Transgenic Models of Tumor Development

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Numerous cancer-prone strains of mice have been created by the introduction of candidate tumor-promoting genes into fertilized eggs. Each transgenic strain is predisposed to develop specific types of tumors, but they usually arise stochastically because of the need for spontaneous mutation of genes that collaborate with the introduced oncogene. These mice are providing insights into the effects of individual oncogenes on cellular proliferation, differentiation, and viability, as well as on oncogene cooperativity. Their predisposed state imposes sensitivity to viral and chemical carcinogenesis, and the mice should prove valuable in tests of potential carcinogens, therapies, and preventive measures.

A NEW ERA OF CANCER RESEARCH DAWNED IN 1984 WHEN mice reared from eggs injected with genes implicated in cancer (oncogenes) proved to develop specific types of tumors. Integrated copies of the T antigen oncogene from simian virus 40 (SV40) elicited brain tumors (*1*), and a *myc* transgene expressed in the mammary gland led to breast cancer (*2*). Shortly thereafter, a T antigen gene designed for pancreatic expression provoked pancreatic tumors (*3*), and the first transgenic reconstruction of a karyotypic alteration proved that an altered *myc* gene primed lymphomagenesis (*4*). Because microinjected DNA often is incorporated into the germ line (*5*), tumor-prone strains were generated. Thus, cancer development in diverse cell types could now be traced from its outset within the living animal.

Malignancy requires multiple steps. A nascent neoplastic clone must bypass its programmed demise, circumvent the need for growth factors from other cells, ignore restraining signals, escape from any immunological surveillance, commandeer its own blood supply (angiogenesis), breach surrounding tissues, and often colonize distant sites (metastasis). The underlying genetic accidents are thought to transcriptionally activate or structurally modify genes that encourage cellular proliferation (the proto-oncogenes) and to delete or inactivate tumor suppressors that normally restrain tumor development (*6*). The evidence implicating many of the genes, however, was indirect: the genes had been discovered in retroviruses or found near viral insertions or karyotypic alterations.

Transgenic studies allow definitive tests of candidate oncogenes and of tumor suppressors if a mutant protein interferes with wild-type function. By varying the attached regulatory sequences, investigators can gauge biological effects in diverse cell types or in specific cell lineages, although unexpected expression patterns sometimes arise because of sequences flanking the random insertion point or because of a novel apposition of regulatory elements (*5*).

In general, the transgene does not directly provoke tumors but establishes a high predisposition, and emergence of a malignant clone requires genetic changes in a rare affected cell. Two rate-limiting steps in normal tumorigenesis, the initial mutation and the expansion of that clone, are superseded by expression of the transgene throughout a cell compartment, but synergistic mutations are still required (Fig. 1). During the preneoplastic phase, any direct effects of the transgene on proliferation or differentiation can be gauged, and the rules of oncogene synergy can be explored by delivery of a second gene.

Over two dozen tumor types have been modeled (Table 1). The 30 or so transgenes encode representatives from each major class of protein implicated in neoplasia (*7, 8*): growth factors [transforming growth factor α (TGF-α), Wnt-1, and Int-2], cytokine receptors [ErbB2 (Neu), and Ret], signal-transducing molecules (the guanine nucleotide–binding Ras proteins, serine/threonine kinase Pim-1, and tyrosine kinases Abl, Fps, and Lck), a cytoplasmic protein involved in cell survival (Bcl-2), and nuclear proteins that serve as transcription factors or directly regulate cell replication (Myc, N-Myc, L-Myc, Fos, Jun, p53, T antigens of papovaviruses, and retroviral Tat). We first discuss the most studied neoplasms and then discuss the influence of genetic background, the preneoplastic state, the susceptibility of different cell types, oncogene cooperativity, and the potential of the mice for testing carcinogens and therapies. Other reviews of oncogene-bearing mice have appeared (*9, 10*).

Breast Cancer

The high incidence of human breast cancer has prompted efforts to model the disease in transgenic mice. The *myc* gene is amplified in some human breast cancers (*11*), and *ras* mutation has been observed

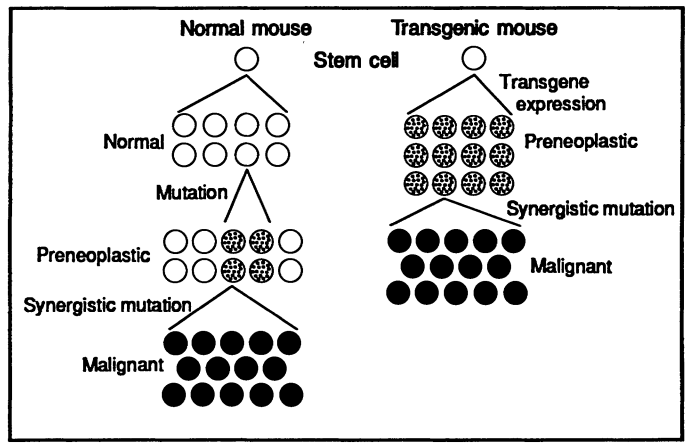

Fig. 1. Tumorigenesis is potentiated in the transgenic mouse by oncogene expression throughout a cell compartment, creating a preneoplastic population from which a malignant clone eventually evolves. In a normal mouse, mutation imposes significant risk only if the affected cell undergoes clonal expansion; expression of a transgene obviates both these steps. Open circles denote normal cells; speckled circles, cells that have acquired one oncogenic alteration, either by transgene expression or mutation; filled circles, malignant cells.

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(*12*). Most transgenic tests have used genes regulated by the long terminal repeat of mouse mammary tumor virus (MMTV), which is stimulated by hormones during pregnancy (Table 1). MMTV-*myc* and MMTV–v-H-*ras* mice both developed transplantable mammary adenocarcinomas (*2, 13*), but disease onset was sporadic and did not involve all the mammary epithelium; hence, neither gene sufficed.

Breast cancer progression often correlates with amplification of the tyrosine kinase receptor gene denoted c-*erb*B2 or c-*neu* (*14*). This receptor is related to that for epidermal growth factor (EGF), but its ligand is unknown. The activated c-*neu* gene, which has a single mutation within its transmembrane domain, was devastating in one MMTV-*neu* strain (*15*): male and female mice both synchronously developed adenocarcinomas encompassing the entire gland. Other strains developed tumors stochastically (*16*).

An autocrine loop (*17*) has been implicated in mouse mammary tumors that express the *int*-1 or *int*-2 gene as a result of MMTV proviral insertion (*18*). The *int*-2 gene is a member of the fibroblast growth factor family, and *int*-1, which is related to the *Drosophila* morphogen *wingless* and is now called *wnt*-1, may be a cell-bound growth factor. Mice bearing MMTV–*int*-1 (*19*) or MMTV–*int*-2 transgenes (*20*) developed mammary hyperplasia, as did mice expressing TGF-α (*21*), a ligand for the EGF receptor. Mammary cancer ensued in most female *int*-1 and some TGF-α mice.

Lymphoid Malignancy

Specific chromosome translocations hallmark many hematopoietic malignancies (*22, 23*). The first to be molecularly defined were those in two B lymphoid neoplasms, Burkitt's lymphoma of humans and plasmacytomas (tumors of immunoglobulin-secreting cells) of rodents, where apposition of the c-*myc* gene and an immunoglobulin locus renders *myc* expression constitutive (*24*). Such translocations were modeled (*4*) by the expression of *myc* from the potent 5′ *Igh* locus enhancer (Eμ), as often occurs in Burkitt's lymphomas (*24*). All Eμ-*myc* mice developed clonal tumors of pre-B or B cells (*4, 25–28*), and treatment with pristane elicited plasmacytomas (*29*). Tumor onset was preceded by a benign polyclonal hyperproliferative phase that generated excess

Table 1. Transgenic models of tumor development. Only the tissue or cell type of the predominant tumor type provoked by the transgene is indicated (appropriate references in parentheses); underlined reference numbers indicate that other tumors were observed. Abbreviations are as follows: SV40 Tag, either the large T antigen gene or the complete SV40 early region; FSFV gp55, the spleen focus-forming virus Env protein, which binds to the erythropoietin receptor; LPV, lymphotropic papovavirus; HTLV-1 *tat*, the trans-activator gene of human T-cell leukemia virus–1; JCV and BKV, human papovaviruses; HIV, human immunodeficiency virus; Py mTag, the middle T antigen gene from polyoma virus; and Ad12, the early region (*E1A* and *E1B* genes) of adenovirus type 12. Regulators include the long terminal repeat from myeloproliferative sarcoma virus (MPSV), Friend spleen focus-forming virus (FSFV), or MMTV; and enhancers and promoters from the genes for immunoglobulin heavy chains (Eμ), metallothionein-1 (MT-1), phenylethanolamine *N*-methyltransferase (PNMT), gonadotropin-releasing hormone (GnRH), leuteinizing hormone β subunit (LHβ), the H-2K antigen of the major histocompatibility locus, whey acidic protein (WAP), and atrial natriuretic factor (ANF).

Tissue or cell type	Gene	Regulator
Hematopoietic		
B (or T) lymphoid	*myc*	Eμ (*4, 25–30, 56, 85*)
	N-*myc*	Eμ (*86*)
	SV40 Tag	Eμ (*25*)
	bcl-2	Eμ (*62–65*)
	*erb*B2 (*neu*)	Eμ (*87*)
	v-*abl*	Eμ (*52, 53*)
	bcr–v-*abl*	Eμ or MPSV (*88*)
	bcr-abl	MT-1 (*89*)
T lymphoid	*myc*	Thy-1 (*90*)
	L-*myc*	Eμ (*91*)
	SV40 Tag	*lck* (*92*)
	N-*ras*	Eμ (*29*)
	pim-1	Eμ or H-2K (*74, 76*)
	lck	*lck* (*93*)
Erythroid	FSFV gp55	β-actin or FSFV (*94*)
Neuronal		
Retinal	SV40 Tag	PNMT (*95*)
Hypothalamic	SV40 Tag	GnRH (*96*)
Retinoblast	SV40 Tag	LHβ (*97*)
Adrenal	JCV early	JCV (*98*)
Perineuronal	HTLV-1 *tat*	HTLV-1 (*99*)
Skin and soft tissue		
Dermal	BPV	BPV (*35, 37*)
	v-*jun*	H-2K (*36*)
Epidermal	c-H-*ras*	Keratin (*32*)
	v-H-*ras*	Zeta-globin (*33*)
	TGF-α	Keratin (*34*)
Adipose	SV40 Tag	α-Amylase (*100*)
Melanocytic	SV40 Tag	Tyrosinase (*101*)
Mesenchymal	HTLV-1 *tat*	HTLV-1 (*102*)
	SV40 Tag	Renin (*103*)
Vascular		
Choroid plexus	SV40 T	SV40 (*1, 82, 104*)
	LPV early	LPV (*105*)
Endothelial	HIV *tat*	HIV (*106*)
	Py mTag	Polyoma (*107*)
Mammary	*myc*	MMTV (*2*), WAP (*55*)
	c-H-*ras*	WAP (*108*)
	v-H-*ras*	MMTV (*13*)
	N-*ras*	MMTV (*58*)
	*erb*B2	MMTV (*15, 16, 87*)
	ret	MMTV (*109*)
	wnt-1 (*int*-1)	MMTV (*19*)
	int-2	MMTV (*20*)
	TGF-α	MMTV (*21*)
Hepatic	SV40 Tag	MT-1 (*110*), urinary protein (*111*), antitrypsin (*112*), albumin (*38*)
	HBV antigen	Albumin (*39*)
	myc	Albumin (*38*), antitrypsin (*113*)
	TGF-α	MT-1 (*40*)
	c-H-*ras*	Albumin (*38*)
	HBx	*HBx* (*41*)
	Growth hormone	MT-1 (*114*)
Pancreatic		
Islet	SV40 Tag	Insulin (*3, 42, 43*)
Acinar	SV40 Tag	Glucagon (*115*)
	SV40 Tag	Elastase (*45*)
	c-H-*ras*	Elastase (*44*)
	myc	Elastase (*46*)
Other tissues	BKV early	BKV (*116*)
Kidney	SV40 Tag	ANF (*117*), protamine (*118*)
Heart	v-H-*ras*	MMTV (*13*)
Lung	c-H-*ras*	Eμ (*25*)
	Ad12 early	MMTV (*119*)
Stomach	SV40 Tag	Amylase (*120*), protamine (*118*)
Bone	c-*fos*	H-2K (*67*)
Cartilage	SV40 Tag	αA-crystallin (*121*)
Lens	SV40 Tag	Vasopressin (*122*)
Pituitary	SV40 Tag	MMTV (*80*), H2-K (*81*)
Diverse types	*myc*	MMTV (*66*)
	p53	p53 (*50*)
	Py mTag	Eμ (*123*)
	v-*fps*	Globin (*124*)

cycling pre-B cells (*30*). Because a malignant clone emerged stochastically only after some 10^{10} cell divisions (*26*), more than one somatic mutation may have occurred. Other hematopoietic models have been reviewed (*10*).

Skin Cancer

Dermal neoplasia has been prominent in experimental carcinogenesis. Such studies (*31*) have revealed an initiation phase, reflecting mutation, and a promotion phase, produced by a strong proliferative stimulus such as a phorbol ester; this scenario presumably echoes the need for oncogene synergy. The tumors that arise in skin painted with carcinogens almost invariably have mutant H-*ras* alleles (*12*), and mutant H-*ras* transgenes expressed in the skin elicited papillomas (*32, 33*), as did a TGF-α transgene (*34*). These benign lesions arose only at sites of wounding or after treatment with a phorbol ester, and some progressed to carcinomas or sarcomas (*33*). Wounding was also implicated in the fibrosarcomas that arose in mice bearing either the bovine papilloma virus (BPV) genome (*35*) or an H-2K–driven v-*jun* gene (*36*). The proliferative stimuli may free the mutant cells from restraints imposed by normal neighbors (*8*), and the increased replication may favor karyotypic changes, such as those in fibrosarcomas of BPV mice (*37*).

Liver and Pancreatic Tumors

Liver carcinogenesis in rodents, the definitive assay for potential carcinogens, frequently involves H-*ras* mutation (*12*). High expression of a mutant H-*ras* transgene in the liver provoked hyperplasia and perinatal death, whereas lower expression produced mild focal dysplasia and occasional frank carcinoma (*38*). A T antigen transgene was tumorigenic in several models (Table 1), whereas *myc* provoked dysplasia but rarely tumors (*38*).

The association of hepatocellular carcinoma in Asia and Africa with previous chronic infection with hepatitis B virus (HBV) appeared puzzling because the virus contained no known oncogene, but two HBV transgenes have proven to be carcinogenic by different mechanisms. Cell death and regenerative hyperplasia preceded the overt aneuploid tumors provoked by a transgene encoding the HBV large surface antigen (*39*); presumably the sustained proliferation, which also prefigured the tumors generated by a TGF-α transgene (*40*), increased the hazard of somatic mutation. In contrast, dysplasia, rather than hyperplasia, preceded the carcinomas generated by the viral *HBx* gene, which activates expression of other genes (*41*). Thus, HBV may predispose individuals to cancer both by altering the hepatocyte differentiation program and by driving regenerative proliferation.

Pancreatic cancer is among the most intractable human tumors. Transgenic models involve either the β cells of the islets of Langerhans or the acinar cells of the exocrine pancreas (Table 1). Several weeks of T antigen expression in β cells rendered half the islets hyperplastic and ablated senescence (*3, 42*); progression of a few islets to frank tumors was heralded by angiogenesis, which may reflect export of basic fibroblast growth factor (*43*). Acinar tumors were engendered by a mutant H-*ras* allele (*44*) or T antigen (*45*), whereas *myc* produced mixed acinar-ductal tumors (*46*). Because *ras* (albeit usually K-*ras*) is mutated in most pancreatic cancers (*47*), it is noteworthy that H-*ras* caused perinatal death due to gross acinar hyperplasia although the cells did not seem malignant (*44*). T antigen may promote karyotypic instability because tetraploidy commonly preceded aneuploid tumor nodules (*45*). Induced karyotypic instability could be central to many forms of tumorigenesis (*48*).

Tumor Suppressors and Genetic Background Effects

Mutations in the tumor suppressor p53, some of which act dominantly, are prevalent in frequent human cancers and are inherited in the rare Li-Fraumeni predisposition to multiple malignancies (*49*). The tumor profile in mice that bear a mutant p53 gene (*50*) resembles that in some Li-Fraumeni families. SV40 T antigen binds to both p53 and the product of *Rb*, a tumor suppressor gene first implicated in retinoblastoma (*6*), and retinoblastoma is among the diverse tumors generated by T antigen transgenes (Table 1). Numerous cell lines have been derived from mice expressing T antigen, and conditionally immortal lines can be obtained with a mutant T antigen active only below body temperature (*51*).

Inbred strains allow tests of whether genetic constitution modulates tumor susceptibility. With an Eμ-*myc* construct expressed in both B and T cells, a C57BL/6 background favored B lymphomas, and a C3H/HeJ background favored T lymphomas, apparently because of differences in supporting cells (*28*). C57BL/6 mice are relatively refractory to tumor induction and that background retarded liver carcinogenesis provoked by an HBV transgene (*39*) and lymphomagenesis elicited by Eμ-*myc* (*26*). The marked susceptibility of BALB/c mice to plasmacytomagenesis, and the *myc* translocations in such tumors, suggests that error-prone immunoglobulin switch recombination in this strain raises the odds of *myc/Igh* recombination (*52, 53*). Undoubtedly, many human alleles also influence tumor susceptibility.

Lessons from the Preneoplastic State

The restriction of expression of the normal *myc* gene to proliferating cells suggests that *myc* helps to govern proliferation (*54*). The elevated cell cycle activity and greater proportion of early B lymphoid cells in pretumorous Eμ-*myc* mice argued that *myc* favored self-renewal over differentiation (*30*). A similar conclusion was reached for mammary epithelium (*55*). The proliferative impetus did not abolish growth factor requirements of Eμ-*myc* B lymphoid cells (*56*). Although *myc* is often called an "immortalizing" gene, autonomous pre-B cell lines emerged only after long-term culture (*27, 56*). Increased proliferation cannot account entirely for the tumorigenic action of *myc* because the gene for the surface antigen Thy-1 promoted as many cycling early B lineage cells as *myc*, but no Eμ-*Thy*-1 mice developed tumors (*57*).

The preneoplastic impact of *ras* has ranged from no discernible effect to the opposing outcomes of increased proliferation or differentiation. Salivary and mammary epithelium appeared unperturbed before tumor onset, whereas the marked hyperplasia in the Harderian lacrimal gland rarely progressed to malignancy (*13, 58*). The proliferation evoked in pancreatic acinar cells (*44*) and liver cells (*38*), at least with high expression of *ras*, contrasts with the increased differentiation that preceded keratinocyte tumors (*32*). Thus, the oncogenic impact of *ras* does not correlate with its ability to increase proliferation, which may reflect its capacity to induce TGF-α (*17*).

A cell producing its own growth factor might seem doomed to malignancy, but transgenic studies indicate that need not be the case. TGF-α caused epithelial hyperplasia in multiple tissues, but frank tumors were common only in the liver and mammary gland (*21, 40*). Similarly, although both the *int*-1 and *int*-2 genes were mitogenic for mammary epithelium, tumors were rare with *int*-2 (*20*). Many hematopoietic growth factor genes tested in vivo perturb hematopoiesis, but even the lethal excess of myeloid cells evoked by granulocyte-macrophage colony-stimulating factor (GM-CSF) or interleukin-3 (IL-3), and of plasma cells by IL-6, was not

considered leukemia because the cells were not transplantable and there was not an increased proportion of immature cells (*10*). Thus, rendering a cell autonomous is insufficient unless other changes impede terminal differentiation (*59*).

The harbinger of a new class of oncogene that maintains cell survival, the *bcl*-2 gene, was discovered by its linkage to the *Igh* locus in follicular lymphoma (*60*), a prevalent but usually indolent human B cell tumor. The gene's survival function was revealed when cells from hematopoietic lines that require IL-3 failed to die on withdrawal of factor if infected with a *bcl*-2 retrovirus although they entered a quiescent (G_0) state (*61*). B lymphoid cells from *bcl*-2 transgenic mice also exhibited enhanced survival in vitro (Fig. 2), and the mice accumulated excess noncycling B cells (*62, 63*). Antibody responses were protracted, and an autoimmune disease eventuated in certain strains (*63*), presumably because B cells that recognized self antigens were preserved. In T cells, *bcl*-2 improved survival in the face of diverse lymphotoxic agents and impeded but did not abrogate the thymic process that eliminates most maturing T cells (*64*). Thus, *bcl*-2 does not inhibit every form of programmed cell death (apoptosis). Because few *bcl*-2 mice developed tumors in the first year of life (*63–65*), *bcl*-2 may contribute to follicular lymphoma by preserving a B cell clone until other changes drive its proliferation (*61*).

Cell Type–Specific Oncogenesis

The oncogenic spectrum of each oncogene varies. The *ras* transgenes have generated diverse tumors (Table 1) but not in every tissue. The *myc* gene is also a versatile but not universal carcinogen (*66*). Although *myc* has provoked tumors at every stage of B cell development (*10*), v-*abl* may be oncogenic only at certain stages because Eμ-v-*abl* mice developed tumors of either a pre-B or plasma cell but never of the intermediate B cell (*52, 53*). One might have expected pleiotropic effects from a *fos* or *jun* transgene because Fos and Jun polypeptides, which are made in almost all cells in response to diverse stimuli, constitute the AP-1 transcription factor that mediates the diverse effects of phorbol esters. Promiscuously expressed *fos* transgenes, however, primarily affected bone development, and only bone and cartilage tumors developed (*67*); a widely expressed v-*jun* transgene generated only dermal tumors (*36*). These neoplasms mirror those produced by the retroviruses that first revealed these genes. Thus, even transcription factors that function in most cells may be oncogenic only in a few.

Oncogene Synergy in Diverse Cell Types

The need for oncogene cooperation in cellular transformation, first established for cultured fibroblasts (*6, 68*), has been extended by transgenic analysis to many cell types. Mutations in the spontaneous tumors have been identified, known oncogenes have been shown to accelerate tumorigenesis, and new genes have been found by insertional mutagenesis (Table 2). A few Eμ-*myc* lymphomas harbored a mutant *ras* gene (*69*), and *ras* oncogenes rendered preleukemic Eμ-*myc* pre-B cells both autonomous and transplantable (*69, 70*). At least 85% of the plasmacytomas of Eμ-v-*abl* mice had c-*myc* rearranged (*52, 53*), seemingly translocated to the *Igh* locus, as in nontransgenic plasmacytomas (*24*). The *myc* gene is often also rearranged in the lymphoid tumors that arise (with long latency) in *bcl*-2 mice (*65*). Hence, *myc* may be the most effective or most accessible partner for both v-*abl* and *bcl*-2.

Delivery of a second oncogene by retroviral infection of newborn transgenic mice showed that v-H-*ras* and v-*raf*, but not v-*abl*, could aid *myc* to transform pre-B cells (*71*), as observed in vitro (*70*). For transoncogenes expressed in the same tissues, a simple cross provides a powerful test for synergy (*13*), but even the most powerful pairs may require somatic mutation. Coexpression of *myc* and v-H-*ras* accelerated mammary tumors, but their focal development implicated additional events (*13*). When the need for *myc* translocation in plasmacytomagenesis was obviated by the provision of both Eμ-v-*abl* and Eμ-*myc* transgenes, plasmacytomas arose rapidly (Fig. 3), but they were oligoclonal, rather than polyclonal, and their origin in the gut wall implicated a role for antigenic stimulation (*52, 53*). Even *myc* plus *pim*-1, which produced a lethal burden of pre-B lymphocytes, may not fully transform (*72*). Although young Eμ-*myc/bcl*-2 mice had copious cycling pre-B and B cells, these cells were not transplantable, and the mice succumbed to tumors of a primitive cell type, perhaps a lymphoid stem cell (*73*).

Because retroviruses that lack an oncogene, such as Moloney murine leukemia virus, primarily promote tumorigenesis by fortuitous integration near a cellular proto-oncogene, thereby affecting its expression, proviral insertion provides a screen for genes that can aid a transgene to induce tumors. Infection of Eμ–*pim*-1 mice greatly

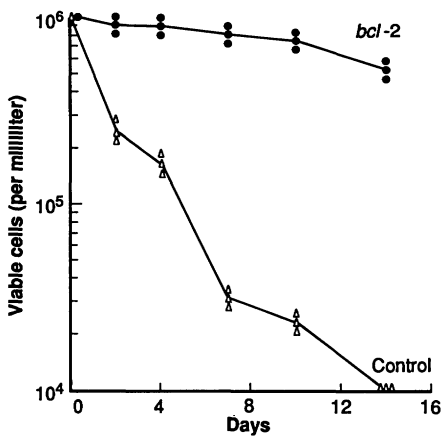

Fig. 2. Enhanced survival of splenocytes from Eμ–*bcl*-2 mice cultured in medium without growth factors. The cells surviving after 2 weeks all proved to be small noncycling B cells (*62, 63*). Control cells were derived from nontransgenic littermates.

Table 2. Oncogene synergy in spontaneous or accelerated transgenic tumors. Appropriate references are given in parentheses.

Transgene	Synergistic gene	Tumor type
	Spontaneous	
v-*abl*	*myc*	Plasmacytoma (*52, 53*)
bcl-2	*myc*	Plasmacytoma (*65*)
myc	N-*ras*, K-*ras*	Pre-B lymphoma (*69*)
SV40 Tag	H-*ras*	Liver carcinoma (*125*)
gp55 gene	p53, *spi*-1	Erythroleukemia (*94*)
	Retroviral delivery	
myc	v-H-*ras*, v-*raf*, N-*ras*	Pre-B lymphoma (*56, 70*)
	Transgenic cross	
myc	v-H-*ras*	Mammary carcinoma (*13*)
myc	v-*abl*	Plasmacytoma* (*52, 53*)
myc	v-*abl*	Pre-B lymphoma* (*126*)
myc	*pim*-1	Pre-B lymphoma (*72*)
myc	N-*ras*	Pro-B, pre-B lymphoma (*126*)
myc	*bcl*-2	Lymphoid progenitor† (*73*)
SV40 Tag	*myc*	Liver carcinoma (*38*)
SV40 Tag	H-*ras*	Gross liver hyperplasia (*38*)
myc	H-*ras*	Liver carcinoma (*38*)
	Proviral insertion	
pim-1	*myc*, N-*myc*	T lymphoma (*74*)
myc	*bmi*-1, *pim*-1	Pre-B lymphoma (*75*)

*Plasmacytoma onset was accelerated when three plasmacytoma-prone Eμ–v-*abl* strains were crossed with Eμ-*myc* mice; a lymphoma-prone Eμ–v-*abl* strain, however, rapidly developed pre-B lymphomas that were not transplantable. †An excess of nontransplantable cycling pre-B and B cells was also evident.

accelerated T lymphoma onset, and every tumor bore a provirus near the c-*myc* or N-*myc* gene, indicating that *myc* genes could cooperate with *pim*-1 (*74*). In infected Eμ-*myc* mice, a few accelerated tumors contained proviruses near *pim*-1, but half the tumors had inserts in a different locus denoted *bmi*-1 and expressed a *bmi*-1 messenger RNA (mRNA) encoding a Zn finger protein (*75*). Because this Zn finger motif is associated with proteins that interact with DNA, the *bmi*-1 product is most likely a nuclear regulatory protein that collaborates with *myc* in oncogenesis. Hence, the rule that a nuclear oncoprotein cooperates with a cytoplasmic one (*68*) seems to have exceptions. Because many tumors contained inserts in more than one locus (for example, *bmi*-1 plus *pim*-1), *myc* probably requires multiple partners to elicit a lymphoma (*75*).

Potential for Tests of Carcinogens and Therapies

Mice primed for tumor onset are sensitive to various insults. The mutagen *N*-ethyl-*N*-nitrosourea prompted T lymphoma development in *pim*-1 mice, which had a sensitivity elevated 25-fold (*76*). Carcinogens also hastened liver cancer in mice bearing HBV (77) and skin carcinoma in H2-K-*fos* mice (*78*). Hence, transgenic mice should provide valuable bioassays for potential carcinogens. Their heightened sensitivity would obviate tests at high doses, where cell toxicity and mitogenicity become predominant (*79*). Mice exhibiting widespread expression of a gene commonly involved in human tumors (for example, p53, *ras*, or *myc*) but having a slow spontaneous tumor onset might be ideal. Preventive agents can also be tested. For instance, retinoic acid inhibited skin papillomas in H-*ras* mice (*33*). The high tumor incidence in many strains also offers possibilities for exploring therapeutic strategies.

Conclusions and Conundrums

Transgenic studies have buttressed molecular oncology by establishing that over two dozen genes implicated in cancer can indeed provoke tumors de novo (Table 1). By allowing tests in relevant cells, the approach obviates reliance on a few readily transfectable fibroblast lines, in which oncogenes of several classes are impotent. It also provides the substantial time frame often needed to generate a malignant clone. In a few models, a relevant mutation has been identified (Table 2), and the mutant gene has been shown to accelerate tumor onset, proving that the mutation was rate limiting. One oncogenic partner, however, may not suffice. Proviral tagging should elucidate some pathways to tumorigenesis but may not mimic base changes or readily disrupt both alleles of a tumor suppressor.

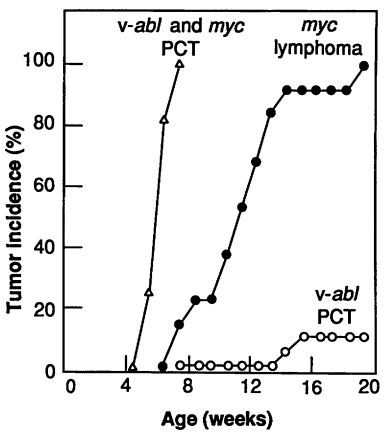

Fig. 3. Accelerated tumor onset in the doubly transgenic progeny of a cross between Eμ-v-*abl* and Eμ-*myc* mice (*52*). All the tumors in these pups were plasmacytomas (PCT) rather than lymphomas [reprinted with permission from *EMBO Journal*].

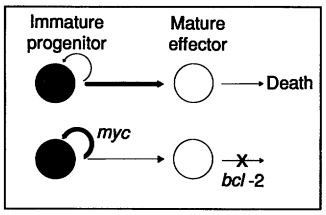

Fig. 4. Differentiation is perturbed in complementary ways by *myc* and *bcl*-2. (**Top**) Normal cells. (**Bottom**) Dysregulated *myc* expression decreases the probability of maturation and increases the proportion of progeny cells that are like the parental cell (self renewal); *bcl*-2 enhances cell survival.

In nontransgenic animals, the initially mutated cell may be subject to restraining signals from normal neighbors (*8*). It has been argued (*68*) that transgenic models do not mimic this step because most neighbors also express the transgene (Fig. 1) and may not therefore provide the inhibitory input. This argument may be pertinent to the few transgenic models (*15, 44*) where tumors seem to arise immediately, but the vast majority of models exhibit stochastic onset, as in normal tumor development.

The oncogenic profile of different oncogenes has varied considerably. The highly cell type–restricted impact of *fos* and *jun* cannot yet be reconciled with the ubiquitous role of the normal gene products. Neither *myc* nor *ras* evoked tumors in every tissue though each is usually assumed to function similarly in all cell types. Even SV40 T antigen, which has been oncogenic in most tissues (Table 1), as might be expected if the widely expressed p53 and Rb products were its principal targets, was much more potent in some cell types (*80–82*). Refractoriness of particular lineages or differentiation stages might reflect absence of molecules that associate with the transgene product or a dearth of requisite substrates. Conversely, susceptibility might reflect increased probability of activation of a cooperating oncogene; the lymphoid gene rearrangement machinery, for example, facilitates translocations (*24*).

The transgenic approach has provided access to the preneoplastic phase. Mitogenesis was promoted by *myc* or T antigen transgenes in many cells and by *fos* in osteoblasts but not by all oncogenes. The *ras* gene could accentuate either proliferation or differentiation, and the cell excess provoked by *bcl*-2 reflects diminished cell death rather than increased production. Some oncogenes (for example, v-*abl*, N-*ras*, and *pim*-1 expressed in lymphocytes) have no obvious direct effect on cell cycling or homeostasis. Thus, their oncogenic potential remains cryptic until other events intervene.

Tests of oncogene cooperativity in vivo (Table 2) indicate that the Myc nuclear oncoprotein can collaborate not only with cytoplasmic oncoproteins (H-Ras, N-Ras, K-Ras, v-Abl, v-Raf, Bcl-2, and Pim-1) but also with the nuclear T antigen and presumably also Bmi-1. The mechanism of collaboration remains elusive (*8*). If the partners provide complementary functions, perhaps related to distinct signal transduction pathways, at least three complementation groups probably are required. Two genes may be needed to establish an indefinite life-span. Both *myc* and *bcl*-2 contribute to clonal longevity: *myc* encourages self-renewal (*30*), and *bcl*-2 impedes cell death (*61–64*) (Fig. 4). The conjunction of the two favors immortalization (*61*) but does not relieve growth requirements (*73*). Most likely p53 and perhaps *bmi*-1 also fall into the immortalization category. A third class of oncogene may reduce growth factor dependence. The *ras* and *raf* genes rendered *myc*-driven pre-B cells autonomous (*70*); hence, these cytoplasmic oncoproteins, and perhaps others such as *pim*-1, *lck*, and v-*abl*, probably mimic a growth factor signal. Rather than supplanting the signal, however, these gene products may convey an unbalanced signal to the nucleus. That might explain why the oncogenicity of genes such as *ras* often appears greater than that of autocrine factor production. The distorted signal might help uncouple proliferation from terminal

differentiation. By disturbing mitotic control, it might also promote karyotypic changes that drive clonal evolution.

In future transgenic studies of cancer, greater control over transgene expression is needed, as illustrated by the varied effects of different amounts of *ras* in the liver (*38*). More consistent expression may be promoted by including locus control regions, which shield the transgene from flanking sequences (*83*), or by exploiting homologous recombination in embryonal stem (ES) cells to modify resident genes (*84*). Better inducible or repressible control systems are needed to clarify, for example, whether a given oncogene is needed to maintain as well as to initiate a tumor. Greater attention will be given to tumor suppressors by creating dominant negative mutations, as demonstrated for p53 (*50*), by using antisense RNA approaches, or by causing gene disruption in ES cells (*84*). To establish whether impaired differentiation is indeed central to tumorigenesis, it will be important to test interference with transcription factors that govern maturation in specific lineages (for example, MyoD). Perturbing expression of DNA replication-repair genes could validate the notion that karyotypic instability is critical. Finally, because many oncogene functions must impinge on cell cycle control, insights should emerge from altering the amounts of key molecules, such as the cyclins and *cdc*2 gene product.

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127. We thank A. Harris, D. Metcalf, R. Harvey, and A. Elefanty for helpful comments and I. Collins for typing the manuscript. Supported by grants from the National Health and Medical Research Council (Canberra), the Victorian Health Promotion Foundation, and the NIH (CA43540 and CA12421).

Viruses in Human Cancers

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Viruses may contribute to the development of human tumors by different mechanisms: indirectly by inducing immunosuppression or by modifying the host cell genome without persistence of viral DNA; directly by inducing oncoproteins or by altering the expression of host cell proteins at the site of viral DNA integration. Human cancers associated with papillomavirus, hepatitis B virus, Epstein-Barr virus, and human T cell leukemia-lymphoma virus infections are responsible for approximately 15 percent of the worldwide cancer incidence. Cancer of the cervix and hepatocellular carcinoma account for about 80 percent of virus-linked cancers. Because experimental and epidemiologic data imply a causative role for viruses, particularly in cervical and liver cancer, viruses must be thought of as the second most important risk factor for cancer development in humans, exceeded only by tobacco consumption.

VIRUSES CAN CONTRIBUTE TO THE DEVELOPMENT OF HUman tumors by a variety of mechanisms that range from genetic stimulation of host cell proliferation to virus-induced immunosuppression that permits the emergence of tumors not directly related to the suppressing virus (Table 1). A patient who is infected with human immunodeficiency virus (HIV) has a substantially increased risk for developing certain cancers, most notably Kaposi sarcomas and B cell lymphomas. These tumors appear to result from immunosuppression caused by HIV infection. Although mice transgenic for the HIV *tat* gene develop tumors similar to Kaposi sarcoma, specific genetic information for *tat* has not yet been found in human Kaposi sarcomas. Herpes simplex viruses (HSVs), on the other hand, have been suspected of contributing to some tumors, particularly anogenital and oral cancers (*1*), on the basis of seroepidemiological studies and reports on in vitro transformation of rodent cells by partially inactivated HSV preparations. Although these viruses are able to induce mutations in host cell DNA and to amplify specific intracellular DNA sequences under conditions of abortive infections (*2*), many recent studies failed to provide evidence for their involvement in human cancers. In this article I will therefore concentrate on those viruses for which tumor development appears to be the direct consequence of a specific infection and where trans or cis effects of viral genome persistence seem to contribute to the stimulation of cell proliferation.

Although members of at least three other groups of viruses exert cell-transforming properties, for example, polyomaviruses (BK, JC, and LPV), adenoviruses (particularly types 12 and 18), and poxviruses (molluscum contagiosum), none of them has yet been regularly documented to be present in human tumors. JC and BK virus genomic DNA in gliomas and insulinomas (*3*) occurs only in a fraction of tumor cells; thus these results are presently inconclusive.

Epstein-Barr virus (EBV), hepatitis B virus, several types of papillomaviruses, and HTLV-I and possibly -II (human T cell leukemia-lymphoma virus) are consistently linked to specific malignancies and will be discussed in greater detail (Table 2). None of these virus infections per se is sufficient to induce cancer. Long latency periods, often lasting several decades, the low number of infected individuals who eventually develop the particular type of cancer, monoclonality of the tumors, and in some instances interactions with chemical or physical factors in carcinogenesis (*4*) point to the requirement for additional modifications in cancer development

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