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Feature Articles

Oncogene Transgenic Mice as Therapeutic Models in Cancer Research

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INTRODUCTION

THE ABILITY to introduce foreign genes stably into the germ line has revolutionised the genetic manipulation of animals. From 1984, mice have been successfully reared from eggs microinjected with genes involved in the development of cancer, and these mice have subsequently developed tumours. The first report involved microinjection of the early region of Simian virus 40 (SV40) into animals which subsequently developed tumours of the choroid plexus [1]. The next model involved expression of the *myc* transgene in the mammary gland [2]. Initially, the mice showed no morphological abnormalities, but at 4 to 5 months of age multiparous female transgene carriers developed solitary mammary adenocarcinomas in a stochastic manner, in areas adjacent to normal tissue expressing the transgene. This suggested that transgene expression is necessary but not sufficient for tumorigenesis, and that additional genetic events are required. Since then, a large number of oncogene transgenic mice have been produced and used to examine the function of a diverse number of oncogenes. Loss of gene function has also been modelled by interrupting coding sequences of particular genes—these are termed "knockout mice".

TRANSGENIC MICE AS MODELS OF MALIGNANCY

A large number of tumour types have now been modelled using oncogene transgenic mice. The transgenes involved are representatives of each major protein class involved in neoplasia: cytokines, cytokine receptors, signal transducing molecules and nuclear proteins which serve as transcription factors or regulate replication.

A number of methods have been used for the production of transgenic mice. These include microinjection into the one-cell embryo [3], infection of preimplantation embryos with retroviral vectors containing foreign DNA [4], infection and transfection of embryonic stem (ES) cells and infection of postimplantation animals [5]. The latter may be advantageous where an oncogene might prove toxic if expressed in every cell. Microinjection is the most widely used and successful method.

The constructs introduced into the germline usually have an oncogene (activated, cellular or viral) or tumour suppressor gene under transcriptional control of a promoter, which is either widely active or of restricted tissue specificity. Normal tumorigenesis involves two events, mutation of a single cell and expansion of the resultant clone. In transgenic mice, the presence of a heterologous promoter expedites the normal process of carcinogenesis by one step. The transgene is usually expressed in a tissue-specific manner, the tissue being determined by the regulatory elements of the hybrid gene. Thus, the promoter region of the mouse mammary tumour virus (MMTV), placed upstream of the coding sequences for c-*myc* and H-*ras*, targets their expression preferentially to tissues in which MMTV is expressed in greatest quantities, i.e. breast, salivary epithelium and lymphoid tissues. For this reason, models of breast cancer often involve the oncogene of interest under the control of the MMTV promoter. However, in most murine transgenic models of cancer, tumours are monoclonal and only become apparent after a variable latency period. It seems highly likely that synergistic mutations are required for tumour formation. Some specific examples of transgenic oncogene mice as models of different malignancies are described below.

Breast cancer (*see Table* 1)

The overexpression or amplification of a number of oncogenes has been demonstrated in breast cancer using Southern and northern analysis and immunohistochemistry of tumour biopsies and cell lines [6]. Nevertheless, transgenic models are better able to address questions about the causal association between expression of a gene and tumour progression.

As outlined above, targeting oncogene expression to mammary epithelium has been possible by using a mammary-specific transcriptional element, such as the MMTV long terminal repeat (LTR) or the Whey acidic protein gene (Wap) promoter [7]. These promoters stimulate high levels of transgene expression in mammary epithelium. There are two models involving the activated c-*neu* oncogene. In the model of Muller and colleagues, tumours arose synchronously in all mice, involved the entire gland and were polyclonal in origin. Development of tumours in these mice appears to involve a single genetic event [8]. This is the only transgenic model of mammary cancer where a single oncogene is sufficient for the malignant transformation of mammary cancer. In mice with the same transgene developed by Bouchard and colleagues, tumours were monoclonal and appeared later in a stochastic pattern in a proportion of mice [9]. Unactivated *neu* oncogene is also associated with the development of mammary tumours in older transgenic mice. This may be of clinical relevance given that overexpression of unactivated

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Table 1. Summary of transgenic mouse models of breast cancer

Transgene	Promoter	Comments	Reference
neu	MMTV	Single step induction. *Neu* oncogene activated	[8]
neu	MMTV	Develop mammary tumours stochastically at 5–10 months	[9]
neu	MMTV	Focal mammary tumours after long latency, many metastasise to lung Unactivated *neu*	[10]
ras	MMTV	Also develop salivary gland tumours	[34]
myc	MMTV	Also develop Sertoli tumors of testis	[2]
myc	Whey acidic protein promoter	80% develop mammary carcinomas	[33]
Wnt-1(int-1)	MMTV	Frequent mammary and less frequent salivary gland adenocarcinomas	[36]
Int-2	MMTV	Pronounced mammary gland hyperplasia, no tumours described	[12]
Has-*ras*	Whey acidic protein promoter	Also develop salivary gland tumours where TG expressed	[31]
Wnt-1 and *int-2*	MMTV	Mammary carcinomas in males and females	[11]
TGF-α	MMTV	Four types of hyperplasia in two lines. Adenocarcinoma in 40% multiparous, 30% of virgin mice by 16 months of age	[19]
Human growth hormone	Mouse metallothionein promoter	3/4 founders developed malignant papillary adenocarcinomas of mammary origin at 27–43 weeks	[35]
TGF-α	Mouse metallothionein promoter	Secretory mammary adenocarcinomas in postlactational mammary gland	[32]
C-*myc*	Whey acidic protein promoter	80% develop mammary carcinomas	[33]

neu is associated with poor clinical outcome in human breast cancer [10].

Mice bearing MMTV *wnt*-1, MMTV *int*-2 and MMTV transforming growth factor (TGF)-α transgenes develop mammary hyperplasia [11, 12, 13]. Double transgenic mice have been used to investigate oncogene cooperativity [7]. The *wnt*-1 and *int*-2 protooncogenes cooperate in mammary carcinogenesis, with tumours arising earlier in the transgenic mice possessing both oncogenes [11]. Mammary tumours arising in mice bearing *neu, ras* or *myc* oncogenes have different and characteristic phenotypes. Most notably their histological patterns can be used to predict the genotype of the mouse [14]. Only 9% of the tumours arising in these three groups of mice could be classified using the standard categories described by Dunn in the Dunn classification, compared with 95% of those seen in tumours in MMTV-infected mice [15]. The remaining 91% were classified as eosinophilic, papillary adenosquamous small cell (SC), basophilic, glandular large cell (LC) and pale nodular intermediate cell (IC). The SC phenotype predominated in *ras* mice, IC in the *neu* mice and LC in the *myc* transgenic mice. This also held true for groups of *ras* and *myc* bigenic animals, where the *myc* phenotype appeared to be dominant, and in trigenic animals with all three oncogenes where again *myc* appeared dominant [13].

Haematological and lymphoid tumours

Representatives of most of the classes of genes implicated in neoplasia and proliferation have been tested for their impact on the haemopoietic system. Oncogene transgenic mouse models of lymphomas, acute and chronic leukaemias and lympho- and myeloproliferative syndromes exist.

The activated c-*myc* oncogene is often implicated in human B-cell neoplasia, particularly Burkitt's and other high grade B cell lymphomas. Transgenic mice have made it possible to test the hypothesis that deregulated *myc* expression is lymphomagenic. An unmodified c-*myc* transgene linked to the 5′ immunoglobulin heavy chain (Igh) enhancer (Eμ), simulating the effect of the translocations characteristic of Burkitt's lymphoma, was very potent with virtually every mouse succumbing to B lymphoid tumours within a few months of birth [16].

N-*ras* and H-*ras* (which along with K-*ras* comprise the *ras* family of oncogenes) have also been assessed for their ability to induce haemopoietic tumours. In mice expressing a mutated H-*ras* human cDNA controlled by CD2 regulatory sequences, high thymic expression of H-*ras* is seen and some lines develop transplantable clonal T lymphoid tumours. However, where H-*ras* is under the control of the Igh enhancer (Eμ), several lines develop lung adenocarcinomas after a short latent period which may antedate any haemopoietic effects [17].

Gastrointestinal tumours

Liver carcinogenesis has been investigated in a number of transgenic models. Two models involving the hepatitis B virus (HBV) large surface antigen and the Hbx gene suggest that HBV may predispose individuals to cancer by altering hepatocyte differentiation and driving regeneration [18]. Sustained proliferation is also a feature of liver tumours seen in TGF-α transgenic mice [19].

Pancreatic cancer has been modelled using β cells of the islets of Langerhans and acinar cells of the exocrine pancreas. The SV40 T antigen is tumorigenic in liver and pancreatic tumours [20]. T antigen expression in the β cells has resulted in the

development of frank pancreatic tumours. The antigen appears to promote karyotypic instability which may be central to many forms of tumorigenesis. *Ras* mutation is often involved in both liver and pancreatic tumours (H-*ras* and K-*ras*, respectively). High expression of a mutant H-*ras* transgene in the liver provoked hyperplasia and perinatal death, but lower expression provoked mild focal dysplasia and occasional frank carcinoma [20]. In the pancreas, a mutant H-*ras* allele [21] or SV40 T antigen [22] were associated with development of acinar tumours, whereas *myc* produced mixed acinar-ductal tumours [23]. Pancreatic tumours are often associated with a mutant *ras* allele, albeit K-*ras*.

Tumour suppressor gene models

Mutations in p53 are frequent in human cancers and are inherited in the Li-Fraumeni syndrome. This is a familial predisposition to cancer, transmitted in an autosomal dominant manner, and characterised by sarcomas in children and a high incidence of early onset carcinoma of the breast in female relatives [24]. Mice bearing a mutant p53 gene develop a spectrum of tumours similar to that seen in some Li-Fraumeni families [25]. SV40 T antigen binds both p53 and the product of Rb, the tumour suppressor gene implicated in retinoblastoma. SV40 T antigen transgenics express T antigen in the retina and develop ocular tumours with ultrastructural features similar to those found in retinoblastoma [26].

IMPORTANCE OF GENETIC BACKGROUND

It is well documented that genetic background modulates tumour susceptibility. Our own experience (detailed below) suggests that background is important and likely to influence incidence, natural history and biology of the tumour. The effect is also demonstrated well in haemopoietic tumours. Studies with Eμ-*myc* transgenes illustrate that the kinetics and tumour type can be influenced as well as tumour susceptibility. On a C57 Bl/6, SJL or BALB/c background Eμ-*myc* transgenes provoked B lymphoid tumours almost exclusively, but seven of eight founder C3H/HeJ transgenic mice developed T lymphomas. In the C57Bl/6 and C3H/Hej mice, an increase in size and numbers of pre-B cells was seen along with similar levels of Eμ-*myc* transcripts in the spleen and thymus [27]. This is consistent with B lymphoid expression of the transgene, suggesting that the difference in tumour type is unlikely to be an effect of the transgene alone. The stromal environment may be important as conventional C3H/HeJ mice repopulated with transgenic C57Bl/6 cells also developed T lymphomas [27]. The effect of genetic background is also seen with transgenic mice harbouring the SV40 large T antigen gene in a C57Bl/6J genetic background. In this model, the level of transgene RNA expression is considerably higher than in transgenic mice harbouring the same transgene in an F1 genetic background. The F1 hybrids are C56Bl mice crossed with NZW mice, which appears to have a dominant negative effect on SV40 large T antigen expression. Choroid plexus papillomas appear later and less frequently, resulting in longer survival of the animal [28].

FEASIBILITY OF USING TRANSGENIC ONCOGENE MICE AS A MODEL FOR CANCER THERAPY

Although mice transgenic for oncogenes have been available for a decade, their use as a model for experimental therapeutics has been limited. They could be useful for preclinical investigation of prophylaxis, primary therapy and adjuvant treatment. The advantages and disadvantages of the model are discussed below and summarised in Table 2.

Table 2. Advantages and disadvantages of transgenic mouse model

Advantages
Non-immunogenic tumours arise in immunocompetent mice
Spontaneous tumours arise in a stochastic fashion
Many tumours resemble human tumours histologically
Metastases are more likely to arise by the same mechanisms as in humans
A broad range of tumours can be studied
Tumours are biologically diverse
The mice can be used for therapy and chemoprophylaxis
Blastocysts can be frozen at any time, so that the line does not have to be maintained
These mice may be of use to test possible carcinogens
In an inbred model tumours can be transplanted between siblings or offspring for experimental therapeutics
Disadvantages
Wastage of mice
Locating the oncogene may be labour-intensive and time-consuming
Experimental therapeutics necessitates a macroscopically visible tumour, ideally with a long natural history
The time of development of tumours is important for experimental therapeutics
An inbred strain is needed for cytokine/immunotherapy experiments
The cytokine may induce the promoter, altering transgene expression artificially
Single 'hit' models, which may develop tumours as a predetermined event, and constitutive expression of the transgene, an artificial first step, must be overcome by any effective therapy
Integration site of the transgene has an uncertain effect on the genome of the animal

ADVANTAGES OF TRANSGENIC MICE AS A MODEL OF CANCER THERAPY

The major advantage of this model is a closer resemblance to the biological mechanisms of the human disease. Non- or weakly immunogenic tumours arise spontaneously and stochastically in immunocompetent mice. This unpredictability provides a better parallel for the development of human malignancy, and makes it more likely that metastases will develop through the same mechanisms as in humans. This in itself is of great potential value in preclinical studies of cancer therapy, adjuvant or maintenance therapy and chemoprophylaxis. In addition, the mice are predisposed to viral and chemical carcinogenesis, and should be of value in tests of putative carcinogens.

Tumours in oncogene transgenic mice have a closer histological resemblance to human tumours, and a model with a natural history closely resembling a particular human malignancy can be sought. A broad and increasing range of tumours can be studied, and these are biologically diverse, both between and within individual mice, like human tumours. An inbred model would also enable tumours to be transplanted between siblings or offspring, making it more valuable for experimental therapeutics. Blastocysts can be frozen at any time, so that the line does not have to be maintained.

DISADVANTAGES OF TRANSGENIC MICE AS A MODEL OF CANCER THERAPY

The main disadvantage of this model is the wastage of mice. This arises for a number of reasons. Firstly, many tumours

(particularly mammary) arise only in one sex. The necessity of heterozygous breeding means that only one quarter of the mice can be used. Attempts to breed homozygous mice have been largely unsuccessful. Even where the mutation is not embryolethal, the animals often develop tumours which are rapidly progressive at an early age. Homozygous mice may also be prone to development of other disorders which limit their usefulness in long-term therapeutic experiments.

Secondly, in most models only a proportion of transgene-positive mice are destined to develop tumours, necessitating larger groups than with other models. With most transgenes, some founder transgenic mice show no biological effect from the presence of the transgene, and up to a third may be mosaic for the transgene. Consequently, greater weight should be attached to effects consistently seen in a number of the progeny and primary animals. On entry into the experiment, animals from the same litter must be allocated randomly between groups to reduce bias. The incidence and natural history of tumour development must be carefully documented in each group. This entails greater space than many animal experiments require.

In many transgenic models, tumours or hyperplasias (especially mammary) may be pregnancy-dependent, regressing when the animal is no longer pregnant or lactating. In addition, some tumours are unlikely to develop in virgin females necessitating continual breeding of the mice. This either involves geometric expansion of the colony or further wastage of mice.

Screening for the transgene makes the model labour-intensive and time-consuming, even if polymerase chain reaction (PCR) is used. Therapeutic experiments are limited to superficial, slowly developing tumours. Many tumours, for example, solid gastrointestinal tract or haematological neoplasms, are not visible macroscopically, and their presence is only diagnosed from the general condition of the animal. By this point the animal is usually too unwell to be recruited into a therapeutic experiment. Such lines are most succesfully used for prophylaxis.

The time taken to develop a tumour is dependent on the promoter, transgene and strain, and needs to be considered when embarking on therapy experiments.

Where transgenic mice are to be used to test therapies which function by modulating the immune system, such as cytokines, the homogeneous genetic background of inbred strains is important. The contribution of different H3 haplotypes may influence results. A genetically inbred colony is necessary and this entails either microinjection of eggs from inbred animals or backcrossing offspring from an F1 hybrid founder for several generations. In practice, it is often difficult to generate isogenic transgenic mice. The eggs from inbred strains are technically more difficult to microinject than the hybrid strains. Inbred mice also tend to have smaller litters, making the establishment of such a colony more problematic. Backcrossing takes a number of generations (ideally 20) to obtain an inbred background, which is a very long-term commitment.

A further complication of the use of transgenic technology for research into cytokine therapies is the possibility that individual cytokines may influence the promoter. For example, tumour necrosis factor is known to induce the SV40 T antigen promoter and this may alter consequent transgene expression. The development of a tumour as a result of constitutive, tissue-specific expression of an oncogene is a 'driven', predetermined event whereas truly spontaneous tumours are not programmed in the same way. As a greater number of transgenic models are identified, this may be overcome with models where additional genetic events are required. The vast overexpression of the transgene which arises as a result of a heterologous promoter may not be relevant to the spontaneous tumour formation seen in humans.

The degree and distribution of transgene overexpression in transgenic animals may stack the odds against a therapeutic modality being effective, as constitutive expression of the transgene, which may be an important early event in the development of a tumour, must be overcome. Another potential problem is the integration site of the transgene and its uncertain effect on the genome of the animal. Different lines of the same transgenic animal can behave differently, not only in respect of tumour natural history, but also in terms of lifespan and viability.

Many of these difficulties have been encountered by companies developing transgenic mice for use in molecular biology and cancer research, and the market has proved disappointing. The Harvard 'oncomouse' was patented in 1988, and licensed exclusively to Du Pont for commercial development. It has now been conceded by the Massachusetts Institute of Technology that, if the primary use of the mouse is basic research, a patent application is not justified [29]. Currently, the most promising commercial use is by environmental protection agencies who are using these mice to assess possible hazards.

FEASIBILITY STUDY

We have established a colony of transgenic mice carrying the activated rat c-*neu* oncogene under the transcriptional control of the MMTV promoter. The founder mice were obtained from Prof. Paul Jolicouer and were reported to develop comedo-type metastatic breast tumours in a stochastic and asynchronous fashion at 7–14 months of age [9]. These mice have been bred on to a Balb/c background for seven successive generations.

Although initial studies reported a tumour incidence of over 40% at 7 months in our own hands the tumour incidence is only 20% in mated female mice at 16 months, and the phenotype appears to be changing with successive generations (Thomas *et al.*, manuscript in preparation). The tumours are morphologically and biologically diverse with a variety of metastatic patterns and histological features. This is influenced by the greater contribution of the Balb/c genotype, underlining the important contribution of genetic background. In later generations, a higher proportion of lymphoma-like tumours have arisen and the age of tumour development appears to be earlier (around 10–12 months, compared with 16–18 months in early generations). Lymphomas have previously been noted in other mice with the *neu* oncogene [30] but not in this founder colony.

Eight of the tumours have been transplanted and successfully passaged in nude mice, and have shown a spectrum of cytokine sensitivity in therapeutic experiments. Long-term experiments using cytokine prophylaxis are currently underway.

CONCLUSION

Our study mentioned above is an attempt to assess the feasibility of using this model for assessing cancer therapy, in particular cytokine therapy. Transgenic mice have the advantage of yielding additional information to existing models. A tumour in an inbred mouse, which is palpable and measurable, and has a natural history making it suitable for therapy, is likely to provide information which is more relevant to events in human cancer.

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