Mouse models for multistep tumorigenesis

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The mouse is an ideal model system for studying the molecular mechanisms underlying the pathogenesis of human cancer. The generation of transgenic and gene-knockout mice has been instrumental in determining the role of major determinants in this process, such as oncogenes and tumor-suppressor genes. In the past few years, modeling cancer in the mouse has increased in its complexity, allowing *in vivo* dissection of the fundamental concepts underlying cooperative oncogenesis in various tumor types. In this review, we discuss how this transition has been facilitated, providing relevant examples. We also review how, in the post-genome era, novel methodologies will further accelerate the study of multi-step tumorigenesis in the mouse.

A TRENDS Guide to
Cancer Biology

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It is widely accepted that tumorigenesis is a multistep process that involves a series of genetic and epigenetic alterations, such as activation of dominantly acting oncogenes and inactivation of tumor-suppressor genes. These mutations accumulate in the cells and change their behavior from normal growth to unrestrained growth and eventually lead to invasion into surrounding tissue and/or metastasis. This multiple-stage process is reflected by a range of observations, including clinical, epidemiological and laboratory experiments. The process can be extremely complex. For example, during the development of colorectal carcinoma, it has been proposed that at least seven sequential genetic alterations occur, including mutation of the adenomatous polyposis coli (*APC*) tumor-suppressor gene, mutation of Ki-*ras* and loss of functional *p53* gene[1]. These sequential mutations of key growth-regulatory genes in the somatic cells and their progeny are generally regarded as 'multiple-hits' in a broader interpretation of the original Knudson 'two-hits' theory, which was based on a statistical analysis of the relationship between age and incidence of retinoblastoma[2].

Over the past decade, our genetic understanding of tumor development has been greatly enhanced by the direct study of human cancer. Studies on rare forms of familial inherited cancer syndromes have led to successful identification of many tumor-suppressor genes that are crucial to the development of distinct forms of cancer. Mutation or loss of these genes has highly penetrant phenotypic consequences, and germline mutations of these genes predispose the carriers to tumor development. However, the study of cancer genetics in humans has certain limitations. For the sporadic forms of cancer in particular, tumor susceptibility in each individual is determined not only by these key players but also by several modifier genes whose activity might also have a key influence on cancer development. The diversity in genetic background and the difficulty in tumor and tissue procurement make it difficult to study tumorigenesis in humans for the purpose of identifying weak tumor determinants. By contrast, the mouse offers several distinct advantages and has proved to be a valuable model system for the study of multistep tumorigenesis at two distinct levels: (i) identification of novel genes involved in cancer pathogenesis; and (ii) experimental *in vivo* assessment of the role of genetic hits in multistep tumorigenesis.

Mice with a controlled genetic background can be raised easily in vast colonies, facilitating the analysis of cancer-relevant genes in tumor development, particularly the low-penetrance genes. The large number of inbred mouse strains that show enormous variation in their susceptibility to different types of tumors provide a good resource for various tumor models. Most importantly, the

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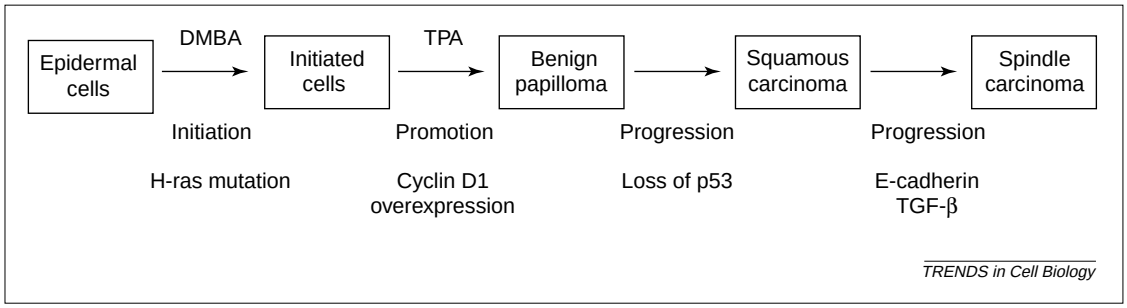

Figure 1. Chemically-induced multistep skin carcinogenesis in the mouse

Epidermal cells are initiated by 7,12-dimethyl-benzanthracene (DMBA) by causing a Ha-*ras*-activating mutation. Treatment with 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA) results in upregulation of cyclin D1 gene expression and promotes the formation of papilloma. Loss of functional *p53* gene accelerates the progression of benign papilloma to malignant carcinoma. Finally, transforming growth factor beta (TGF-β) enhances the conversion of squamous carcinoma to metastatic spindle cell carcinoma, accompanied by downregulation and delocalization of E-cadherin.

similarity between the mouse and human genomes, and particularly between the histological stages and genetic pathways underlying tumor development in human and mouse, make the mouse an ideal model system for the study of human cancer pathogenesis. Furthermore, it is now possible to manipulate the mouse genome either by overexpressing a dominantly acting oncogene in transgenic mice or by deleting a tumor-suppressor gene from the germline of knockout mutants in a tissue- and cell-type-specific manner. Many of these transgenic and knockout mutants now provide faithful model systems for most human cancers and will also permit analysis of the roles of strong and weak determinants and establish a link between individual gene mutations and specific cellular changes that lead to tumor development.

Molecular carcinogenesis in the mouse: the skin as a paradigmatic model system for the study of multistep tumorigenesis

One of the best-established multistage tumorigenesis models is mouse skin carcinogenesis. The sequential application of a carcinogen, followed by treatment with a non-carcinogenic promoter can effectively induce skin tumors. Studies of chemically induced mouse skin tumors established the three stages of tumor development: initiation, promotion and progression[3,4] (Fig. 1). Initiation is generally accomplished by topical application of a single subcarcinogenic dose of skin carcinogen, such as 7,12-dimethyl-benzanthracene (DMBA). The treatment causes irreversible DNA damage, resulting in mutation of the Ha-*ras* oncogene in epidermal cells. Subsequent studies demonstrated that the type of mutation of Ha-*ras* was dependent on the chemical initiator, suggesting that the initiator has a direct effect on the Ha-*ras* gene[5,6]. The promotion is achieved by repeated application of skin promoters, most commonly the phorbol esters, such as 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA). Although most tumor promoters do not bind covalently to DNA and are not mutagenic *per se*, they cause a range of cellular and biochemical changes, such as elevated activation of protein kinase C and increased expression of transforming growth factor alpha (TGF-α), c-Jun and c-Fos, which have an obvious and direct connection with the regulation of cell growth and differentiation[3]. This promotes the selective clonal expansion of initiated epidermal cells and leads to the formation of multiple squamous papillomas. The progression stage is generally a spontaneous process. It is characterized by a high level of genetic instability, particularly chromosomal alterations resulting in elevated expression of genes encoding Ha-Ras and cyclin D1, as well as loss of expression of the tumor-suppressor p53 (Ref. 7). These changes confer to papilloma cells a further growth advantage, leading to the conversion of the papillomatous lesion into malignant squamous carcinoma. A further advanced stage in mouse skin carcinogenesis is the progression of squamous carcinoma to spindle carcinoma. This stage is associated with the changes of epithelial differentiation markers, such as decreased expression of E-cadherin, keratins K1 and K10, and increased expression of α6β4 integrin[8].

The mouse skin carcinogenesis model not only establishes a link between genetic pathways and histological stages of tumor development but also serves as a model system to study the functions of many other genes in the modulation of the tumorigenic process. For instance, DMBA–TPA treatment of cyclin D1-deficient mice resulted in a decrease in skin tumor development, indicating that cyclin D1 is an important target of the Ha-Ras pathway in skin tumorigenesis[9]. By contrast, p53-deficient mice displayed an accelerated progression of papilloma to carcinoma upon treatment, suggesting that p53 plays an important role in the tumor-progression phase[10]. The involvement of the c-*fos* gene in malignant conversion in the skin is corroborated by studies on the c-*fos* knockout mice[11]. The promyelocytic leukemia (*PML*) tumor-suppressor gene has also been found to antagonize the initiation phase of skin tumorigenesis, resulting in increased tumor development in PML-null mice[12]. This model system was also used to elucidate the dual functions exerted by the gene encoding TGF-β in skin tumorigenesis[13,14]. TGF-β transgenic mice were more resistant to the induction of benign papilloma, but the malignant conversion rate was greatly increased, and most of the carcinomas progressed further to invasive spindle carcinomas. More recently, the epidermal growth factor (EGF) receptor has also been

shown to play an essential role in SOS-dependent skin tumor development. In fact, SOS transgenic mice bearing a disrupted gene for the EGF receptor do not develop skin tumors[15]. Finally, the mouse skin carcinogenesis model has been of great use in defining the concept of haplo-insufficiency in tumor suppression. For instance, skin carcinogenesis studies show that $p27^{kip1}$ exerts the tumor-suppression function in a dose-dependent manner. The nullizygous and $p27^{kip1}$ heterozygous mice are both predisposed to tumorigenesis, and the heterozygous mutants do not lose the wild-type allele[16]. Further studies in other mutant mouse models suggest that the $p27^{kip1}$ gene is not an exception, and that many other tumor suppressors are haplo-insufficient in their function, such as PML (see below)[17].

Interspecific crosses of knockout and transgenic mutants for the study of multistep tumorigenesis: the APL paradigm

One of the most effective approaches for the study of cooperative tumorigenesis in the mouse is to cross a specific tumor-prone transgenic or knockout line with other transgenic or knockout mutants, thus allowing the assessment of whether these additional mutations can accelerate tumor development. The selection of 'additional' gene mutations can be divided into two categories: (i) genes that are involved in the molecular biochemical pathway that is thought to control the development of a tumor of a specific histological origin; and (ii) genes that maintain genome integrity. The first category includes genes that regulate cell proliferation, differentiation or apoptosis. Genes that fall into the second category are those involved in DNA repair and the control of genome stability.

A compelling example of the power of transgenic and knockout methodology in the study of multistep carcinogenesis is provided by the attempt to dissect acute promyelocytic leukemia (APL) pathogenesis in the mouse. APL is associated with reciprocal chromosomal translocations, which always involve the retinoic acid receptor (RAR)α gene variably translocated and fused to five distinct genes: PML, promyelocytic leukemia zinc finger (PLZF), nuclear mitotic apparatus protein (NuMA), nuclear phosmin (NPM) and STAT5b (Ref. 18). As a consequence of these translocations and in view of their reciprocity, two fusion genes are generated, encoding two distinct fusion proteins, that coexist in the APL blast.

Transgenic mice expressing PML–RARα under the control of a promyelocytic-specific promoter develop a form of leukemia that closely resembles human APL[19–21]. However, the incidence of leukemia development in these mice is low and the latency is long. These results suggest that PML–RARα translocation is necessary but not sufficient to induce leukemia and that additional genetic events are probably required. Furthermore, transgenic mice expressing another APL-specific fusion gene, PLZF–RARα, display a very different phenotype[22]. Leukemia that develops in PLZF–RARα transgenic mice more closely resembles human chronic myeloid leukemia (CML). This leukemia is characterized by an expansion of myeloid cells that can terminally differentiate, whereas classic APL is characterized by a distinctive block in myeloid differentiation at the promyelocytic stage. Once again, leukemia in PLZF–RARα transgenic mice develops after a long latency. Thus, like PML–RARα, PLZF–RARα does not seem to be sufficient for APL leukemogenesis, and additional genetic alterations have to occur in order to cause full-blown leukemia. The phenotypic difference displayed by the two transgenic models indicates that PML–RARα and PLZF–RARα differ in their oncogenic activities and represent distinct RARα mutants in spite of their identity in the RARα portion, suggesting that, in APL harboring PLZF–RARα, other genetic events are required to block the differentiation at the promyelocytic stage.

As the APL-specific chromosomal translocations are reciprocal, RARα–PML and RARα–PLZF transgenic mice have also been generated. Interestingly, although RARα–PML transgenic mice do not develop leukemia, RARα–PML acts as a tumor modifier, whose presence significantly increases the penetrance of PML–RARα and accelerates leukemia development (Fig. 2). Double transgenic mice coexpressing PML–RARα and RARα–PML reciprocal fusion genes develop early onset APL-like leukemia[23]. The PML gene also behaves like a tumor-suppressive modifier of PML–RARα-induced leukemogenesis. Inactivation of both, or even one, PML allele dramatically increases the incidence of APL-like leukemia in $PML^{-/-}$/PML–RARα or $PML^{+/-}$/PML–RARα double mutants[17]. The data also demonstrate that PML is haplo-insufficient in antagonizing the function of PML–RARα. In this respect, it must be emphasized that, in the APL blast, the dose of the two genes involved in the translocation is, in fact, reduced to heterozygosity, because one allele is involved in the translocation.

However, coexpression of PLZF–RARα and RARα–PLZF in double transgenic mice resulted in a completely different outcome. Rather than accelerating leukemia development, the presence of RARα–PLZF transformed the biological features of the disease from a CML-like leukemia in single TM to an APL-like leukemia in double TM (Fig. 2). In contrast, leukemia onset was unaffected. Thus, RARα–PLZF did not function as a classic tumor modifier, but rather as a tumor 'metamorphoser'[24]. Similarly, loss of PLZF function also metamorphoses, once again in a dose-dependent manner, the CML-like leukemia in PLZF–RARα mice into APL-like leukemia in $PLZF^{-/-}$/PLZF–RARα mice[24].

The leukemia incidence and phenotype in PML–RARα or PLZF–RARα transgenic mice in an RARα-null background has not yet been reported. It will be interesting to see whether partial or complete loss of RARα function also plays a role in leukemogenesis.

Taken together, these studies in transgenic and knockout models demonstrate that the two translocation products, as well as the inactivation of the genes involved in these translocations, are essential for the development of a leukemia with APL features. However, given the fact that the reciprocal translocation product is not expressed in 100% of human APL patients, and that the earliest leukemia onset observed in double transgenic mutants is still approximately six months, it is possible that further genetic events are required. Several approaches could be applied to such APL mouse models in the search for these additional genetic events, as we will discuss below. These additional hits could participate in the pathogenesis of human APL and also in the pathogenesis of other human cancers.

Identification of tumor-modifier genes in the mouse

Mouse models of cancer not only enable the determination of the role of specific genetic events in multistep tumorigenesis, as discussed above, but also help in identifying novel genes involved in this process, particularly tumor modifiers, which are notoriously difficult to discover by performing population-based genetic studies. A successful example of the potential of this approach is represented by the identification of Mom-1. This tumor modifier was identified in an attempt to characterize genetic factors that influence tumor incidence in the *Min* mouse, a strain that harbours a point mutation in the *APC* gene and that is prone to develop multiple adenomatous polyposis in the small intestine[25]. The incidence of polyposis in the *Min* mouse varies considerably depending on the strain background. In a C57BL/6 background, *Min* mice develop a large number of tumors, whereas an AKR background is resistant to the effects of the APC mutation. Genetic mapping linked a locus on chromosome 4 to the resistance. As this locus could change tumor susceptibility triggered by the *Min* allele, it was named 'modifier of *Min*', or Mom1. Subsequent studies led to the identification of the gene encoding secreted phospholipase 2a (Pla2g2a) as the gene encoding Mom-1[26]. Pla2g2a is highly expressed in the resistant strains, but weakly expressed in the sensitive strains. Furthermore, mutation analysis in the sensitive and resistant strains identified a thymidine insertion that results in aberrant Pla2g2a splicing in the sensitive strain. Reintroduction of a functional *Pla2g2* gene into a sensitive strain renders the mice resistant to tumor development[27].

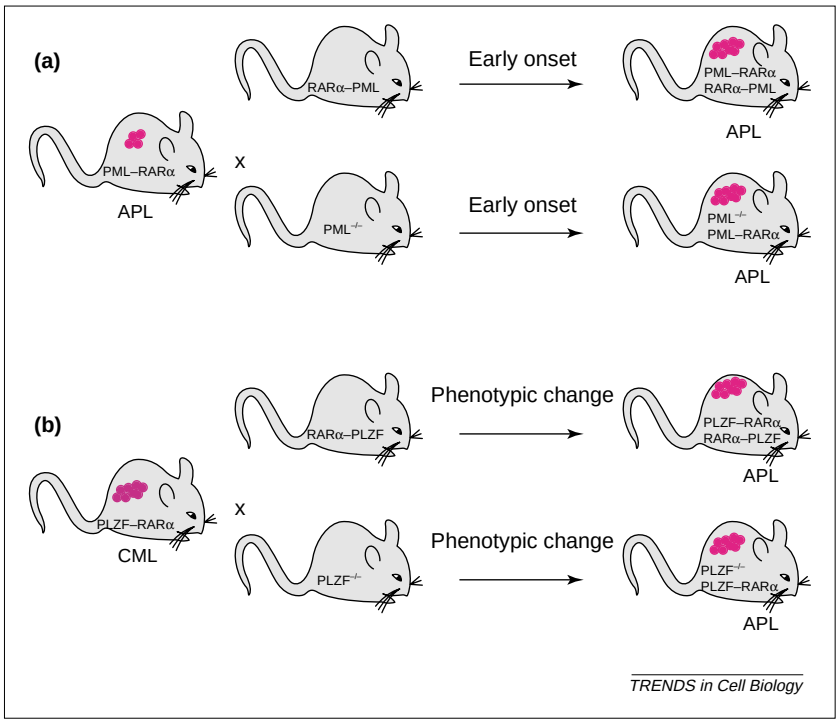

Figure 2. Effect of additional genetic hits in mouse models of APL

(a) Crosses between promyelocytic leukemia (PML)–retinoic acid receptor α (RARα) transgenic mice and RARα–PML transgenic mice or PML-deficient mice result in acceleration of leukemia onset and also a higher incidence of leukemia. Thus, RARα–PML and PML appear to function as tumor-development modifiers. (b) By contrast, crosses between promyelocytic leukemia zinc finger (PLZF)–RARα transgenic mice and RARα–PLZF transgenic mice or PLZF-deficient mice metamorphose the chronic myeloid leukemia (CML)-like phenotype observed in PLZF–RARα transgenic mice into acute promyelocytic leukemia (APL)-like leukemia. Thus, RARα–PLZF and PLZF function in APL as tumor phenotype modifiers or tumor metamorphosers.

The molecular identification of Mom-1 represents a paradigm and exemplifies the power of mouse genetics for the analysis and identification of tumor modifiers. So far, the loci of many more tumor modifiers have been identified, and their influences on tumor development extend from affecting the size and number of the tumors to the latency and even the morphology of the tumors[17,24,28]. Moreover, strong genetic interactions between tumor modifiers have also been implicated in several linkage studies in various mouse models of human cancers[29,30].

New approaches to identify and validate the role of additional genetic events in mouse models of human cancer

SKY and CGH microarray in the mouse

Novel methodologies are allowing for more comprehensive study of the multistep process towards full-blown transformation and metastasis in animal model systems. Additional genetic events occurring throughout cancer evolution can now be studied in mouse models at various different levels with greater sensitivity.

- At the chromosomal level by spectral karyotyping (SKY). SKY has been developed to detect particular chromosome translocations, regional chromosomal amplifications and deletions[31,32]. SKY is based on hybridization of metaphase chromosomes with chromosome-specific probes differentially labeled with a combination of fluorophores. Utilizing a combination of conventional fluorescent light microscopy, Fourier-transform spectroscopy and CCD-camera, fluorescence-emission spectrum of each metaphase chromosome is measured simultaneously and their spectral images are visualized.
- At the gene locus level. Many methods have been applied, such as standard fluorescent *in situ* hybridization (FISH), comparative genomic hybridization (CGH)[33], genetic linkage and loss of heterozygosity (LOH) analysis, whereas the development of oligonucleotide and cDNA microarrays allow the detection of changes in gene expression throughout tumor progression[34–36].

The combination of all of these approaches makes them extremely powerful for the precise location of genetic changes during multistep tumorigenesis. For example, CGH and FISH can refine the region of translocation breakpoint after SKY narrows the search to a particular chromosomal region. Such combination has allowed the identification of a recurrent translocation on chromosome 14 in lymphomas in the ataxia-telangiectasia mutated (ATM)-deficient mouse[37]. Similar analysis in PML–RARα and RARα–PML transgenic mice revealed that deletion of chromosome 2 is a frequent event in leukemia development[38]. Furthermore, the combination of CGH and microarray technology (CGH microarray) makes it possible to analyse the whole genome for DNA copy-number variation at higher resolution[39,40]. Conventional CGH, which was developed to compare the changes of DNA copy number across the genome, is based on hybridization of tumor genomic DNA to the normal metaphase chromosomes. Therefore, the resolution is low and the technique can only been used to determine regions of chromosomal amplification and deletion. By contrast, DNA microarray-based CGH increases the resolution of CGH to such an extent that, not only can it detect changes in known genomic regions, but it can also directly identify candidate genes that are important to tumor development[41,42]. Moreover, the combination of array technology and genomic mapping makes it possible to study single-nucleotide polymorphism (SNP) in a high-throughput manner, thus speeding-up enormously the mutational analysis and the linkage mapping of cancer-associated genes[43,44].

Insertional mutagenesis

Retroviral insertional mutagenesis has been successfully used to identify various cancer-associated genes in the mouse. Random insertion of proviral sequence into the mouse genome can in fact alter gene expression. Some of these insertions can result in overexpression of proto-oncogenes or silencing of tumor-suppressor genes, thus leading to acceleration in tumor development. As an example, the proviral insertion resulting in elevated expression of a truncated *Notch1* gene led to accelerated development of T-cell tumors in c-*myc* transgenic mice[45]. More recently, a newly improved method has been developed that uses inverse polymerase chain reaction (PCR) to allow quick identification of the tag sequences of affected genes[46]. The availability of the mouse genome sequence and expressed sequence-tagged databases will further facilitate the detection of cancer-associated genes.

TVA-based retroviral gene delivery system for the study of cooperative oncogenesis in the mouse

A novel retroviral gene delivery system has been proven to be extremely useful for the study of cooperative oncogenesis in the mouse[47]. This system is based on the utilization of the receptor of subgroup A avian leucosis virus, TVA. Mice do not harbour a TVA gene homolog and are therefore resistant to avian virus infection. However, in transgenic mice the expression of TVA receptor gene under the control of tissue- or cell-specific promoter render them susceptible to avian retroviral infection, allowing genes of interest to be introduced somatically in a tissue- and cell type-specific manner[48]. In practice, the gene of interest is cloned into an avian Rous sarcoma virus-derived replication-competent cloning vector (RCAS). The proviral vector is transfected into chicken fibroblast cells to produce a high titer of infectious viral stock. The virus can then be delivered to TVA-expressing transgenic mice by injecting the virus-producing cells or by injecting the virus itself (Fig. 3). The TVA-based retroviral gene delivery system offers several advantages over current transgenic and knockout mouse models. First, the virus is replication competent and can be consistently propagated in avian cells to produce a higher titer of viral stock. Second, infected murine cells do not produce infectious virus. The viral receptor in infected transgenic mouse cells remains available for multiple rounds of infection. Therefore, several genes of interest can be introduced into the same cells simultaneously or sequentially, allowing analysis of the phenotypic consequence of genetic mutations, both individually and in combination. Furthermore, TVA transgenic mice can be crossbred with other transgenic or knockout mice to study the role of

cooperative genetic mutations in accelerating tumor development and metastasis. A recent study on the mechanisms of gliomagenesis demonstrates the power of the TVA-based approach for dissecting cooperative oncogenesis in the mouse[49]. To investigate the role of epidermal growth factor receptor (EGFR) mutation in glioma pathogenesis, transgenic mice were generated that expressed the TVA receptor under the control of the astrocyte-specific glial fibrillary acidic protein (GFAP) promoter. Infection of GFAP–TVA transgenic mice with the virus encoding a constitutively active mutant EGFR did not result in glioma development. However, coinfection of GFAP–TVA transgenic mice with viruses carrying CDK4 and mutant EGFR or infection of GFAP–TVA transgenic mice deficient in INK4a–ARF with the mutant EGFR virus triggered gliomagenesis, suggesting that genetic alterations of EGFR and INK4a–ARF are two crucial genetic events in gliomagenesis[49].

Inducible transgene and conditional knockout

Recent advances in tissue-specific manipulation of targeted genes achieve a tighter spatial and temporal control of gene expression. These overcome the limitations encountered by generating conventional mouse mutants, such as embryonic lethality in the knockout approach or constitutively high levels of gene expression in transgenic mice.

Inducible systems, originally developed for *in vitro* cell-biology studies, have been applied more recently *in vivo* in transgenic approaches to regulate gene expression in a controlled manner. One of the most widely used systems is the tetracycline system, which uses a tetracycline-responsive promoter to drive the expression of the gene of interest[50]. Another widely utilized method takes advantage of the ability of the estrogen receptor (ER) to shuttle from the cytosol to the nucleus, upon binding to the ligand or analogues such as tamoxifen. The generation of an in-frame fusion between the protein of interest (e.g. a transcription factor) and the ER renders the activity of the fusion protein tamoxifen dependent[51].

As far as gene knockout is concerned, the *Cre–loxP* system has been widely applied to create a conditional disruption of gene expression in a specific tissue[52]. In essence, a first mutant mouse is generated where *loxP* sites flank the target gene, or part of it. These are short sequences that are recognized only by the Cre (cyclization recombination) bacteriophage recombinase. *LoxP* sites are usually introduced by homologous recombination within introns of the target gene, so that they do not interfere with its proper translation. Next, a transgenic line is generated where the *Cre* gene is under the control of a tissue-specific promoter. Cre recognizes and catalyzes recombination and excision of the region located between *loxP* sites. Crosses of these two murine lines create a double mutant where the gene of interest is now disrupted in a tissue-specific manner. Several tumor-suppressor gene mutants have been generated using the *Cre–lox* system in order to study the consequences of their inactivation in a given tissue (e.g. the intestinal-specific inactivation of *APC* or the mammary-specific inactivation of *BRCA1*[53,54].

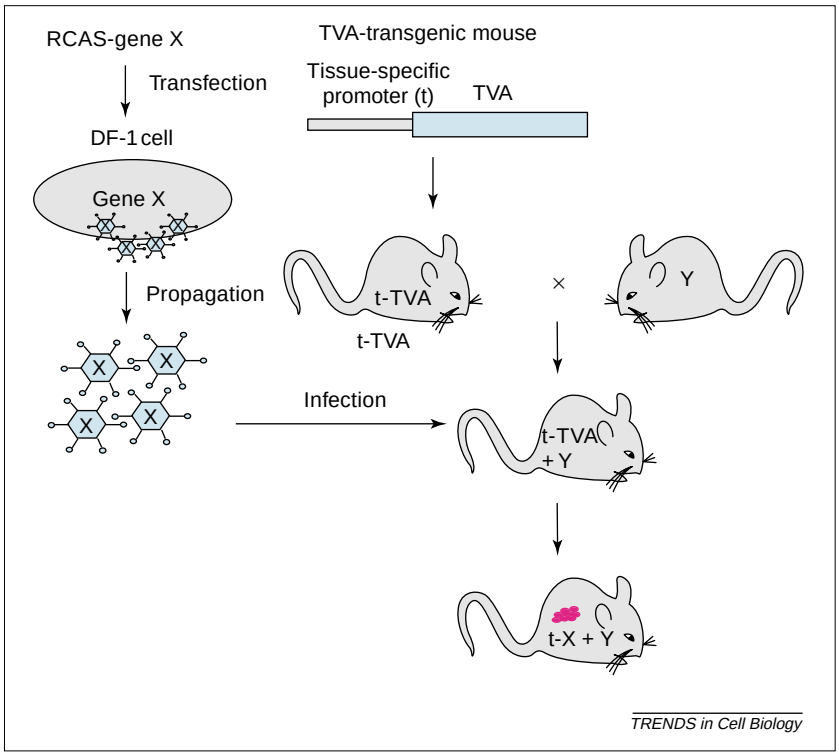

Figure 3. TVA-based gene delivery approach

Chicken DF-1 cells are transfected with a plasmid encoding the replication-competent subgroup A avian viral vector, Rous sarcoma virus-derived replication-competent cloning vector (RCAS), and a gene of interest (gene X). The retrovirus is produced and propagated in DF-1 cells to obtain high-titer virus particles. Next, the virus is used to infect transgenic mice expressing the TVA receptor under the control of a tissue- or cell-type-specific promoter (t). Mice do not harbour a TVA gene homolog and thus are resistant to avian viral infection. Only cells engineered to express TVA receptor can be infected. As a result, the retroviral infection enables the introduction of gene X into a specific cell-type or tissue (t-X). Mice carrying multiple genetic alterations can be obtained by crossing t-TVA transgenic mice with knockout or transgenic mutants (Y). The Y mutant harbors one mutation and additional mutations can be delivered by retroviral infection. The cooperative interaction between gene X and Y mutations can result in the development of tumors in the mouse.

Recombinant congenics for the identification of cancer genes

Recombinant congenic (RC) strains provide an additional useful tool for cancer study[55]. RC strains are produced by limited backcrossing between a common 'donor' inbred strain and a common 'background' inbred strain, before proceeding with inbreeding of the progeny derived from the initial backcross. In this way, a series of strains is generated, each of which contains a random small subset of genes from the donor strain and the

majority of genes from the background strain. Typically, the RC strains carry approximately 12.5% of the donor genome and 87.5% of the background genome. As a result, the individual gene of the donor strain that might play a role in tumorigenesis can be segregated and its contribution to the multiple complex traits can be mapped and studied separately. This approach has proved effective in identifying new cancer-susceptibility loci, particularly for lung and colon cancers in the mouse. So far, nine new loci, Scc1–Scc9, have been identified that are involved in tumorigenesis in the colon[56,57], whereas four new loci, Sluc1–Sluc4, influence the susceptibility to lung cancer in the mouse[29]. This system does have a few disadvantages, however. These include the length of time required to generate each series of RC strains, and limited family members available for each RC series. In addition, the genetic mapping resolution can be poor in RC strains in limited cases in which there are a large number of segregated loci. Despite these limitations, in the future this approach might provide a substantial contribution to the identification of cancer-susceptibility genes, tumor modifiers and metamorphosers.

Concluding remarks

In the past few years, modeling human cancer in the mouse has already proven incredibly instructive for studying the mechanisms underlying cancer pathogenesis, the function of cancer genes and their intricate interactions in multistep tumorigenesis. The future completion of a proper annotation of the human and mouse genome, as well as the advent of novel technologies for the study of cooperative oncogenesis in the mouse and for the identification of genetic events throughout tumor progression, will certainly markedly speed up this learning process. This acceleration is very much needed in order to disentangle the overwhelming complexity that such a flurry of genetic information will impose on our analytical capacity.

Acknowledgements

This work is supported by the NCI, the De Witt Wallace Fund for Memorial Sloan-Kettering Cancer Center, the Mouse Model of Human Cancer Consortium (MMHCC), and National Institutes of Health Grants to Pier Paolo Pandolfi and the Lymphoma & Leukemia Society, of which Pier Paolo Pandolfi is a Scholar. We thank Alan Balmain and Ming Hu for their suggestions and critical reading of the manuscript. Finally, we are indebted to all the past and present members of the laboratory of Molecular and Developmental Biology (MADB) at Memorial Sloan-Kettering Cancer Center, working on mouse models of cancer and related subjects.

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