Chapter 5
Experimental Tumour Models in Mice

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Abstract The complex multistage processes of tumour initiation, progression and metastasis challenge the methods that are used in basic cancer biology research and anticancer drug development. Experimental tumour modelling in mice provides means for observing tumour development, identifying target molecules and pathways and designing and testing novel strategies for diagnosing and treating cancer in a manner that is not possible in in vitro systems or in human patients. To gain maximal benefit from the use of mouse tumour models one should be aware of the possibilities and limitations of each approach, and pay careful attention to selection of the model and planning of the experiments. We aim in this review to give the reader some basic information on experimental mouse tumour models that have evolved from simple chemical treatments to extremely complex genetic models, and to discuss their advantages and disadvantages. We discuss some species-specific differences between mice and humans, and also between the inbred mouse strains, that can affect the various processes of tumorigenesis. Finally, we try with a few examples of cancer studies involving the p53 and retinoblastoma tumour suppressors, and the extracellular matrix protein collagen XVIII and its antiangiogenic endostatin fragment to illustrate the importance of evaluating data from various tumour models in order to achieve a proper understanding of the function of a given molecule or pathway in tumour development.

5.1 Introduction

Tumour initiation, progression and metastatic dissemination to distant sites are complex multifactorial processes. Besides the multiple genetic alterations that occur in a stepwise manner in cancer genes and their modifier genes during malignant transformation, a number of other factors affect primary tumour growth and metastasis (Hanahan & Weinberg, 2000; Hahn & Weinberg, 2002). These include several

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epigenetic regulatory mechanisms such as DNA methylation, microRNA regulation and histone modifications (Sharma, Kelly, & Jones, 2010). Cancer progression also involves numerous interactions between transformed cells and their constantly evolving microenvironment, consisting of the extracellular matrix (ECM), vasculature and stromal cells (inflammatory cells and cancer-associated fibroblasts) which produce growth factors, cytokines, inhibitors, proteases and ECM components to affect the ultimate process of tumour growth (Hanahan & Weinberg, 2000; Hahn & Weinberg, 2002). This complexity in tumour development places high requirements on the methods that are used for cancer research.

Tumour modelling in mice has significantly contributed to our current understanding of cancer and its treatment. Existing and novel hypotheses on carcinogenesis are currently being tested in novel in vivo model systems, particularly using genetically modified mice (Frese & Tuveson, 2007). Experimental cancer research can roughly be divided into two categories: basic cancer biology studies which focus on the molecules and mechanisms underlying tumour growth and metastasis, and the development of cancer therapies and their preclinical testing in animals. The latter also involves important pharmacological and toxicological issues and specific practices and criteria for evaluating tumour response, as discussed in detail elsewhere (Bibby, 2004; Schuh, 2004; Suggitt & Bibby, 2005; Talmadge, Singh, Fidler, & Raz, 2007). Nevertheless, for both purposes the choice of relevant tumour models and appropriate experimental conditions is absolutely critical.

5.2 The Mouse as a Model Organism in Cancer Research

In vitro cell and organotypic culture systems are powerful and constantly improving methods for studying many aspects of tumour growth, but they do not allow us to investigate entire physiological systems and their interactions, nor processes such as angiogenesis and metastasis. Therefore in vivo animal models are indispensable for cancer research. The laboratory mouse (*Mus musculus*) is considered the most accurate and accessible model organism for cancer studies for several reasons. First, it has a similar organ system and systemic physiology to humans and shares genetic features with humans. The majority of the human genes have murine counterparts, and the functions of these genes are in most cases closely related. Gene mutations that cause certain diseases in humans often, but not always, cause similar diseases in mice (Paigen, 2003; Peters et al., 2007). Furthermore, mice are small in size and easy to house in the laboratory. They have a relatively short breeding cycle and can produce a large number of offspring. An additional, one unique advantage over other higher laboratory animals is associated with the mouse, namely our ability to manipulate the mouse genome. Thus, transgenic mice can be created to overexpress activated oncogenes, tumour suppressor genes can be inactivated (knock-out models) or selected cancer genes can be mutated in a precise manner to create replicas of the genetic defects that cause cancer in humans (knock-in models). These methodologies have led to a number of key discoveries in cancer pathogenesis and are continuously producing increasingly sophisticated in vivo models for cancer studies (Rangarajan & Weinberg, 2003; Frese & Tuveson, 2007).

There are several important species-specific differences (genetic, molecular, cellular, developmental and metabolic) between humans and mice, however, which can affect tumour development and which it is thus critical to understand in order to design experiments and interpret their results. These differences include the species' susceptibility for developing cancer over their lifetime. Humans have a significantly longer lifespan, are larger in size and have larger numbers of cells undergoing division, so that in theory they have an increased risk of DNA damage which may ultimately lead to cancer. Yet they show markedly lower cancer rates upon ageing than mice. It has been estimated that an accumulation of 4–7 mutations is needed to transform a human epithelial cell, whereas fewer changes are required for murine cells. This can at least partly be explained by differences between the mouse and man in the retinoblastoma (Rb) and p53 pathways and telomere function that control the division and lifespan of cells, as discussed in detail elsewhere (Hanahan & Weinberg, 2000; Hahn & Weinberg, 2002; Rangarajan & Weinberg, 2003).

The tumour spectrum and karyotype also vary between the two species. Laboratory mice tend to develop mesenchymal tumours such as sarcomas and lymphomas, whereas humans are more prone to epithelial carcinomas (Rangarajan & Weinberg, 2003; Anisimov, Ukraintseva, & Yashin, 2005; Maddison & Clarke, 2005) These human epithelial tumours show highly abnormal karyotypic profiles characterized by changes in chromosome number and multiple, non-reciprocal translocations, features that are uncommon in murine tumours (Rangarajan & Weinberg, 2003). The tumours that develop in telomerase-deficient mice in old age show similar translocations, however, and it has been suggested that telomere dysfunction in human cancer cells might also be linked to abnormal cytogenetic profiles (Artandi et al., 2000).

5.3 What Constitutes an Optimal Mouse Model for Cancer Research?

Mouse tumour models have progressed from chemical carcinogen models and transplantable murine tumours to advanced genetic models with increased susceptibility to tumour development. Each of these approaches has its own advantages and limitations, which should be considered when choosing the most appropriate model for a particular question. By paying careful attention to the possibilities of a given model and experimental design (e.g. mouse strain and sex, immunological status of the host, method and site of tumour implantation, selection of endpoints), one can extract the most reliable information from the experiment. As a general rule, an experimental model in basic cancer research should mimic the counterpart human disease in clinical, genetic and histopathological terms, whereas in drug testing it is faithful recapitulation of the targeted neoplastic event that is crucial. Nevertheless, one should always bear in mind that all mouse models are more or less imperfect when it comes to recapitulating human malignancies (Schuh, 2004; Frese & Tuveson, 2007).

One should also consider several practical issues such as convenient monitoring methods, penetration rates and tumour latency periods which all contribute to a manageable mouse cohort and the cost-effectiveness of the model. It should be noted that in rapidly growing tumours with high penetrance, such as genetic tumour models with strong oncogene expression and concomitant loss of a tumour suppressor, the evolution of the microenvironment and acquisition of secondary oncogenic mutations (imperative for neoplastic transformation in human cells) are not necessarily simulated accurately (Frese & Tuveson, 2007).

One extremely important issue is the ethics of the tumour model. According the principle of the 3 R's (replacement, reduction and refinement) (Russel & Burch, 1992), when replacement of a murine model with an alternative in vitro method or lower animal species is not possible, the number of mice can be potentially reduced (without losing important information) by using genetically homogenous inbred mouse strains instead of hybrids (still recognising that humans are not genetically homogenous), and by careful experimental design and controlled conditions. The experiment should also be refined, which ensures that the mice are treated with care, and that the pain, discomfort and stress that the animals experience are minimized. In tumour experimentation this includes limitation of the tumour burden, determination and respect of humane end points, the use of non-invasive in vivo monitoring methods, and the technical competence of the laboratory personnel.

5.4 Transplantable Tumour Models

A major breakthrough in experimental cancer modelling was the development of transplantable rodent tumours (Corbett, 2002). Numerous immortalized cell lines of human and mouse origin are tumorigenic in mice and routinely used in cancer research (see www.sanger.ac.uk/perl/genetics/CGP). These cells are maintained and amplified in culture, and usually implanted by simple subcutaneous (s.c.) injection into the flank of the mouse. Transplantable tumour models have a number of advantages over other experimental mouse models. The immortal cell lines are readily available, easy to maintain and in many cases the specific mutations in cancer genes are known (www.sanger.ac.uk/perl/genetics/CGP). Tumour implantation is fast and simple and does not require any specific expertise. Tumour growth is synchronized and generally rapid, and tumour monitoring by palpation and caliper measurements in the s.c. location is easy (Schuh, 2004; Becher & Holland, 2006). The emerging non-invasive in vivo imaging systems for fluorescent protein or luciferase-tagged tumour cells (also readily available, see www.metamouse.com) represent a major methodological and ethical improvement in transplantation models (Hoffman, 2005). Moreover, transplantation is highly reproducible and a relatively small number of mice is needed which contributes to the cost-effectiveness of this tumour model.

One of the main drawbacks in the use of transplantable tumorigenic cell lines is that they have been passaged for many generations in culture and, due to selection pressure under in vitro conditions, accumulate additional mutations and do

not represent the original tumour in its native state. This means that they may display altered growth characterisitics, protein expression profiles and drug sensitivity, and also altered histomorphology when inoculated into mice. Moreover, implanted tumours model cancer as if it was a disease of a homogenous pool of cells, and lack the morphological and cellular complexity of in vivo tumours.

Quality control is also an important issue in the context of transplanted tumour cells. Many cell lines have undocumented source and passage histories, and subclones in different laboratories may diverge from the parental cell line, causing altered responses when inoculated into mice (Schuh, 2004; Becher & Holland, 2006). This is exemplified by a study of collagen XVIII/endostatin, related to our own work, where implanted murine Lewis lung carcinoma and B16F10 melanoma cells producing antiangiogenic endostatin showed equivalent tumour growth rates and microvessel densities in collagen XVIII/endostatin knock-out and control mice, whereas a variant B16F10 subclone lacking expression of this collagen displayed increased angiogenesis and grew faster in the knock-out mice (Sund et al., 2005). This clearly indicates that tumour growth is determined not just by the genetic defects accumulating in the cancer cells but also by host-derived factors, and that these host-derived effects can sometimes be masked by the proteins produced by the implanted tumour cell. This example also highlights the importance of testing various tumour cell lines, and preferably also other experimental cancer models.

5.4.1 Xenograft Models

The term xenograft refers to the transplantation of cultured human tumour cells or human tumour explants into immunodeficient mice which do not reject the graft. The most common immunodeficient mouse strains used in xenograft models are hairless nude mice (nu/nu), which completely lack the thymus, and SCID (severe combined immunodeficiency) mice, in which the differentiation of haematopoietic stem cells into mature T and B lymphocytes is hindered. These mice have normal innate immune cells such as macrophages and natural killer cells, however, which can affect tumorigenesis and metastatic spread in xenograft systems (Jacob, 2004; Schuh, 2004).

The first successful xenotransplant of cultured human melanoma cells in nude mice was achieved almost 40 years ago (Giovanella, Yim, Stehlin, & Williams, 1972). Today a wide variety malignant human tumour-derived or genetically engineered cell lines are readily available, some backed by an extensive literature and others with reporter gene expression. Xenograft models are generally thought to be of only limited value in basic cancer research, but since human tumour cells with human cellular processes are used in this system, they are particularly suitable for preclinical anticancer drug validation after an in vitro pre-screen with highly sensitive human tumour cell lines such as MCF-7 breast carcinoma, NCI-H460 lung carcinoma and SF-268 glioma (Suggitt & Bibby, 2005).

Unfortunately, there are some gross disadvantages associated with xenograft models. Nude and SCID mice have severe defects in their immune systems which

can either promote or restrict tumour growth or limit their capacity to tolerate various treatments (Schuh, 2004). Xenograft tumours may also show increased response to anticancer agents. In addition, even though human tumour cells can be implanted into immunodeficient mice, the system is not ideal because the tumour microenvironment is still of murine origin and does not allow perfect interactions with neoplastic human cells. This limitation can be partially overcome by using humanized mice, such as NOD/SCID (non-obese diabetic/SCID) or Rag (recombination-activating gene) null mice engrafted with human haematopoietic cells or tissues (Schuh, 2004; Shultz, Ishikawa, & Greiner, 2007).

5.4.2 Syngeneic Models

In a syngeneic model cultured murine tumour cells are implanted into the mouse strain from which the tumour cell originates. This system overcomes the problems of incompatibility between the tumour cell and the murine stroma of the host and the immunodeficiency of the host, but still possesses most of the other limitations, and obviously also the advantages, associated with transplantable tumour models. Thus, a syngeneic microenvironment in the host allows the essential interactions between tumour cell receptors and stromal ligands and the functioning immune system of the host creates a more realistic milieu for tumour development and also facilitates drug testing (Corbett, 2002). On the other hand, murine tumour cells may show profound differences in cellular processes compared with the corresponding human tumour cells, as discussed above, such as the differences in telomere function.

There are numerous C57BL/6-derived tumour cells available from different depositors, but FVB/N-derived tumour cells, for example, are rather unusual. While many of the early-established murine tumour cell lines were carcinogen-induced and others developed naturally in old inbred mice, the later tumorigenic cell lines are often genetically engineered or derived from tumours of genetically modified mice (Corbett, 2002). One example is the widely used C57BL/6-derived B16 melanoma, which can be employed to follow the processes of solid tumour formation and metastatic spread. The B16 melanoma cell line originates from a spontaneous mouse skin melanoma, and after s.c. implantation colonizes the lung, liver and spleen. Intravenous injections of parental B16 cells and subsequent serial in vivo−in vitro selection processes have resulted in different subclones with specific metastatic potentials in the lungs (B16F10), brain (B16B19b, B16B10n) and ovaries (B17-010) (Alvarez, 2002), for example.

5.4.3 Orthotopic Models

Anticancer therapies showing promising responses in preclinical testing with mice often fail in clinical use. This has been the case with collagen XVIII-derived endostatin, for example, which proved extremely efficient in reducing angiogenesis and primary tumour growth in experimental mouse models (Folkman, 2006) but showed

no antitumour activity in human clinical trials using standard response criteria (Kulke et al., 2006; Moschos et al., 2007). One reason behind this disparity is that the conventional s.c. xenograft and syngeneic systems used in preclinical testing do not represent advanced disease. Indeed, the lack of metastasis from the subcutaneous growth site often limits the use of these approaches. This has led to the development of clinically more relevant and accurate cellular and surgical orthotopic models in which human or mouse-derived neoplastic cells or intact small tumour explants are injected or surgically implanted (surgical orthotopic implantation, SOI) into the anatomical site of the primary tumour (Manzotti, Audisio and Pratesi, 1993; Hoffman, 1999; Bibby, 2004; Schuh, 2004). The realistic microenvironment provided in orthotopic models enhances primary tumour growth and enables local invasion and angiogenesis studies to be carried out in a clinically relevant location. Orthotopic tumours also show increased metastatic potential and they often disseminate in a similar manner and to the same distant organs as the primary human tumours. Recent observations indicate that the tumour site used in an experimental model also affects the response to therapeutic agents (Bibby, 2004). Due to the complex surgical techniques, however, which require special technical expertise and enable only a small number of mice to be used per experiment, orthotopic models are employed mainly in basic cancer biology research and are not widely used in anticancer drug development or preclinical testing (Hoffman, 1999).

5.5 Autochthonous Models

An autochthonous tumour is described as an endogenous or *in situ* tumour that develops from the animal's own normal cells. Autochthonous models include spontaneously occurring tumours in mice and cancers induced by chemicals, which will be discussed here in more detail. Physical treatments, e.g. with UV light, asbestos fibres, viruses or their genes, including murine polyoma and simian virus 40 T antigen or human papillomavirus E6/E7 antigen, or with microbes such as *Helicobacter*, can also be used to evoke tumour formation in mice (Suggitt and Bibby, 2005; Frese & Tuveson, 2007; Talmadge, Singh, Fidler, & Raz, 2007). It is generally thought that autochthonous models mimic tumour development more precisely than transplanted tumours. The advantageous properties of these models include an orthotopic growth site, an opportunity to monitor different stages in tumour development from hyperplasia to advanced malignant cancers, and authentic metastasis routes through the blood and lymphatic vessels (Berger, 1999). Autochthonous models have contributed to many basic discoveries in cancer biology and are also of importance as a source of many tumour cell lines used in in vivo transplantation models and in vitro studies (Schuh, 2004), but for several reasons their use in drug development is limited and they are only occasionally used for advanced or phase II drug screening (Berger, 1999). The significant limitations associated with autochthonous models include long tumour latency periods in many models and substantial variability in

tumour incidence and multiplicity, resulting in the need to use a large number of animals (Schuh, 2004; Frese & Tuveson, 2007; Talmadge, Singh, Fidler, & Raz, 2007).

5.5.1 Spontaneous Tumour Models in Mice

As discussed earlier, the spectrum of age-related spontaneous tumours differs considerably between humans and mice, and the genetic background also plays a significant role in dictating the exact tumour spectrum in different mouse strains. The most frequent tumour type in the widely used C57BL/6 strain, for example, is malignant lymphoma/leukaemia, whereas old FVB/N mice often develop lung adenocarcinomas but seldom lymphomas (Mahler, Stokes, Mann, Takaoka & Maronpot, 1996; Paigen, 2003; Anisimov, Ukraintseva, & Yashin, 2005; http://www.informatics.jax.org/external/festing). Additional spontaneous, induced (often with the mutagen ethylnitrosourea, ENU) or genetically engineered mutations in inbred mouse strains can predispose them to new spontaneous tumours in selected organs or tissues, or change the existing strain-specific tumour pattern (DePinho, 2000; Attardi & Donehower, 2005). Such mouse strains can be used as models for studying particular human cancers. A classical example of a spontaneous tumour model is the $Apc^{Min/+}$ mouse, in which an ENU-induced mutation in the adenoma polyposis coli (*Apc*) gene leads to the formation of multiple intestinal adenomas and provides a mouse model for human familiar colon cancer syndromes caused by mutations in the human *APC* gene (Moser, Pitot, & Dove, 1990). In another example, old telomerase-deficient mice that were heterozygous for the p53 tumour suppressor showed a shift in the tumour spectrum from lymphomas and sarcomas to carcinomas of the breast, colon and skin with cytogenetic profiles typical of human carcinomas (Artandi et al., 2000).

5.5.2 Chemical Models

Certain environmental factors such as coal tar, metals and dyes increase tumour incidence in exposed humans. The pioneer study of experimental chemical carcinogenesis was conducted in 1915 by painting rabbits' ears with coal tar, which caused the formation of epithelial skin tumours. It was only much later, however, that the DNA-targeting carcinogenic molecules in the coal tar and other environmental chemicals and their mechanisms of action were identified. Coal tar was shown to contain several polycyclic aromatic hydrocarbons (PAHs), such as dibenzanthracene and benzopyrene, that form covalent adducts with DNA and induce cancer in mouse skin. A wide variety of chemicals can be used to induce cancers in laboratory animals, affecting different organs depending on the administration routes and protocol. Diethylnitrosamine (DEN), for example, can be used to produce tumours in the liver, lung and oesophagus, and 7,12-dimethylbenz[α]anthracene (DMBA) in the skin, mammary gland and colon (Luch, 2005; Loeb & Harris, 2008).

The two-stage model of mouse skin carcinogenesis on exposure to a specific initiation-promotion regimen is a widely used chemical cancer model. In

this approach tumour initiation is achieved with a single dose of the carcinogen DMBA, which leads to somatic *H-Ras* mutation and oncogene activation in a few selected keratinocytes. These cells undergo clonal expansion upon repeated applications of the tumour promoter 12-O-tetradecanoylphorbol-13-acetate (TPA), which stimulates the production of growth factors and chronic inflammation, leading to proliferation of the mutant cell population, and precipitating spontaneous secondary mutations in other cancer genes such as *p53*. As the DMBA-initiated keratinocytes can persist in the skin for a long time without forming tumours unless TPA is given, they are thought to represent the epidermal stem cell population, or 'transit amplifying cells' that can divide a small number of times before undergoing terminal differentiation (Owens, Wei, & Smart, 1999; Owens & Watt, 2003; Perez-Losada, & Balmain, 2003) The chemical treatments lead to epidermal hyperplasia and the outgrowth of highly differentiated benign papillomas. Approximately 10% of these papillomas progress to malignant squamous cell carcinomas (SCC), which can disseminate into the sentinel lymph nodes via the lymphatic vessels and further to distant organs via the lymphatic and blood vessels. A small portion of the SCCs can go through an epithelial to mesenchymal transition to the most aggressive and invasive phenotype, spindle cell carcinoma (Yuspa, 1991).

There is significant variability in papilloma formation between mouse strains in the DMBA-TPA model. The outbred SENCAR strain, for example, is highly sensitive to the initiation-promotion regimen, whereas the inbred FVB/N strain shows intermediate sensitivity and C57BL/6 mice are completely resistant to this protocol. On the other hand, both FVB/N and C57BL/6 mice are susceptible to a complete carcinogenesis protocol with repeated DMBA applications (Hennings et al., 1993). The gender disparity often seen in human cancers, and also in experimental cancer models, applies to the DMBA-TPA model as well, as male mice show a higher tumour incidence than females (Balbin et al., 2003; personal observation).

The DMBA-TPA protocol is one of the oldest experimental cancer models. It has provided the data from which many basic concepts of carcinogenesis have been formulated, and despite the long duration and labour-extensiveness of the experiments, still continues to play an important role in understanding the multistage nature of cancer and the mechanisms of inflammation-associated tumour growth (Loeb & Harris, 2008). By subjecting mice modified genetically for collagen XVIII and endostatin to a DMBA-TPA regimen we have found that, besides inhibiting angiogenesis and primary tumour growth, collagen XVIII/endostatin can regulate tumour initiation, tumour-associated inflammation, lymphangiogenesis and lymph node metastasis (Brideau et al., 2007 and unpublished data).

5.6 Genetic Models

The development of advanced molecular technologies that allow manipulation of the mouse genome has provided enormous opportunities for investigating the functions of specific genes in cancer and to create mouse models that mimic human cancers. Genetic tumour models are now available for many common human cancers,

some of which faithfully simulate the human disease (see http://emice.nci.nih.gov or http://tumor.informatics.jax.org/mtbwi/index.do). These can be classified simply as transgenic models in which oncogene or dominant-negative tumour suppressor gene expression is driven by ectopic promoter and enhancer elements and models with targeted gene alterations generated by homologous recombination in which tumour suppressor genes are inactivated or selected mutations are introduced in cancer genes or their modifier genes (reviewed by Hahn & Weinberg, 2002; Herzig & Christofori, 2002; Rangarajan & Weinberg, 2003; Maddison & Clarke, 2005; Frese & Tuveson, 2007).

5.6.1 Transgenic Models

The first transgenic tumour models were established in the mid-1980's by overexpression of single viral or cellular oncogenes under tissue-specific promoters (Macleod & Jacks, 1999; Becher & Holland, 2006). Classical examples of these are the models for mammary tumours and B-cell lymphomas in which c-Myc was expressed under the control of the murine mammary tumour virus (MMTV) promoter and immunoglobulin enhancers, respectively (Stewart, Pattengale, & Leder, 1984; Adams et al., 1985). The most relevant of these early transgenic models, such as the RIP-Tag mouse pancreatic tumour model in which the SV40 large T antigen is expressed under the rat insulin promoter (Hanahan, 1985) are still widely used and have also been appropriately revised to allow more precise evaluation of the stepwise progression of tumour development (Du, Lewis, Hanahan, & Varmus, 2007). These rather simple transgenic models have given valuable information on many principles of tumorigenesis, and, importantly, prepared the ground for more sophisticated genetic models.

Universal problems associated with all traditional transgenic mouse models are the random nature of transgene integration and the use of minimal regulatory elements to drive transgene expression, both of which may result in inaccurate regulation of the target gene. This may cause incomplete penetrance of the desired trait, for example, and/or variability in transgene expression levels. A problem often confronted in cancer research is constitutive expression of the transgene with a potential for affecting other cellular processes and mouse development. This disadvantage can be overcome by means of inducible promoters that allow cancer gene expression to be controlled spatially and temporally, and currently also reversibly. The most widely used system for generating mutant mice with inducible transgene expression employs the tetracycline and doxycycline-controlled transcriptional regulatory elements of *E. coli* (Zhu, Zheng, Lee, Homer & Elias, 2002), while another way is to fuse the oncogene with the oestrogen receptor (ER), making its expression regulatable via oestrogen or tamoxifen ligands (Frese & Tuveson, 2007). These models have been used to demonstrate the necessity for oncogene expression in tumour maintenance, for example (Maddison & Clarke, 2005; Frese & Tuveson, 2007). Suitable transgenic models for our topic of interest, SCC of the skin, include the K14-HPV16 model, in which the early region of human papillomavirus type

16 is targeted to the basal cells of the squamous epithelium (Arbeit, Munger, Howley, & Hanahan, 1994), and the inducible Involucrin-c-myc^{ER} model, targeting the suprabasal epidermal keratinocytes (Pelengaris, Littlewood, Khan, Elia, & Evan, 1999).

5.6.2 Models with Targeted Gene Alterations

Targeted inactivation of tumour suppressor genes, including *p53* and *Rb*, by homologous recombination in embryonic stem cells was achieved in the early 1990's (Donehower et al., 1992; Jacks et al., 1992). Loss of these tumour suppressors in the mouse often resulted in a rather different tumour spectrum from that observed in humans, however. For example, mutations in the human *RB1* gene cause retinoblastoma in childhood followed by osteosarcomas and small cell lung cancer at an adult age, but inactivation of the *Rb* locus in mice led to embryonic lethality, and heterozygous mice developed pituitary and thyroid tumours. Retinal tumours were nevertheless observed in mutant mice when both *Rb* and the closely related *p107* gene were disrupted (Classon & Harlow, 2002).

Similarly, germline inactivation of *p53* in the mouse resulted in an increased risk of developing mainly lymphomas and soft-tissue sarcomas, whereas the spectrum of human tumours with *TP53* mutations also includes breast, brain, colon and lung tumours (Jacks et al., 1994; Hahn & Weinberg, 2002). Numerous genetic tumour models involving ubiquitous or tissue-specific transgenic overexpression of p53 in a dominant-negative manner, and also total or conditional knock-out or knock-in models in which common human p53 mutations have been introduced into the mouse genome, have subsequently shown that the dosage of p53 protein and the type of mutation have profound effects on disease phenotypes. Moreover, p53 knock-out crosses with other tumour-prone strains either overexpressing oncogenes (c-Myc, Wnt-1, Ras) or lacking tumour suppressors (Rb, Nf1, Apc, Brca-1/2) markedly affect tumour susceptibility and the tumour spectrum (Attardi & Donehower, 2005). As mentioned above, telomerase-deficient mice that are heterozygous for *p53* develop breast, colon and skin carcinomas upon ageing instead of the lymphomas and sarcomas which are common in the absence of the *p53* allele alone (Artandi et al., 2000). These mice with aberrant expression of tumour suppressors p53 and Rb, represent just a few examples of classical targeted tumour models. An ample repertoire of genetic models involving changes in the critical cellular processes in tumour development (including self-sufficiency/proliferation, apoptosis/survival, genomic instability, senescence, angio/lymphangiogenesis, invasion and tissue remodelling) and a number of cancer genes have been generated during the last 20 years, some of which have resulted in a very different tumour spectrum from that expected (Hahn & Weinberg, 2002; Herzig & Christofori, 2002; Maddison & Clarke, 2005; Frese & Tuveson, 2007; see also http://emice.nci.nih.gov and http://tumor.informatics.jax.org/mtbwi/index.do).

There are certain severe limitations associated with basic models with targeted gene alterations (and obviously also with transgenic models) in which a single

genetic change is introduced into the mouse genome in order to mimic human cancer. As discussed above, tumour development requires multiple stepwise genetic and epigenetic changes in epithelial cells, which cannot be achieved in constitutive knock-out models. In addition, most human tumours result from sporadic mutations in proto-oncogenes or tumour suppressor genes in somatic cells, whereas in traditional mouse models the modifications are introduced into the germline and may thus influence mouse development as well (Herzig & Christofori, 2002; Maddison & Clarke, 2005).

Conditional mutagenesis, which allows spatiotemporal mutations to be introduced into the mouse genome, has partially resolved these shortcomings. Conditional mutants are generated by means of Cre-Lox or yeast-derived FLP-FRT systems, in which bacterial Cre or yeast-derived FLT recombinase, driven by a tissue-specific promoter, catalyses a recombination event that results in the desired mutation in selected cells at a controlled point in time. These systems can be further fine-tuned by controlling Cre expression through ligand-induced systems such as CreER, or by delivering Cre into the tissue using adenoviral vectors, for example (Macleod & Jacks, 1999; Maddison & Clarke, 2005; Frese & Tuveson, 2007). Gut-specific homozygous inactivation of the *Apc* tumour suppressor gene which circumvents the embryonic lethality of total Apc deficiency represents a pioneer work aimed at producing a conditional model for tumour development (Shibata et al., 1997). Other improvements in genetic models include the generation of a mouse strain in which *K-ras* oncogene activation is achieved in just a few cells by means of spontaneous recombination (the "hit and run" strategy), mimicking the sporadic formation of human cancers (Johnson et al., 2001). Restricted somatic mutations can also be achieved by delivering oncogenes by retroviral infection to transgenic mice engineered to express a receptor for avian retrovirus (tumor virus A, TVA) under cell-type-specific promoters. In this way multiple genetic changes can be sequentially introduced into somatic cells by multiple rounds of retrovirus infection, thereby mimicking more accurately the multistage nature of human cancers and the consecutive changes involved. This approach has been used to study the cooperation between activated K-ras and Akt oncogens in glioblastomas (Holland, 2000). Furthermore, the retrovirus strategy has been successfully combined with traditional genetic tumour models such as RIP-Tag to allow detailed studies on multistep cancer progression (Du, Lewis, Hanahan & Varmus, 2007).

5.7 Concluding Remarks

Experimental tumour modelling in mice has been extended from simple chemical carcinogenesis models and transplanted tumours to complicated genetic models generated by advanced molecular technologies and with a potential for more accurate recapitulation of the changes that occur in human cancers. Some of the models can also be used to investigate tumour metastasis, while others are more suitable for monitoring primary tumour growth only, an issue discussed in detail elsewhere (see Khanna & Hunter, 2005; Kim & Baek, 2010). Common or novel mutations that are

found in human tumours, or their combinations, can be introduced into the mouse genome to validate their contribution to tumour formation and progression in vivo. These models can be used to explore the detailed molecular mechanisms underlying tumour development, and also to an increasing extent for targeted drug testing. Nevertheless, even if the novel genetic models appear to provide better opportunities to study different aspects of tumorigenesis, the traditional mouse tumour models are most likely to maintain their position in many applications, particularly for large-scale drug testing. In addition, some of the old models have been reintroduced and combined with transgenic and knock-out models, and have proved very useful in intricate and challenging cancer studies. Examples of these include the classical DMPA-TPA protocol for mouse skin carcinogenesis applied to mice with mutations for cancer genes to explore the contribution of these to tumour-associated inflammation and skin cancer of stem cell origin. As discussed thoroughly above, all experimental models have their strengths and weaknesses, and eventually it is the biological or translational problem that dictates which model or models should be employed to obtain reliable answers to that particular question.

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