelicci, P.G.; and Ley, T.J. (1997). *Blood*, **89**, 376-387.
- [21] Brown, D.; *et al.* (1997). *Proc. Natl. Acad. Sci. U.S.A.*, **94**, 2551-2556.
- [22] Early, E.; *et al.* (1996). *Proc. Natl. Acad. Sci. U.S.A.*, **93**, 7900-7904.
- [23] Dobie, K.; Mehtali, M.; McClenaghan, M.; and Lather, R. (1997). *TIGS*, **13**, 127-130.
- [24] Castellanos, A.; *et al.* (1997). *Blood*, **90**, 2168-2174.
- [25] Corral, J.; *et al.* (1996). *Cell*, **85**, 853-861.
- [26] Yergeau, D.A.; *et al.* (1997). *Nature Genet.*, **15**, 303-306.
- [27] Okuda, T.; Cai, Z.; Yaug, S.; Lenny, N.; Lyu, C-J.; van Deursen, J.M.A.; Harada, H.; and Downing, J.R. (1998). *Blood*, **91**, 3134-3143.
- [28] Castilla, L.H.; *et al.* (1996). *Cell*, **87**, 687-696.
- [29] Fairbairn, L.J.; Cowling, G.J.; Reipert, B.M.; and Dexter, T.M. (1993). *Cell*, **74**, 823-833.
- [30] Ramírez-Solis, R.; Liu, P.; and Bradley, A. (1995). *Nature*, **378**, 720-724.
- [31] Smith, A.J.H.; De Sousa, M.A.; Kwabi-Addo, B.; Heppell-Parton, A.; Impey, H.; and Rabbitts, P. (1995). *Nature Genet.*, **9**, 376-385.
- [32] Choo, Y.; Sánchez-García, I. and A. Klug. (1994). *Nature*, **372**, 642-645.
- [33] García Hernández, B.; Castellanos, A.; López, A.; Orfao, A. and Sánchez-García, I. (1997). *Proc. Natl. Acad. Sci. U.S.A.*, **94**, 13239-13244.