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Oncogenes and Tumour Suppressor Genes in Transgenic Mouse Models of Neoplasia

Deborah J. Fowlis and Allan Balmain

INTRODUCTION

THE STUDY of the molecular mechanisms of carcinogenesis has been greatly enhanced in recent years by the advent of transgenic mouse technology. It is now possible to introduce cloned genes directly into the germ line of mice in such a way that the genes are inherited in a stable Mendelian fashion. This approach enables the study of the *in vivo* function of genes thought to play important roles in the control of cell growth, development and differentiation. This short review will concentrate on the application of these techniques to investigate mechanisms of neoplastic development in specific tissues.

It is now acknowledged that proto-oncogenes, present in all normal cells, play a pivotal role in many human and animal cancers after they have undergone a genetic alteration leading to aberrant expression or function of the gene product [1]. However, from studies of human tumours it is difficult, if not impossible, to determine whether such changes are the cause or consequence of neoplastic development. These questions can only be addressed using animal model systems in which the various steps of tumour initiation and progression can be reproduced, either by mutation of the appropriate genes using chemical carcinogens [2, 3], or by direct introduction of mutant genes into the germline of mice [4]. Transgenic mice offer the possibility of investigating the effects of proto-oncogenes or their activated counterparts *in vivo*, when expressed using their

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own promoter sequences or after forcing their expression in particular organs using strong tissue-specific promoter elements. There are obvious advantages of using *in vivo* systems such as these over *in vitro* systems: cells transformed *in vitro* tend to follow selection pressures dictated by the particular growth conditions used, and these do not necessarily reflect those pertaining *in vivo*, where complex cell interactions and hormonal influences act on tumour cells. In addition, there is also a more practical advantage. The ability to target a specific gene, which is known to be involved in particular forms of human cancer, to the appropriate organ or cell type in the mouse offers new avenues to the development and testing of novel therapies [5, 6]. Such approaches have not yet been exploited in the clinic, but the range of tumour types which can now be generated in mice by tissue-specific expression of 'relevant' genes is already very wide (see Table 1), and it is certain that models which reflect the human situation even more accurately will be developed in the future.

TISSUE-SPECIFIC NEOPLASIA IN TRANSGENIC MICE

Table 1 shows a list of recent studies on tumour development in transgenic mice. Other more comprehensive lists of previous investigations have already appeared elsewhere [4, 7]. Basically, two approaches have been used to study the effects of oncogene expression *in vivo*, involving the use of promoter elements which direct expression to specific tissues, or which should allow more generalised expression in a variety of different target sites. This is illustrated by the early studies of Palmiter *et al.* [8], who expressed the SV40 large T antigen in mice under the control of its own (viral) promoter. Somewhat surprisingly, since this promoter is expressed in many cell types, the mice mainly developed tumours of the brain. In contrast, expression of the same oncogene from the promoter of the insulin gene, normally expressed only in the pancreas, led to the formation of pancreatic cell tumours [9].

These studies and many others carried out subsequently have confirmed that tissue specificity is normally dictated by the regulatory sequences 5′ to the oncogene. On the other hand, different oncogenes placed under the control of the same regulatory region can result in different carcinogenic potentials.

Haematopoietic system

Early studies in patients with Burkitt's Lymphoma had shown that the condition is associated with translocations involving the c-*myc* proto-oncogene on chromosome 8 and loci of the immunoglobulin (Ig) heavy or light chain genes on chromosomes 14, 2 or 22 [10]. These translocations result in aberrant expression of the c-*myc* gene under the control of Ig gene regulatory elements.

Many transgenic lines have now been generated expressing a variety of genes in B cells using the Ig heavy chain enhancer (Eμ). Transgenic mice expressing an oncogene such as c-*myc* linked to Eμ frequently incur B or pre-B cell lymphoma [7]. Lymphomas so derived were found, as judged by their Ig gene rearrangements, to be monoclonal. Also, they arose with variable latency and in many cases, only a small proportion of mice developed tumours. Similar conclusions have been reached using transgenic mice which develop other tumour types [11, 12]. A general theme has emerged that a single oncogene is not by itself sufficient for tumour induction, since the majority of tumours are clonal in origin and must, therefore, have been selected from the population of oncogene-carrying cells.

This, of course, agrees with the classical concept of carcinogenesis as a multi-step process in which successive biological changes are accomplished by altering different critical target genes. Studies using transgenic mice have supported and extended these concepts by showing that oncogenes can act synergistically *in vivo*, since mice carrying two oncogenes (conveniently obtained by cross-breeding lines which have single transgenes) frequently develop tumours at a much faster rate than either of the parental lines. This form of cooperation was first shown for *ras* and *myc* genes in induction of tumours of the mammary gland [13], but has also been shown to apply to other genes [14] and to other malignancies [15] (for review, see [16]).

In some cases, the co-operating oncogene can be delivered exogenously, by infection with retroviruses carrying specific genes [17, 18]. For example, Langdon *et al.* [17] found that the development of Eμ c-*myc* B lymphoid tumours was accelerated when retroviruses carrying v-Ha-*ras* or v-*raf* genes were used to infect neonatal Eμ c-*myc* mice, whereas retroviruses containing c-*abl* did not accelerate tumour development. In other cases, it has been shown that mutations in cellular *ras* genes could arise as spontaneous or carcinogen-induced second events during lymphomagenesis induced in Eμ *myc* or Eμ *pim*-1 transgenic mice [19, 20]. These results suggested that the cytoplasmic protein products of either *ras* or *raf* genes, which incidentally have recently been shown to lie at different points on the same cellular signalling pathway [21], could cooperate with the nuclear *myc* protein to transform cells of the B lymphocyte lineage. This confirms and extends previous observations on oncogene cooperation *in vitro* (reviewed in [16]).

More recent experiments have taken this approach one stage further, and used retroviruses, which integrate more or less at random into the mouse genome, leading to gene activation at the integration site, to localise and isolate genes capable of co-operating with oncogenes carried by single transgenic mice. By using the MoMuLV provirus as a tag, two groups have identified common sites of integration in tumours which led to the isolation of several new genes, one of which encodes a novel type of zinc finger protein [22, 23]. These approaches have already led to the identification of genes which are important in the control of growth and differentiation of cells of the haemopoietic lineage, and similar techniques are presently being applied to other cell types.

Another widely studied haemopoietic malignancy is chronic myeloid leukaemia (CML). This disease, together with a subset of cases of acute lymphoblastic leukemia (ALL) is associated with the presence of the Philadelphia chromosome, which generates a fusion product between the *bcr* and *abl* genes on chromosomes 22 and 9, respectively. Attempts have also been made to produce transgenic mice carrying *bcr-abl* fusion genes in order to study animal models of these specific tumours [24]. These studies are still continuing, but the specific restricted phenotypes seen in patients carrying particular *bcr-abl* fusion genes (i.c. CML or ALL) have not yet been fully reproduced in mice [25]. On the other hand, the pathology of human follicular lymphoma associated with the t(14;18) translocation which activates the *bcl*-2 oncogene can apparently be recreated in transgenic mice using *bcl*-2/Ig fusion genes [26].

Skin

Tumours of the skin are among the most frequently occurring human cancers. Although the genetic alterations in human skin tumours have not been described in detail, the H-*ras* oncogene does appear to be activated in a proportion of the cases investigated [27]. Interestingly, the same H-*ras* oncogene is activated

Table 1. Tissue-specific tumour development in transgenic mice

Regulatory region	Oncogene	Phenotype	Reference
Haematopoietic system			
IgEμ	c-*myc*	B or pre-B lymphoma	[20, 12, 15, 17]
IgEμ	*pim*-1	T-cell lymphoma	[19]
IgEμ	*bcl*-2	Extended B-cell survival/follicular proliferation	[26]
bcr	*bcr/abl*	Acute leukaemia (myeloid or lymphoid)	[24]
GP91 p Hox	SV40 T Ag	Histiocytic lymphoma	[64]
Skin			
Keratin 10	Ha-*ras*	Papilloma	[29]
Zeta-globin	v-Ha-*ras*	Papilloma/carcinoma	[34]
Polyoma virus	BNFL-1	Epidermal hyperplasia	[36]
Keratin 14	TGF-α	Epidermal hyperplasia/papilloma	[37]
Keratin 6	HPV-1 early region	Epidermal hyperplasia	[39]
H2K MHC	v-*jun*	Epidermal hyperplasia Dermal fibrosarcoma	[40]
BPV-1	BPV-1	Fibrosarcoma	[41]
Tyrosinase	SV40 T Ag	Melanocytic hyperproliferation Malignant melanoma	[42, 43]
Mammary gland			
MMTV LTR	c-*myc*	Mammary carcinoma	[11]
MMTV LTR	v-Ha-*ras*	Mammary carcinoma Harderian gland tumour	[13, 60]
MMTV LTR	N-*ras*	Mammary carcinoma Harderian gland tumour Male infertility	[44]
MMTV LTR	*int*-1	Mammary carcinoma Salivary gland tumour	[48]
MMTV LTR	*int*-2	Mammary carcinoma Prostate gland tumour	[47]
MMTV LTR	TGF-α	Mammary carcinoma	[49]
MMTV LTR	c-*neu*	Mammary carcinoma	[51, 52]
Whey acidic protein	c-*myc* + Ha-*ras*	Mammary carcinoma	[53]
Brain			
SV40 or metallothionine	SV40 T Ag	Choroid plexus tumours	[8]
Polyoma early region	Polyoma T Ag	Pituitary tumours	[69]
Liver			
α-1-Anti-trypsin	SV40 T Ag	Liver carcinoma	[54]
Metallothionine	TGF-α	Liver carcinoma	[55]
Albumin	Hepatitis B virus	Liver carcinoma	[56]
Bone			
Protamine 1	SV40 T Ag	Bone tumour Heart tumour	[59]
Various	c-*fos*	Bone tumour	[61]
Small intestine			
Intestinal fatty acid binding protein	SV40 T Ag	Crypt proliferation	[63]
Large intestine			
Glucagon	SV40 T Ag	Colon carcinoma	[62]
Lung			
IgEμ + SV40 promoter	*ras*	Lung adenomas	[12]
Albumin	H-*ras*	Lung adenocarcinomas	[65]
Thyroid			
Thyroglobulin	SV40 T Ag	Adenocarcinoma	[66]
Moloney LTR	*mos*	C-cell thyroid tumours/pheochronocytomas (MEN II)	[67]
Kidney			
Renin	SV40 T Ag	Vascular hyperplasia	[68]
Pancreas			
Insulin	SV40 T Ag	Pancreatic carcinoma	[9]

in chemically induced mouse skin tumours [2]. In this model system, the proportion of tumours which have activated H-*ras* genes, and the specific mutations which result in gene activation, depend on the nature of the initiating carcinogen [28].

The role of the H-*ras* oncogene has now been tested in transgenic mice, using the promoters of keratin genes to direct high level expression of the transgene to the epidermis. Bailleul *et al.* [29] reported hyperkeratosis of the skin and subsequent papilloma formation in transgenic mice expressing a mutant human H-*ras* oncogene from a suprabasal keratin promoter (K10). The papillomas developed initially behind the ears or at the tail-base areas that are often scratched or bitten, suggesting that the secondary event in carcinogenesis in these animals may be a mild wounding stimulus. Earlier studies were complementary to these. Brown *et al.* [30] applied Harvey murine sarcoma virus to mouse skin and found that papillomas did not develop unless the tumour promoter, 12-0-tetradecanoyl-phorbol-13-acetate (TPA), was subsequently applied to the skin. The wounding event necessary for papilloma formation in the studies of Bailleul *et al.* [29] may be functioning as a tumour promoter—indeed wounding has been shown to be an efficient promoting stimulus [31].

An interesting aspect of this study was that the *ras* gene induced generalised hyperkeratosis before the appearance of the benign tumours, suggesting that the same gene may control both growth and differentiation in epidermal cells. Moreover, the fact that tumours arose as a result of expression of the oncogene in suprabasal cells indicates that cells which have left the stem cell compartment are nevertheless capable of giving rise to at least benign tumours. This is compatible with the observation that terminally benign human keratocanthomas have a relatively high incidence of H-*ras* mutations [27]. In the latter study, the specific mutation observed, an A:T → T:A transversion causing a glutamine to leucine alteration at codon 61, is identical to the mutation found in rodent skin tumours induced by dimethylbenzanthracene (DMBA) [2] and in the majority of spontaneously transformed epidermal cells in culture [32]. There would appear to be a specific property of *ras* genes carrying this mutation which enables terminally differentiating cells to be reactivated into the proliferative cycle. This property may be related to the high affinity of this particular mutant for GTP [33], and subsequent highly efficient interaction with an effector molecule which mediates the proliferative response.

An unexpected specificity for expression in the skin was seen in transgenic mice expressing a v-Ha-*ras* oncogene under the control of a zeta-globin promoter which is normally only expressed in cells of the erythroid lineage [34]. The transgenic mice also developed papillomas only at sites of mechanical irritation. These mice were termed promoter-sensitive since the initiation step—activation of the *ras* oncogene—had already been accomplished and they required only application of a promoter to the skin for papillomas to develop. These mice, together with transgenic lines which carry several copies of the normal human H-*ras* gene under the control of its own promoter [35], show very high sensitivity to treatment with initiators and promoters of carcinogenesis, suggesting that they may be of use in the detection and classification of environmental carcinogens.

Other studies have described transgenic mice which develop hyperplastic skin conditions, if not complete neoplasia, and which may be useful models of human skin disease [36–39]. Expression of Epstein–Barr virus latent membrane protein in the skin of transgenic mice led to induction of a condition resembling psoriasis, increased epidermal thickness and hyperproliferation [36]. Similarly, mice expressing transforming growth factor α (TGF-α) from a basal keratin promoter also developed hyperproliferation, with subsequent appearance of papillomas at the base of the tail, similar to the phenotype observed by Bailleul *et al.* [29] using the keratin 10/*ras*-construct. These results support the suggestion [29] that TGF-α may mediate some of the effects of *ras* in these transgenic animals.

Skin tumours of a different kind were observed in mice transgenic for v-*jun*, driven by the H-2K major histocompatibility class I antigen gene promoter [40]. V-*jun* mice have normal skin until a wounding stimulus is applied, which results in formation of benign hyperplastic granulation tissue. After a few months, the hyperplastic tissue progresses to malignant dermal fibrosarcomas in a proportion of the mice. Again, it would appear that secondary (wounding) and unknown tertiary events are necessary for carcinogenesis.

Fibrosarcomas were also induced in transgenic mice expressing bovine papilloma virus type 1 [41]. In one of the few studies of its kind to date, the authors investigated the nature of possible second events occurring during progression of benign fibromas to malignant fibrosarcomas. They found that subsequent numerical chromosomal alterations could be detected in cell lines from the malignant tumours, but not from their benign counterparts. A frequent observation was monosomy of chromosome 14, which carries the retinoblastoma tumour suppressor gene. Whether or not this gene is functionally altered in these tumours has not yet been determined.

Approaches to a transgenic mouse model for melanoma formation have recently been described [42, 43]. The strategy used was to employ the promoter of the tyrosine gene, expressed at high levels in melanocytes, to drive expression of the SV40 large T antigen. The resultant mice showed widespread deregulation of melanocyte growth control, and in some cases, progression to malignant melanoma [43].

Breast

Cancer of the breast is one of the foremost causes of death in women, and there has been a great deal of effort invested in determining the nature of the target genes involved and in generating animal models of the disease. The mouse mammary tumour virus (MMTV) has proved to be an extremely useful tool in this regard, since the long terminal repeat (LTR) of the virus contains sequences which induce expression of linked genes in the mammary gland. This promoter has, therefore, been used to direct expression of a number of oncogenes and growth factors to the mammary gland, with consequences ranging from mild hyperplasia to frank adenocarcinoma.

In early studies using this system it could be shown that both c-*myc* [11] and mutant H-*ras* [13] can induce the formation of adenocarcinomas in transgenic mice. Interestingly, transgenics expressing an N-*ras* oncogene from the same promoter develop mammary tumours and additionally show compromised male fertility [44]. This suggests distinct physiological actions for the very closely related individual *ras* family members.

MMTV is known to induce mammary tumours in mice by insertional activation of a number of genes encoding growth factor-related polypeptides [45]. These observations prompted several groups to test whether the same genes might be involved in human breast tumour development, with equivocal results. One of the genes at a common virus integration site (*int*-2) is, in fact, amplified in a subset of human carcinomas, but recent evidence suggests that the crucial gene is not the *int*-2 gene itself but a closely linked gene of the cyclin family (cyclin D1/*prad*-

1/*bcl*-1) which is amplified and over-expressed in the same tumours [46].

What is clear is, however, that *int*-1 and *int*-2 genes, when driven from the MMTV promoter, can induce epithelial growth and mammary tumours in transgenic mice [47, 48]. In more recent experiments, it has been shown that cross-breeding of these animals leads to more rapid tumour formation in the double transgenics, indicating that the two genes can cooperate to induce mammary neoplasia [14]. Whether expression of the cyclin D1 in mammary glands of transgenic mice has any phenotypic consequences remains to be determined.

Other growth factors and/or receptors have also been shown to act as oncogenes in MMTV-transgenic mice. Matsui *et al.* [49] found that expression of a MMTV TGF-α construct induced a range of abnormalities from simple hyperplasia to mammary adenocarcinoma in females, whereas male mice did not undergo any morphological changes.

It has been shown that the *neu* oncogene, encoding a growth factor receptor related to the EGF receptor, is amplified and/or over-expressed in a proportion of human breast carcinomas [50]. More importantly, the expression of this gene is claimed to have prognostic significance, since over-expressors tend to have relatively shorter average survival times. Studies using transgenic mice have supported the idea that this oncogene has potent tumour-inducing effects in mammary tissue. Expression of the c-*neu* oncogene from the MMTV promoter resulted in polyclonal mammary adenocarcinomas that developed synchronously [51]. It was suggested that expression of the c-*neu* oncogene, in the mammary gland at least, was sufficient to induce carcinogenesis in a single step, in apparent contradiction to the concept of multistage carcinogenesis. Later, however, Bouchard *et al.* [52] found that MMTV/c-*neu* mice only developed tumours after a longer latency period, suggesting the requirement of additional events. The reasons for this discrepancy have not yet been clarified.

The whey acidic protein (WAP) gene promoter, which is induced hormonally in mid-pregnancy, was used to observe the effects of oncogenes on a well-defined differentiation process in WAP/c-*myc* and WAP/Ha-*ras* double transgenic mice [53]. Co-expression of these genes resulted in hyperplastic growth and impairment of function in mammary glands. Tumours were only observed 3–4 months after the induction of oncogene expression, suggesting once again that additional events were required for carcinogenesis, even in the presence of two activated oncogenes.

Liver

In humans, liver cancer occurs at high frequency in areas where infection by hepatitis B virus is prevalent. Because of the high rates of infection in many parts of the Far East and Africa, liver cancer has become a major health problem in many developing countries.

Since alpha-1-antitrypsin is synthesised predominantly in hepatocytes, its gene promoter was used to direct expression of SV40 T antigen to the liver in transgenic mice [54]. Liver carcinoma was observed in most founder animals and some also developed stomach or pancreatic cancer. The tumours developed in discrete stages, with additional events being necessary for full neoplasia. Jhappan *et al.* [55], expressing TGF-α from the inducible metallothionein promoter, induced carcinoma in liver and impeded normal development in mammary glands. This work, as well as many others in Table 1, illustrated that a gene can be oncogenic in one tissue whilst having a disrupting influence on normal development, or in some cases, no obvious consequences at all in another, i.e. the response of different tissues to the same gene product is often phenotypically distinct.

In what may be a more accurate reflection of the development of liver cancer in humans, Chisari *et al.* [56] generated transgenic mice expressing hepatitis B viral constructs from an albumin gene promoter. They observed a series of changes in the liver reminiscent of the stage-specific pathology of human hepatoma development, including the existence of a chronic preneoplastic phase before the outgrowth of hepatocellular carcinoma. Interestingly, although mutations in the p53 tumour suppressor gene have been detected in human liver tumours, particularly in those associated with aflatoxin exposure [57], no p53 mutations were found as second events in transgenic mouse liver tumours [58].

Other tissues

A number of lines of transgenic mice have been reported which develop tumours or show growth deregulation in specific tissues [59–70] (Table 1). Tissues in which neoplasia or growth abnormalities have been induced include the lung [65], large [62] or small [63] intestine, thyroid [66, 67], kidney [68], pituitary [69] and bone [61]. In some cases, the specific phenotypes observed are surprising consequences of fortuitous integration at particular sites within the genome or of the combination of promoter and gene sequences used. For example, heart and bone tumours have been produced in transgenic mice using SV40 T antigen fused to the mouse protamine 1 gene regulatory sequence [59]. The protamine gene is normally transcribed exclusively in spermatids, so expression seen in heart and bone of transgenic mice was surprising. Such unexpected transcription patterns have also been observed when the MMTV promoter directs oncogene (v-Ha-*ras*) expression to lungs, kidney and lymphoid organs as well as to mammary glands, resulting in neoplasia in these tissues [60].

TRANSGENIC APPROACHES TO THE FUNCTIONS OF TUMOUR SUPPRESSOR GENES

It is evident that inappropriate expression of many oncogenes can result in tumour development, supporting the theory that the dominantly acting oncogenes are a necessary step in carcinogenesis. However, a critical feature of oncogenes is that, alone, they appear to be insufficient for tumour formation. The necessary second event may involve, in some cases, the functional loss of tumour suppressor genes. According to the classical definition of such genes [71], alterations at suppressor loci are recessive, and have no phenotypic consequences unless both alleles are lost or inactivated. Several tumour suppressor genes have now been cloned and many more candidate loci have been identified in the human genome [72, 73].

Clearly, animal models for these recessive, loss of function, mutations would be fascinating but are more difficult to create than the dominant gain of function oncogene models, since the former involve somehow eliminating both normal suppressor genes. Nevertheless, the ultimate goal of knocking out tumour suppressor gene function *in vivo* is now within reach. The development of sophisticated techniques for the manipulation of genes in embryonic stem (ES) cells by homologous recombination has made it possible to introduce mutations into single alleles at any given locus in order to inactivate the target gene [74]. ES cells carrying mutant alleles can then be used to generate chimaeric mice after injection into blastocysts and re-implantation into pseudo-pregnant hosts. Mice showing germline transmission of mutant genes can be bred to give mice

homozygous at the defective locus. Many laboratories are now using this approach to knock out putative tumour suppressor loci. The results so far are tantalising, but incomplete. A knock-out experiment has been successfully carried out on the p53 gene [75]. Amazingly, mice without any functional p53 gene are phenotypically normal indicating that p53 alleles are, at the least, not required for normal development. However, animals homozygous for the null p53 allele develop tumours spontaneously at around 3 months after birth, and most are dead by 5 months. The tumours which develop are predominantly lymphomas, but include also sarcomas and testicular tumours [75].

Recent unpublished studies from this laboratory (C.J. Kemp, P. Burns and A. Balmain, in collaboration with L. Donehower and A. Bradley) have established that lack of functional p53 is crucially important at the benign–malignant transition in skin tumours. Lack of p53 did not increase the frequency or growth rate of early papillomas after treatment of the knock-out mice with initiators and promoters of carcinogenesis, but markedly increased the progression rate to carcinomas. This is compatible with studies on chemically induced mouse skin tumours, which showed that p53 mutations appear to occur at the benign–malignant transition [76].

The mechanistic basis for this apparent role for p53 in malignant progression is not yet clear, but may be related to its putative function in control of genomic integrity [77]. Lack of normal p53 may facilitate the accumulation of genetic defects which give a strong selective advantage to developing tumour cells. Interestingly, transgenic mice carrying a mutant form of p53 (which is known to act in a dominant negative fashion to inactivate the normal p53 function [78]) developed a wide spectrum of malignancies of different tissues [79]. A similar range of tumour types was also seen in individuals from families exhibiting Li-Fraumeni syndrome. Two groups, therefore, identified the p53 gene as a candidate gene for Li-Fraumeni syndrome, and confirmed its involvement by directly sequencing the gene from affected individuals [80, 81]. This constitutes another example of the parallels between mechanisms of carcinogenesis in mouse and man.

CONCLUSIONS AND FUTURE PERSPECTIVES

The transgenic systems described here appear to model many human cancers and thus may provide a way of defining individual stages in carcinogenesis and pin-pointing the oncogenes and tumour suppressor genes that induce transitions between these stages. It has become clear recently that the loss of growth suppressor genes has just as much importance in carcinogenesis as the acquisition of oncogenes. The ability to control both the genetic background of the host and the aetiology of tumour induction, i.e. by a single transgenic oncogene, means that the likelihood of observing reproducible genetic second events in transgenic mice may be high. Approaches to the detection and analysis of loss of suppressor genes in mice have already been made using standard chemical carcinogenesis systems [82]. The strategy employed was to induce tumours in F1 hybrid mice (or interspecific *Mus musculus/Mus spretus* crosses) which are amenable to analysis for loss of heterozygosity because of the existence of discrete genetic polymorphisms distinguishing the parental alleles. This approach is eminently suited to the analysis of loss of tumour suppressor genes in transgenic mice, by breeding the transgene into the F1 hybrids. The approach can be further refined by cross-breeding with animals in which tumour suppressor genes such as p53 or Rb have been deleted by homologous recombination. The use of transgenic mouse technology may, therefore, lead not only to answers to many questions related to the true *in vivo* roles of oncogenes or tumour suppressor genes, but to the detection of novel genetic loci of importance in cell growth and differentiation.

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