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Forty years of cancer modelling in the mouse

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Abstract

Mouse models of human cancer have played an important role in formulating modern concepts of multistage carcinogenesis, and are providing us with a new armoury of tools for the testing of novel therapeutic approaches to cancer treatment. The development of inducible and conditional technologies provide us with greater opportunity to generate mouse models which faithfully recapitulate human tumorigenesis, in terms of both the biology and the genetics of this disease. It is now feasible to control, in time and space, the development of tumours in almost any mouse tissue, such that we now have available mouse models of all major human cancers. Moreover, novel non-invasive approaches to tumour imaging will enable us to follow tumour development and metastasis *in vivo*, as well as the effects of candidate therapeutic drugs. Such new generation tumour models, which accurately emulate the disease state *in situ*, should provide a useful platform with which to experimentally test drugs targeted to specific gene products, or combinations of genes that control rate-limiting steps of tumour development.

Keywords: Cancer; Mouse models; Transgenic; Knockout; Carcinogenesis; Genetic susceptibility; Tumour promotion; Imaging; Preclinical

1. Introduction

The first sentence of an article on mouse cancer models published in 1966 in the *European Journal of Cancer* [1] states: ''The transplantable tumour has been the principal tool in the experimental evaluation of anticancer agents''. Sadly, in spite of the enormous advances made in understanding the mechanisms of tumour initiation and progression, and in modelling cancer development in the mouse, this statement is just as relevant today as it was 40 years ago. Even in these days of designer drugs targeted to specific gene products in important signalling pathways, the *in vivo* testing is still primitive, and does not in any way reflect the sophistication of the drug development process. The favoured preclinical model still involves transplantable human tumours growing in immunodeficient mice. The purpose of this article is to summarise briefly the advances made in the development of animal models of cancer over the past 40 years, and to illustrate the potential of such sophisticated models (as well as some simple ones) not only to foster a deeper understanding of human cancer, but to also provide realistic possibilities for the selection of drugs and drug combinations that have a higher success rate in the clinic.

2. Chemical carcinogen models

By the mid-1960s, many of the concepts of initiation, promotion and progression during multistage carcinogenesis had been established, largely due to the pioneering efforts of Berenblum, Shubik, Boutwell and others from 1940 onwards [2,3]. While a great deal of knowledge was accumulated about carcinogens and their metabolism, tumour biology, and the biochemistry of malignant cells, virtually nothing was known about cancer genetics until the advent of molecular biology and the era of gene cloning [4]. Many advances in our knowledge of genetic mechanisms of carcinogenesis followed the identification of cellular oncogenes by Stehelin and coworkers in the mid-1970s [5], and the first demonstrations that DNA isolated from tumour cells carrying mutations in cellular proto-oncogenes, or ret-

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roviruses could directly lead to cell transformation [6–8]. These studies led directly to the cloning of members of the *ras* family as transforming oncogenes that could be activated by single point mutations [9–11]. The identification of activated *H-ras* genes in chemical carcinogen-induced primary tumours established a solid link between carcinogen exposure and mutations in DNA that could contribute to multistage carcinogenesis [12–15]. Subsequent studies have demonstrated many parallels between tumour development in mouse and human systems (reviewed in [16]), confirming earlier suggestions that the mouse is in fact a very good model system for the study of human cancer.

3. Germline manipulation and mouse cancer models

The field underwent another major revolution when the capacity to manipulate the mouse germline [17] allowed the first oncogenes to be introduced into normal mice, leading to the development of tumours as a result of aberrant expression of single genes [18–20]. Parallel studies on gene knockouts [21,22] in turn were followed by seminal experiments on the inactivation of tumour suppressor genes by gene knockout technology [23,24]. The development of these wonderful new tools and reagents provided new avenues of research for chemical carcinogenesis studies. The effects of specific carcinogens could now be studied not just in normal mice, but in animals lacking specific genes involved in different stages of carcinogenesis [25,26]. The emphasis in mouse models of cancer gradually shifted away from classical chemical or radiation models to these more sophisticated approaches, with almost limitless power to manipulate the mouse germline.

A wide variety of transgenic and knockout models have been developed to study the effects of different oncogenes and tumour suppressors (reviewed in [27]), and these models have proved valuable in further understanding molecular mechanisms and pathogenesis. However, some limitations exist with these mouse models, in which the genetic defect is present in every cell in the animal from the start of development. Many of these models, therefore, do not recapitulate sporadic forms of human cancer, but have more similarities with familial cancers caused by "high penetrance" mutations in critical genes. Many tumour suppressor gene mutations cause embryonic lethality in the homozygous state, or a spectrum of tumours and other abnormalities and surprising phenotypes, which at first glance are different from their human counterparts. Simple heterozygous knockouts of *Brca1* or *Rb1*, do not give rise to malignant breast cancers or retinoblastomas, but further germline manipulations to introduce additional defects have led to more accurate mimics of the equivalent human conditions [28,29].

Many of the limitations of standard transgenic/knockout models are now being overcome by more regulatable and targeted systems (reviewed in [30]). Switch on/off systems to regulate gene expression include interferon, [31], tetracycline, which has been used widely to control numerous transgenes ([32], reviewed in [33]) and hormonally regulatable fusion constructs such as the myc-oestrogen receptor (myc-ER) system [34] which has been used to look at myc expression in the suprabasal layer of the skin [35]. Conditional gene expression systems include the Cre-Lox and Flp-FRT recombinase systems [36,37], which can allow the spatial and temporal control of somatic mutations, and be used to target gene disruption as well as activating gene expression, in specific tissues [38–41,54,56,57]. This has allowed study of the loss of genes such as *Brca1* and *Brca2* in mammary gland epithelium, which are normally lethal in the homozygous state. Conditional mutation of either of these genes in mammary tissue produces mammary tumorigenesis [42,55]. Controlled Cre-expression, achieved by adenoviral delivery of Cre recombinase, can more accurately model sporadic mutational events in a subset of cells, and has been used to generate lung or colon cancer in conditional *Kras2* or *Apc*-mutant mice [43–45]. The development of Cre-Lox technology has also allowed larger disruption to be made, such as chromosomal rearrangements, deletions and duplications [46,47,52], and progress is underway to model translocations found in human leukaemias [48].

An elegant approach to look at the spontaneous somatic expression of oncogenes was demonstrated recently by a model of *Kras* activation in which mice are engineered to express activated Kras2 at random in somatic cells with low frequency [49]. This strategy was based on a variation of the hit and run strategy, first developed by Bradley and co-workers [50], which usually consists of two distinct homologous recombination steps in embryonic stem (ES) cells. However, this new approach allows the second recombinational excision (run) step to occur *in vivo*, resulting in random, both spatial and temporal activation of the mutant Kras2 allele. As such, this model represents a major advance in that it mimics the sporadic nature of human cancers, which in most cases develop in clonal fashion from single cells.

Retroviral gene delivery systems based on the avian leucosis virus had made use of the fact that mice do not express. TVA, the receptor which is required for delivery of the virus. A model of gliomablastoma has been developed in mice engineered to express the receptor in a tissue-specific fashion, either under the nestin promoter, which is active in neural and glial progenitors, or in astrocytes with a glial fabrillary acidic protein (GFAP) promoter. Retroviral delivery (RCAS vector delivery) of combinations of specific oncogenes and mutated growth

factors in these cells produces either glioblastoma or gliomageneis [51,53].

4. Molecular imaging of cancer in the mouse

The usefulness of mouse models for pre-clinical testing has been augmented by the ability to visualise tumour development and progression in small animals [58]. This has been achieved with the development of smaller versions of imaging equipment commonly used in humans such as magnetic resonance imaging (MRI), and an increasing number of reporter systems. Recently, MRI was successfully used to monitor tumour formation and development in a tet-inducible Kras2 model of lung cancer [59]. A higher resolution form of position emission tomographic (PET) imaging, which measures pre-administered decaying nuclides emitted from the subject, has been developed for small animal use [60]. 18F-labelled probes, such as glycopeptides, have been utilised to study proliferation, metabolism and cell surface expression in xenografts in mice [61]. A number of PET reporter probe (PRG) systems are being gradually characterised (reviewed in [62]), which are commonly enzyme- or receptor-based, such as the mutant herpes simplex virus thymidine kinase reporter system, or dopamine type 2 receptor [63]. However, PET imaging is a costly procedure, and more inexpensive and rapid methodologies, such as fluorescence imaging using luciferase and green fluorescence protein (GFP) are becoming prevalent, using relatively cheap charged-coupled device (CCD) capture technology. Bioluminescence imaging based on *in vivo* expression of luciferase has the advantages of being rapid and exhibiting low backgrounds. It has been used in a number of studies to visualise tumours, initially in transplanted tumours [64], and more recently, pituitary tumours in a conditional *RB1* mouse model engineered to express Cre and luciferase under the control of the intermediate lobe-specific pro-opiomelanocortin (POMC) promoter [38].

5. Tumour promotion and environmental stress

However, in spite of the power and elegance of these models, it is critical not to lose sight of the fact that environmental agents play a major role in the development of human cancers. Rodents in which whole signalling pathways have been knocked out have given us invaluable information on the wiring diagrams of the cell, but, with few exceptions, do not report on the mechanisms by which the major environmental agents influence human cancer. Chemical and physical carcinogens influence tumour development in many ways, acting as initiators (normally mutagens), promoters, or as agents that induce oxidative damage, leading to genomic stress and genetic instability. In animal models, tumour promoters play a major role in the selection of the initiated cell, and promoting its outgrowth to first benign and then malignant lesions [65,66]. Although the study of tumour promotion and chemical carcinogenesis, in general, became unfashionable with the development of genetically determined models, which offered much greater control, many important questions remain to be answered that are most appropriately studied using models involving exogenous stress. Recent data indicating that the nature of the promoting agent has a major effect on the selection of particular initiated cells should help to re-awaken interest in this important field. For example, the tumour promoter 12-*o*-tetradecanoyl-13-phorbol acetate (TPA) selects dimethylbenz[a]anthracene (DMBA) treated skin cells carrying mutant *Hras* genes, and promotes their expansion to papillomas. Promotion using a different agent, for example mezerein [67], or transgenic overexpression of ornithine decarboxylase [68] leads to the formation of papillomas with *Kras* mutations. In mouse liver, exposure to a carcinogen followed by phenobarbitol promotion gives tumours with β-*catenin* mutations, whereas promotion by partial hepatectomy selects initiated cells carrying *ras* mutations [69]. It is possible that the selectivity of specific *ras* mutations in human tumours, for example Ki-*ras* in colon and pancreas, *Hras* in tumours of stratified squamous epithelia, *Nras* in melanoma or leukaemia, may be a reflection not of the frequency of tissue-specific mutations in these genes, but of the local environmental conditions that promote the outgrowth of the initiated cells.

Early studies on the inhibition of the effects of tumour promoters, and the consequences for tumour development, have presaged modern chemoprevention in humans. It has been known for many years that inflammation of the skin is an important determinant of the tumour promotion process, and that inhibition of this inflammation using steroidal or non-steroidal drugs could prevent the development of papillomas [70]. More recently, not only has it been shown that tumour promoters induce expression of members of the cyclo-oxygenase family, such as Cox-2, but that loss of this enzyme prevents tumour progression *in vivo* [71], and small molecule inhibitors are rapidly becoming important chemopreventive agents in humans [72].

6. Mouse models of genetic susceptibility to cancer

Rare mutations or polymorphisms, which have major effects on tumour growth or survival, contribute to only a small fraction of tumours in the human population. Highly penetrant mutations in genes such as *BRCA1/2* are responsible for a proportion of cancers that show

familial aggregation, and mouse models of such genes have provided many fundamental and unexpected insights into the tumorigenic process. However, the genetic basis of susceptibility to the majority of cancers, which have no obvious familial segregation is almost completely unknown [73,74]. Current methodologies for identifying low penetrant cancer susceptibility genes in humans are less than satisfactory due to the complexity of human populations and current methodologies. Mouse models offer one important approach to the study of these alternative models of susceptibility [75], and provide an opportunity to study the effect of gene-environmental effects. Studies on mice have revealed that tumour predisposition in different strains is controlled by multiple loci which control fundamental processes such as the tumour growth rate, ability to stimulate angiogenesis, or the risk of malignant progression. This has led the way to the development of a number of very sophisticated genetic tools for the analysis of complex genetic traits, including recombinant inbred strains, recombinant congenic strains, and intra- or inter-specific backcrosses (expertly reviewed by Demant [76]). Using such approaches, a large number of resistance/susceptibility genes for cancers of all of the major tissue types including the lung, colon, skin and haematopoietic system have already been mapped in the mouse genome [77]. An important feature of mouse genetics is the ability to detect interactions between genetic variants, which may lead to the identification of a number of genes, which act synergistically or in combination in cancer resistance. It has been shown that specific alleles at different loci, when inherited in combination in mice, exert much greater effects on tumour development than would be expected from their individual "strength" as tumour predisposition loci. Such genetic interactions were originally demonstrated in plants, and, more recently, in recombinant congenic strains [78] or in interspecific backcross mice [79]. Until recently, only a few susceptibility genes had been identified, mostly because standard linkage analysis generally allows mapping to a 10–30 cM region. Generation of congenic mice, containing fragments of the region, or fine mapping can refine these regions to 1–2 cM, but is a laborious and expensive process. Multistep approaches have been proposed which include both linkage analysis and linkage disequilibrium in heterogeneous mouse crosses [80], and recently led to the identification of *Stk6*/*STK15* as a candidate low-penetrance tumour-susceptibility gene in mice and humans [81]. The use of recombinant congenic strains also identified *Ptprj* as a candidate for the mouse colon cancer susceptibility locus, *Scc1* [82]. Increased knowledge of the mouse genome sequence, together with new genomic tools, such as expression arrays, will greatly aid in the identification of potential candidate susceptibility genes.

7. New mouse models for pre-clinical drug evaluation

It might be expected, in view of the explosion of new and ever more accurate mouse models of cancer, that the pharmaceutical industry would have taken advantage of these possibilities for testing of novel candidate therapeutic drugs. This is, however, with a few exceptions, and not the case. It seems counter-intuitive to invest vast sums in drug discovery, only to go into clinical trials and spend even larger sums on drugs that are doomed to fail because they have not been appropriately tested. On the other hand, the use of poor models may have led to the demise of many potentially very important drugs because they are negative in poor pre-clinical animal model tests [83].

What are the reasons for this reluctance to adopt a more rigorous approach to the testing of new drugs for cancer? Apart from inertia, one oft-quoted reason is the expense involved in generating primary tumours in mice. There is no doubt that such models may be more expensive, but it could be argued that if just one major drug failure is prevented by the foresight provided through informed animal studies, the tens or even hundreds of millions of dollars saved would more than compensate for the added costs of primary tumour models. We now know much more about the tumour microenvironment and its importance as a determinant of tumour growth [84]. Human tumour xenografts growing subcutaneously in mice that are devoid of T cells (which are known to exert both positive and negative effects on tumour growth; [85–87]) are clearly woefully inadequate models of the real disease. Individual responses to drugs vary enormously in human cancer patients, and it is extremely naive to expect that responses of a few xenografts growing under these highly artificial conditions will predict the human situation. However, even primary tumour models in inbred strains are still not ideal, as they do not reflect the genetic heterogeneity of the human population. Models that can in addition take account of genetic heterogeneity in drug responses, for example, using advanced intercross lines or outbred, wild-derived mouse populations [88], would be a better approach, but this may be asking too much at this stage. Finally, a major impediment to the use of modern animal models for the preclinical testing of chemotherapeutic drugs is the restrictive application of patents obtained years ago on the use of genetically manipulated mice for drug testing [89]. These restrictive practices have prevented even those companies with a desire to move in this direction, from taking advantages of the sophisticated technologies now available. It is to be hoped that discussions of these issues [90,91] will lead to a resolution that enables the field to move out of a stagnant period.

Finally, the authors of the 1966 paper cited at the beginning of this article [1] were obviously aware, even

at that early stage, of the limitations of xenograft models for chemotherapy testing, stating that, ''questions may nevertheless be raised as to the appropriateness of the use of transplantable tumours''. They further suggested that primary tumour models would provide a more realistic test of potential therapies, in agreement with studies carried out 10 years earlier by Scholler and coworkers [92], who showed that spontaneous primary tumours are more resistant to chemotherapy than are similar transplanted tumours. Obviously, these cries have for decades fallen on deaf ears. Primary mouse tumour models, both new and old, offer the possibility of testing drugs either individually or in combination to target rate-limiting steps of tumour development in ways that have not hitherto been possible. The requirement for development of these pre-clinical approaches is clear from the numbers of cancer drugs in clinical trials. Hundreds of experimental drugs are now in the clinic, but it is likely that even those targeted at specific gene products involved in cancer, such as imatinib mesylate (Glivec; Gleevec) which inhibits the kinase activity of BCR-ABL, will ultimately fail due to the development of drug resistance [93,94]. To overcome these problems, combinations of drugs that simultaneously or sequentially block interacting pathways may be required. Such studies will be impossible to carry out in humans because of the large number of patients that would be essential to test even a small proportion of the possible combinations. Possibly in the next 40 years, we will begin to see some of these exciting mouse models of cancer being used constructively for the benefit of human cancer patients.

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