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Analyses of tumor suppressor genes in germ-line mouse models of cancer

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Abstract

Tumor suppressor genes are critical regulators of growth and functioning of cells, whose loss of function contributes to tumorigenesis. Accordingly, analyses of the consequences of their loss of function in genetically engineered mouse models have provided important insights into mechanisms of human cancer, as well as resources for preclinical analyses and biomarker discovery. Nowadays, most investigations of genetically engineered mouse models of tumor suppressor function use conditional or inducible alleles, which enable analyses in specific cancer (tissue) types and overcome the consequences of embryonic lethality of germline loss of function of essential tumor suppressor genes. However, historically, analyses of genetically-engineered mouse models based on germ-line loss of function of tumor suppressor genes were very important as these early studies established the principal that loss of function could be studied in mouse cancer models and also enabled analyses of these essential genes in an organismal context. Although the cancer phenotypes of these early germline models did not always recapitulate the expected phenotypes in human cancer, these models provided the essential foundation for the more sophisticated conditional and inducible models that are currently in use (see Chapters XXX). Here, we describe these “first generation” germline models of loss of function models, focusing on the important lessons learned from their analyses, which helped in the design and analyses of “next generation” genetically-engineered mouse models.

Introduction

Analysis of cancer phenotypes in genetically engineered mice has its origins in transgenic models based on expression of dominantly-acting oncogenes, which were described in Chapter XXX. Following from these early successes in the generation of these transgenic models, and following from the advancement in technologies for gene recombination in mouse embryonic stem cells (Thomas and Capecchi 1987), the next stage in the generation of mouse models of cancer focused on the loss of function of tumor suppressor genes based on their germline deletion in mice – or as they are often called “knockout” mouse models.

Indeed, more than 20 tumor suppressor genes have been analyzed in germline loss of function mouse models (Jacks 1996). In general, tumor suppressor loss in human cancer is

recessive in nature, such that sporadically arising tumors have loss of one allele, which is usually accompanied by inactivation of the second allele. Accordingly, one would expect that it should be necessary to study the consequences of tumor suppressor loss in mouse models homozygous for inactive alleles. However, in practice, many homozygous tumor suppressor alleles are embryonic lethal (*e.g.*, *Pten* and *RB*), yet analyses of the heterozygous state has proven to be informative either because the other allele undergoes loss of function in the tumor context (*e.g.*, *Pten*), or because analyses of the heterozygotes display a haploinsufficient phenotype (*e.g.*, *p27*).

Among the principal benefits of analyzing tumor suppressor genes in germline “knock-out” mice: (i) the technology for generating these models is very straightforward (especially by today’s standards); (ii) homozygous knockout mice may provide insights into the essential roles of tumor suppressors in embryonic development and/or normal physiology that may be masked in conditional alleles; (iii) heterozygous knockout mice have provided useful models for cancer since they may reveal unexpected roles and inform on the necessity of loss of the second allele; (iv) intercrossing of two or more tumor suppressor knockout alleles to generate mice with compound deficiencies has provided insights into cooperativity of tumor suppressor function *in vivo*; and (v) generation of primary cells lacking tumor suppressors (either in embryonic stem cells or embryonic fibroblasts) have provided useful models for *in vitro* cell culture analyses.

On the other hand, analyses of tumor suppressors in germline mouse models has several important limitations, including: (i) the inability to study the consequences of tumor suppressor function as homozygotes because of embryonic lethality (*e.g.*, *Pten* mutant mice); (ii) the low penetrance of the cancer phenotype in some cases (*e.g.*, *Nf* mutant mice); (iii) the dissimilarity between the cancer phenotypes that prevail in the mouse model with the expected consequences based on analyses of human cancer (*e.g.*, lack of retinoblastoma in *Rb* mutant mice); and (iv) confounding phenotypes that make it difficult to study phenotypes of interest (*e.g.*, prevalence of sarcomas in the *p53* mutant mice and lymphomas in the *Pten* mutant mice).

This chapter provides a historical overview of germline models based on loss of tumor suppressor gene function. We describe the relevance of the mouse models and their phenotypes, as well as important lessons learned from their analyses that set the stage for the more sophisticated models that currently prevail (Table 1).

***p53* mutant mice — the story unfolds**

Functional inactivation of the *p53* tumor suppressor gene is an almost universal feature of many human cancers. Given its importance in cancer suppression and the prevalence of its inactivation in cancer, the generation of *p53* mutant mice was an obvious priority and among the first such knock-out alleles to be described (Donehower et al. 1992; Jacks et al. 1994a). Indeed, analyses of *p53* germline mutant mice provided some of the first evidence that loss of function of tumor suppressor genes could give rise to cancer phenotypes in genetically engineered mouse models, which set the stage for many subsequent studies.

Unlike other tumor suppressor alleles (see below), *p53* homozygous null mice are phenotypically normal, which was somewhat surprising at the time since it had been anticipated that *p53* would be required for embryonic development (Donehower et al. 1992). Nonetheless, *p53* homozygous null mice were prone to develop tumors by 3–6 months of life although they were mostly lymphomas and sarcomas, rather than adenocarcinomas that are the most prevalent consequence of *p53* mutation in human cancer (Jacks et al. 1994a). Heterozygous animals also had an increased cancer risk, although the distribution of tumor types in these animals differed somewhat from homozygotes, and in most cases, the resulting tumors are accompanied by loss of the wild-type *p53* allele (Jacks et al. 1994a).

At the time, the description of the phenotype of the *p53* mutant mouse was ground-breaking, since it demonstrated the potential for analyses of cancer phenotypes in loss of function mouse models. However, many aspects of the *p53* mutant mouse phenotype were unexpected, since these mice were phenotypically normal aside from the cancer phenotypes and since the spectrum of tumors that these mice developed, particularly the sarcomas, did not recapitulate the spectrum of tumors that result from *p53* inactivation in human cancer. Nonetheless, these landmark studies paved the way for many future analyses of loss of function mouse models of cancer.

***Rb* mutant mice — mouse cancer phenotype may not parallel human cancer spectrum**

Another important early focus in the generation of germline loss of function mouse models of cancer was the retinoblastoma susceptibility gene, *Rb*, which is inactivated in sporadic retinoblastoma, as well as in osteosarcomas, soft tissue sarcomas and carcinomas of the breast, lung and bladder. Analyses of germline mutant mice revealed that *Rb* is an essential gene since homozygous null mice display embryonic lethality at mid-gestation due to defects in the development of the hematopoietic and nervous systems (Clarke et al. 1992; Jacks et al. 1992; Lee et al. 1992). Notably, *Rb* heterozygous mice do not develop retinoblastoma, although they do develop pituitary adenomas (Jacks et al. 1992). Together with the analyses of *p53* mutant mice, these analyses of *Rb* mutant mice provided the proof of principle that tumor suppressor loss could be modeled in genetically engineered mice, but underscored concerns about the relevance of these models for human cancer, since the phenotypes observed in the mouse models did not parallel the expected phenotypes as occur in human cancer. In fact, it was many years later that Mike Dyer's group explained the absence of retinoblastoma in *Rb* mutant mice as a consequence of compensation by the related protein *p107* (Donovan et al. 2006).

***Pten* mutant mice — tumor spectrum may be obscured by “faster growing tumors”**

Similar to *p53*, inactivation of *PTEN* (phosphatase and tensin homologue deleted from chromosome 10) tumor suppressor gene occurs very frequently in many types of human cancers by gene inactivation or aberrant regulation of its expression (Parsons and Simpson 2003). *PTEN* germline mutations are also found in three related human autosomal dominant

disorders, Cowden disease (CD), Lhermitte-Duclos disease (LDD) and Bannayan-Zonana syndrome (BZS), characterized by tumor susceptibility and developmental defects (Di Cristofano and Pandolfi 2000).

Loss of function of *Pten* in germline mutant mice results in early embryonic lethality, which demonstrates its essential role in development (Di Cristofano et al. 1998; Stambolic et al. 1998). *Pten* heterozygous mutant mice display dysplasia and/or cancer in several tissues including prostate, skin, endometrium, and colon (Di Cristofano et al. 1998; Podsypanina et al. 1999). However, the predominant phenotype in *Pten* heterozygotes is the frequent development of T cell lymphomas, which is a major cause of their lethality and tends to obscure slower-growing tumors, such as those of the breast and prostate. In some cases, tumors occurring in *Pten* heterozygous mice retain the wild type allele, although *Pten* function tends to be lost regardless (Di Cristofano et al. 1998; Stambolic et al. 1998). Thus, analyses of *Pten* germline mice reinforced the significance of analyzing loss of function phenotypes in mutant mice, although the difficulties in analyzing the phenotypes of particular interest provided the impetus for subsequent studies using a conditional allele (see chapter XX).

APC mutant mice — modeling tissue-specific genetic alterations in germline mutant mice

Genetic analyses of familial adenomatous polyposis (FAP) led to the discovery of the APC (adenomatous polyposis coli) gene, a tumor suppressor protein that acts as an antagonist of the Wnt signaling pathway, which is responsible for familial adenomatous polyposis as well as sporadic cancers of the colon, stomach, and esophagus. Interestingly, the first Apc-deficient mice were generated by mutagenesis with ethylnitrosourea (Moser et al. 1990). The resulting Min mice (for Multiple intestinal neoplasms) are embryonic lethal (Moser et al. 1993), while Min heterozygotes develop intestinal tumors and rarely live beyond 5 months of age due to secondary effects of the tumors (Moser et al. 1990). *Apc* germline mutant mice have similar phenotypes as Min mice (Fodde et al. 1994; Oshima et al. 1995). In particular, homozygous mice display early embryonic lethality, while heterozygotes develop multiple polyps throughout the intestinal tract, mostly in the small intestine (Oshima et al. 1995). In general, *Apc* germline mutant and *Min* mutant mice have been an excellent resource for studying intestinal tumorigenesis, although many studies now are performed with the corresponding conditional alleles.

***Nf1/2* mutant mice— another example of modeling tissue-specific genetic alterations in germline mutant mice, but less efficiently**

Neurofibromatosis are genetic disorders that primarily affect growth of neural tissues; the most common type is neurofibromatosis type 1 (*NF1*), which encodes a protein that stimulates Ras GTPase activity. *Nf1* null mutant mice display embryonic lethality at mid-gestation due to defects in cardiac development (Brannan et al. 1994; Jacks et al. 1994b). *Nf1* heterozygotes do not develop neurofibromas, which are the most prevalent consequence

of *NF1* loss in humans, however they do develop pheochromocytomas, which are derived from the neural crest (Jacks et al. 1994b).

Neurofibromatosis type 2 (NF-2) is also associated with benign tumors of the nervous system. Germline null alleles for the *Nf2* locus are embryonic lethal (McClatchey et al. 1997), while *Nf2* heterozygous mice develop a variety of malignant tumors (McClatchey et al. 1998), in contrast to the narrow spectrum of benign tumors observed in human NF2 patients. Thus, *NF* germline mutant mice represent another example in which the tumor spectrum in the mutant mice does not parallel that observed in humans.

***Ink4a* mutant mice — distinct tumor spectrum in germline mice versus humans may reflect a need for collaborating factors**

The *INK4a* tumor suppressor locus contains two transcripts, *p16INK4a* and *p19ARF* (for Alternative Reading Frame), both of which encode proteins that regulate cellular proliferation. *INK4a* mutant mice have been generated that delete the functions of both *p16* and *p19ARF* function (Serrano et al. 1996). The resulting homozygous null mice develop normally and are fertile, but the majority develop spontaneous tumors, mostly sarcomas and lymphomas, by approximately 30 weeks of age. However, *Ink4a* null mice do not develop melanomas, despite the fact that germline *INK4a* mutation predisposes humans to melanoma, another example in which the tumor spectrum of the germline allele does not parallel the human situation.

Furthermore, homozygous null mice deficient for *p19ARF* but expressing functional *p16INK4a* develop tumors, primarily fibrosarcomas and lymphomas, with limited penetrance (Kamijo et al. 1997). However, treatment of these mutant mice with the carcinogen dimethylbenzanthracene (DMBA) treatment resulted in skin tumors (Kamijo et al. 1997). These findings suggested that the absence of an expected tumor spectrum in germline models of tumor suppressors may reflect the need for collaborating factors, in this case a carcinogen.

***p27(Kip1)* mutant mice — haploinsufficiency of tumor suppressor function**

p27 is another cell cycle regulator that has been studied in germline mutant mice. *p27* mutant mice are large in size, due to hypercellularity of the pituitary, and they often develop pituitary tumors (Fero et al. 1996; Kiyokawa et al. 1996; Nakayama et al. 1996). Additionally, analyses of *p27* heterozygous mutant mice provided one of the first examples of haploinsufficiency of tumor suppressor function *in vivo*, since tumors developing in these mice do not show inactivation of the wild type allele of *p27* (Fero et al. 1998). Another notable early example of haploinsufficiency of tumor suppressor function in cancer is that of the *Nkx3.1* homeobox gene, whose haploinsufficient loss of function in germline mutant mice predisposes to prostate cancer (Bhatia-Gaur et al. 1999). Indeed following from these early studies, it is now widely accepted that inactivation of both alleles of a given tumor suppressors is not always necessary for tumorigenesis.

***Brca1/2* mutant mice — cancer phenotypes masked in the germline mouse alleles**

The tumor suppressor genes, *BRCA1* and *BRCA2*, are associated with an inherited predisposition to breast and ovarian cancer. Analyses of their germline loss in mouse models was complicated by the fact their homozygous loss of function results in early embryonic lethality in most cases, and by the fact that *Brca1* and *Brca2* heterozygous mice do not exhibit any cancer phenotype (Hakem et al. 1996; Liu et al. 1996; Connor et al. 1997; Suzuki et al. 1997). Thus, here is an example in which the tumor phenotype was not evident in the germline mutant mice, but in which conditional deletion of *Brca1/2* did result in relevant mammary tumor phenotypes (See Chapter XXX).

Conclusions

While germline mutant models for investigating the functional consequences of loss of tumor suppressor gene function have largely been supplanted by conditional targeting strategies, these early studies provided an essential foundation that set the stage for all of the subsequent more “sophisticated” models that are now widely utilized. First, the proof of principle concept that loss of tumor suppressor function can lead to cancer phenotypes was established by analyses of germline mutant mouse models. Second, these analyses revealed that many tumor suppressors are essential genes that function in important aspects of normal development and differentiation. Third, another important concept learned from these analyses was that the tumor spectrum resulting from loss of function in mice was not necessarily the same as observed in humans. Nevertheless, this does not necessarily reflect a lack of relevance of these models, but rather the presence of confounding factors, analyses of which have provided insight into the nature of tumor suppressor function. Finally, we learned from these early studies that the classic tumor suppressor model in which loss of both alleles is necessary for tumorigenesis is not always the case. These are among the important lessons learned from analyses of germline models that have paved the way for many future studies.

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Table 1

Lessons learned from analyses of germline mouse models of cancer

Tumor suppressor	Major lessons
<i>p53</i>	First example showing that loss of function of a tumor suppressor can result cancer phenotypes in mice, although the spectrum of tumors was not necessarily what one would predict from its role in human cancer
<i>Rb</i>	One of the first examples in which even if the homozygous null allele results in embryonic lethality, the heterozygotes may develop tumors, although they did not develop retinoblastoma (subsequently shown to be due to compensation)
<i>Pten</i>	An important example of a heterozygous mutant allele that displayed tumor phenotypes, although the wild type allele was not always lost
<i>p27</i>	One of the first examples of haploinsufficiency of tumor suppressor function <i>in vivo</i>
<i>APC</i>	A key example in which targeted deletion and mutagenesis results in similar cancer phenotypes
<i>Ink4a</i>	The occurrence of tumor phenotypes in the relevant tissue (skin) was evident only with treatment with carcinogen treatment
<i>Brca1/2</i>	An example in which the homozygotes are embryonic lethal but the heterozygotes do not develop cancer phenotypes