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Animal models of tumor immunity, immunotherapy and cancer vaccines

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Reliable animal models are critical for evaluating immunotherapies and for defining tumor immunology paradigms. Tumor immunologists are moving away from traditional transplantation tumor systems because they do not adequately model human malignancies. Transgenic mouse models in which tumors arise spontaneously have been developed for most cancers. The models use one of three technologies: tissue-specific promoters to drive expression of SV40 large T antigen or tissue-specific oncogenes; deletion of tumor suppressor genes by gene targeting; or, conditional deletion of tumor suppressor genes or activation of oncogenes via Cre-lox technology. Knockin mice expressing human tumor antigens and gene-targeted mice with deletions for immunologically relevant molecules have been integral to advancing knowledge of the tumor–host relationship. Although animal models are becoming more sophisticated, additional improvements are needed so that more realistic models can be developed.

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Abbreviations

Apc	adenomatosis polyposis coli
ARR_2	androgen-receptor-regulated promoter region
MMTV	mouse mammary tumor virus
Py	polyoma virus
Rb	retinoblastoma
T ag	SV40 large T antigen
t ag	SV40 small t antigen

Introduction

Animal models have played a critical role in establishing basic paradigms of tumor immunology because they provide an *in vivo* milieu that cannot be reproduced *in vitro*. As novel immunotherapies and cancer vaccines have been developed, animal models have also played an important role in pre-clinical testing for therapeutic efficacy. Historically, investigators have used transplantable tumor models, in which inbred animals are inoculated with tumor cells derived from the same genetic strain. The tumors were initially derived from spontaneously occurring malignancies or induced by chemicals or irradiation, and maintained either by *in vivo* or *in vitro* passage. As the tumor immunology field has moved towards developing cancer vaccines and other novel cancer immunotherapies, the same transplantable tumor models have been used to test therapeutic efficacy. Unfortunately, many of these tumor models are not good predictors for human clinical trials, as numerous therapies that look promising in experimental animals have turned out to be ineffective in patients. Although immunotherapy and cancer vaccine studies are moving away from using transplantable tumor models, they remain a mainstay for immunologists examining issues of basic tumor immunology. This review will briefly describe the pros and cons of transplantable tumor models and then focus on the recently developed transgenic mouse models in which tumors develop spontaneously. A brief overview of other mouse models that have been useful in defining basic principles of tumor immunology will also be discussed.

Transplantable tumor models

Although transplantable tumors have long been integral to tumor immunology research, they have several characteristics that limit their applicability to human disease and make them less than optimal for predicting immunotherapy efficacy in patients. First, most transplantable tumors were derived many years ago, and today's 'syngeneic' mouse strains may no longer be fully syngeneic with these tumors. In addition, some transplantable tumors have picked up endogenous viruses and express viral antigens not expressed by their mouse hosts. Therefore, many transplantable tumors may be partially histoincompatible with their 'syngeneic' mouse host and/or contain viral epitopes that make them significantly more immunogenic than naturally arising human tumors. Second, transplanted tumors are typically inoculated subcutaneously or intravenously and therefore do not grow in the anatomically appropriate site. As a result, the animal model does not mimic the organ-specific physiology characteristic of the tumor and the immune system is not exposed to the tumor in a manner comparable to that of naturally occurring malignancies in patients. Third, transplantable tumors generally progress very rapidly following inoculation, whereas spontaneous human tumors usually develop more slowly through a gradual series of cellular changes from pre-malignant to malignant pathologies. Therefore, the immune system of patients is slowly acclimated to tumors, whereas the immune system

of experimental animals with transplanted tumors is abruptly exposed. These kinetic variations may lead to different immunological outcomes, such as tolerance versus activation. Fourth, for patients with solid tumors, disseminated metastatic disease is frequently the predominant cause of death, and many cancer vaccines and immunotherapies are aimed at reducing and/or preventing metastasis. Most transplantable mouse tumors, however, are not spontaneously metastatic, so vaccine efficacy studies using these models are not particularly relevant for human metastatic disease.

Despite these obvious limitations, some transplantable tumors have distinct experimental advantages. For example, when inoculated in the mammary fat pad of syngeneic mice, the mouse 4T1 mammary carcinoma is spontaneously metastatic to the same sites as human mammary adenocarcinoma. If the primary tumor in the mammary gland is removed, then this transplantable tumor serves as an excellent model for the treatment of established, disseminated metastatic disease in a post-surgery setting [1–3].

Transplantable tumors have also been derived from spontaneous tumors that arise in genetically engineered mice. Because these recently derived tumors are syngeneic with their spontaneous tumor counterparts, they have been used in conjunction with the spontaneous models. For example, experiments with such transplantable tumors have demonstrated that older mice are significantly less responsive to cancer vaccines than younger mice [4•], and that combination immunotherapy consisting of passive administration of tumor-antigen-specific antibodies plus a cell-based vaccine provides more effective immunity than either therapy alone [5•].

Models for testing immunotherapy and cancer vaccines

In developing better animal models for both immunotherapy and cancer vaccine studies, investigators have tried to address the problems associated with transplantable tumors and to develop experimental systems that more closely mimic human malignancy. Efforts have been directed towards developing transgenic mouse models in which tumors develop spontaneously and progress through the known pre-malignant and malignant stages; defined human tumor antigens are expressed so that the host is tolerized to tumor-encoded molecules; and, the timing of tumor onset can be controlled so that tumors arise when the host has a mature immune system, as they do in humans.

SV40-driven transgenic models

Numerous transgenic mice have been generated by placing the transforming genes of the SV40 or polyoma virus early regions under the control of a tissue or cell-specific promoter. These mice spontaneously develop tumors in the targeted tissue. Table 1 includes some of these models and summarizes their characteristics by target organ. These models are useful because the mice develop organ-localized tumors, and, in some cases, also develop metastatic lesions. Most of these transgenic mice develop prostate cancer [6,7] or mammary carcinoma [8–10]; however, pancreatic [11,12], ovarian [13] and melanoma [14] models have also been reported.

The SV40 early region contains both large T and small t antigens (SV40 T ag and SV40 t ag, respectively). SV40 T ag inactivates the p53 and retinoblastoma (Rb) tumor-suppressor genes and the t ag activates cyclin Dp, which alters the mitogen-activated protein kinase (MAPK) and stress-activated protein kinase (SAPK) pathways. The original prostate cancer model, called the transgenic adenocarcinoma mouse prostate (TRAMP) mouse, was generated using the entire SV40 early region [7]. However, there has been concern that the multiple perturbations induced by the SV40 early region are not consistent with human prostate cancer, so another model called the 'LADY' mouse, containing only the T ag was developed [6].

A limitation of the SV40-driven prostate models is that the resulting tumors do not morphologically or phenotypically resemble human prostate tumors. For example, TRAMP mice develop seminal vesicle and stromal tumors, and LADY mice develop neuroendocrine tumors, whereas most human prostate cancers (adenocarcinoma) are of epithelial origin. In addition, tumor progression in many of the SV40 models is very rapid and therefore differs from development of human tumors, which typically progress more gradually. These characteristics have led some investigators to question the physiological relevance of SV40-driven transgenic models [15].

Organ-specific oncogene-driven transgenic models

Because of the desire to generate animal models in which the mechanism of tumor induction more closely parallels that of human disease, transgenic models using tissue or cell-specific promoters driving tumor-specific oncogenes have been developed. These models utilize a cell or tissue-specific promoter driving an oncogene that is thought to be causative of tumorigenesis. Table 1 includes some of these models and gives their characteristics. Most of these models involve oncogenes such as Her2/neu (ErbB2), which is driven by mammary tissue-specific promoters such as the Her2/neu endogenous promoter or mouse mammary tumor virus (MMTV) promoter [5•,16–21,22•,23]; however, prostate [24] and intestinal models [25] have also been reported. Several characteristics of these tumors demonstrate their similarity to human malignancies. Tumors in these models progress as they do in humans from pre-malignant lesions to invasive tumors and in some cases metastatic disease. Tumor progression in one of the Her2/neu models

Table 1

Selected transgenic mouse models of spontaneous malignancies.

Target organ	Model name	Promoter/ transgene	Genetic background	Percent mice with tumors	Metastasis	Comments	References
Breast	neuNT	MMTV/rat activated Her-2/neu	FVB	100%		Palpable mammary masses by ~13–14 weeks.	[44]
Breast	BALB/c neuT	MMTV/rat activated Her-2/neu	BALB/c	100%	Lung mets in older mice (~week 33)[a].	Mammary hyperplasia at ~8–13 weeks; DCIS at ~8–17 weeks; 1 palpable mass by ~20 weeks; 10 palpable masses by ~week 30.	[17]
Breast	neuN	MMTV/ unactivated Her-2/neu	FVB	~75%	Lung mets in ~72% of mice >8 months of age.	DCIS at ~37 weeks; 1 palpable mass by ~41–49 weeks; ~2.5 palpable masses thereafter; less disease than BALB/c-neuT mice; tolerant to neu.	[16,17]
Breast	MMT	(MMTV LTR/PyMT) × MUC1 Tg	C57BL/6	100%		Focal hyperplasia at ~4 weeks; palpable mammary tumors by day 65; 50% of mice have tumors by day 80–90; rapid progression.	[8]
Breast	MT	MMTV/PyMT	FVB	100%	Lung mets.	Multifocal mammary adenocarcinoma; rapid progression.	[9]
Breast	neuNT	(MMTV Cre) × loxP activated neu with endogenous promoter	BALB/c	100%		MMTV/Cre transgenics were bred with transgenics containing an inducible activated neu gene under its endogenous promoter; mammary tumors appear by ~8 months.	[18]
Prostate	TRAMP	Truncated rat probasin/SV40 T + t	C57BL/6 and FVB	100%	100% to lymph nodes and/or lungs; less common to kidney, adrenal gland, bone.	Prostate intraepithelial hyperplasia by 10 weeks; invasive neuroendocrine tumors by 20 weeks.	[7,45]
Prostate	LADY (12T-10)	Large probasin/ SV40 Tag	CD-1	100%	88% at 9 months; liver and lung most common; also to lymph nodes, bone.	Low grade prostatic intraepithelial neoplasia (PIN); invasive neuroendocrine tumors by 22 weeks; androgen receptor negative.	[6]
Prostate	$Pten^{-/-}$	Cre-lox conditional knockout	(C57BL/6 × DBA/2)F1 × (129/BALB/c)	100%	~50% with mets to lymph nodes, lungs.	Prostate hyperplasia at 4 weeks; PIN at 6 weeks; invasive prostate adenocarcinoma by 9 weeks; tumors are androgen receptor negative.	[29•]
Prostate	$Nkx3.1^{+/-}$ $Pten^{+/-}$	Double knockout	129/Sv × C57BL/6	84%	25% to lymph nodes after 1 year.	High grade PIN; invasive adenocarcinoma after 1 year; androgen independent; Pten is a tumor suppressor gene; Nkx3.1 is homeobox gene that is prostate-specific.	[30]
Prostate	Lo Myc or Hi Myc	Lo Myc: rat probasin/myc Hi Myc: ARR_2-probasin/myc	FVB	100%		PIN by 2–4 weeks; mice with high levels of myc expression develop invasive prostate adenocarcinoma by 3–6 months; mice with low levels of myc by 10–12 months.	[24]
GI/colorectal	Apc 1638	Truncated Apc gene	B6.129	90%		Colon polyps develop and progress to adenomas and colon carcinoma; 1–7 foci per mouse; mice are heterozygous for the truncated gene product.	[25]

Table 1 Continued

Target organ	Model name	Promoter/ transgene	Genetic background	Percent mice with tumors	Metastasis	Comments	References
GI/colorectal	CEA.Tg/ MIN	CEA.Tg × Apc mutated	C57BL/6	100%		Multifocal; tolerant to CEA.	[34,46]
Pancreas	MET	Rat elastase/ SV40 Tag 1-127 × MUC1.Tg	C57BL/6	50%		Pancreatic dysplasia at birth progressing to microadenomas and acinar cell carcinomas by week 9; by week 12 up to 9 tumor foci per mouse; the shortened SV40 Tag eliminates potential SV40 viral antigens.	[12]
Ovary	Tg MISIIR Tag	Mullerian inhibitory substance type IIR/SV40 Tag	B6C3F1	50%	Ascites	Poorly differentiated ovarian carcinoma.	[13]
Ovary	Ad	Cre-adenovirus/ $p53^{+}$Rb1 floxed recipients		97% by day 227 if both alleles are inactivated.	Ascites; mets to lung and liver.	Cre-adenovirus is inoculated intrabursally in the ovary; 5% of mice get tumors outside of the ovary.	[32•]
Melanocytes	Tyr-SV40E	Mouse tyrosinase/ SV40 T+t	C57BL/6	100%	61% of mice with eye tumors get mets.	Earliest melanomas are in the eye; skin melanomas are later and less frequent.	[14]

CEA, carcinoma embryonic antigen; DCIS, ductal carcinoma *in situ*; GI, gastrointestinal tract; mets, metastases; mo, month; PIN, prostate intraepithelial neoplasia. [a]Piero Musiani and Guido Forni, unpublished.

correlates with increasing tumor-induced immune suppression of the host, a situation that also occurs in patients with malignancies [26••]. Gene expression profiling of mammary tumors regulated by the endogenous Her2/ neu promoter shows similarities to human mammary carcinoma [27•].

Although these transgenic models have a high incidence of spontaneous cancer, and are therefore very useful experimentally, investigators have questioned the physiological relevance of those models in which the oncogene is driven by a strong viral promoter such as MMTV [18]. Another limitation of some of these models is that they simultaneously develop multiple primary tumors, unlike their human counterparts in which typically a single primary tumor arises.

Tumor-suppressor-gene knockout models

Many human malignancies are associated with mutations in tumor suppressor genes. Because such mutations are considered causative of malignancy, tumor-suppressor-gene targeted mice ('loss-of-function') have been developed, either with or without co-activation of oncogenes. Table 1 includes some of these transgenic models and gives their characteristics. The most commonly targeted tumor suppressor gene is p53, and these mice typically develop tumors in multiple tissues (e.g. lung, skin, intestine, brain, thymus, lymphocytes and connective tissue). Two prostate cancer transgenic models have also been developed based on loss-of-function of Pten, a tumor suppressor gene that also has anti-apoptotic activity [28,29•,30].

Cre-lox conditional expression models

Traditional knockin and knockout transgenic mouse technology has provided numerous models of spontaneous tumorigenesis; however, these models share a major limitation. Unlike human malignancies, which typically develop after birth, the targeted/transgenes in these mouse models are altered during embryonic development. Therefore, disease onset is much earlier than in humans, and the kinetics of tumor progression do not parallel those of human malignancies. To overcome this problem, mouse models are being developed based on the ability of the bacterial recombinase Cre to activate genes that are flanked by LoxP sites. Typically, one strain of mice will contain a tissue-specific promoter upstream of a floxed oncogene or inactivator of a tumor suppressor gene, and a second strain will contain the Cre recombinase regulated by an inducible promoter. When the two strains are interbred and the F1 mice are given the inducer, then the targeted gene is affected. Using this approach, tumor-inducing genes can be manipulated at any time during the life of the mouse [31].

In an adaptation of the Cre-lox approach, Flesken-Nikitin and colleagues [32•] have devised a novel method for inducing localized ovarian tumors. Instead of mating Cre and floxed mice, they inoculated the ovarian bursa of mice with floxed versions of the *p53* and *Rb1* tumor suppressor genes with adenovirus encoding the bacterial Cre recombinase. The resulting mice developed predominantly ovarian tumors that progressed and metastasized in a similar way to human ovarian carcinomas.

Table 2

Selected transgenic mice expressing human tumor antigens.

Model name	Promoter/tumor antigen	Genetic background	Comments	References
PSA1Tg	Endogenous human/PSA	BALB/c	PSA expressed on prostate ductal epithelium; immune response to immunization with PSA.	[33,47]
Muc1.Tg	Endogenous human/Muc1	C57BL/6	Muc1 tissue distribution similar to human Muc1; no immune response to MUC1-expressing tumor cells or MUC1 protein.	[36]
(CEA Ge)18FJP	Endogenous human/CEA	C57BL/6	CEA expressed in the cecum, colon, gastric foveolar cells, and on 20% of luminal epithelial cells; no circulating CEA; immune response to immunization with CEA.	[46]
hHer-2 Tg	Whey acidic protein/ErbB-2	B6C3 backcrossed to C57BL/6	ErbB-2 expressed constitutively in Bergman glia cells (brain) and in secretory mammary epithelia during pregnancy and lactation.	[48••]

CEA, carcinoma embryonic antigen; PSA, prostate-specific antigen.

Transgenic mice expressing human tumor antigens

Many human tumor antigens are expressed by non-malignant cells, so investigators developing cancer vaccines must study the immunogenicity and host responsiveness to endogenous molecules. Therefore, transgenic mice expressing human tumor antigens have been generated. Some of these models and their characteristics are listed in Table 2. Such models are particularly useful for human tumor antigens, such as prostate-specific antigen (PSA), for which there is no mouse counterpart [33]. In some cases, tumor antigen transgenic mice have been crossed to mice that contain oncogenes, resulting in mice that develop spontaneous tumors expressing relevant tumor antigens (e.g. carcinoma embryonic antigen [CEA]/adenomatous polyposis coli $[APC]^{+/-}$ mice; [34]). In some cases, the tumor antigen itself is an oncogene and causes spontaneous tumor formation. Examples are the neuT and neuN transgenic mice, although both of these models use a rat her-2/neu gene rather than a human gene [16,17,35]. These models have provided valuable information on the challenges of inducing anti-tumor immunity to self antigens for which the host has varying degrees of tolerance [5•,23,36–40].

Gene-targeted (knockout) mice

The availability of knockout mice has allowed investigators to identify many molecules that are pivotal in tumor immunity. Knockout mice have been used in at least two types of scenarios. First, they are inoculated with a transplantable tumor derived from the genetic background of the knockout, and the mice are followed for tumor progression. As most gene-targeted mice are on a C57BL/6 or BALB/c background, experiments are limited to transplantable tumors derived from these strains (for an example of this approach see [41] and [42]). In an alternative experimental design, mice that have increased tumor resistance have been bred with knockout mice and the resulting offspring intercrossed or

Table 3

Websites for animal models.

Website URL	Content
http://emice.nci.nih.gov/	Mouse Models of Human Cancer Consortium.
Http://cancermodels.nci.nih.gov/	These National Cancer Institute (NCI) sites include a database of mouse cancer models, relevant publications and a listing of mice available from the NCI. Models are listed by affected organ and there are minireviews for each organ.
Http://www.jax.org/ Http://jaxmice.jax.org/library/models/cancer.pdf	The Jackson Laboratory. This site provides information and availability on the many mouse models distributed and/or developed at The Jackson Laboratory — the world's largest private supplier of inbred strains of mice.
http://ccr.cancer.gov/tech_initiatives/animalmodels/default.asp	NCI-sponsored Animal Models Initiative. (Password needed to access this site).
Http://tbase.jax.org/	The Jackson Laboratory transgenic/targeted mutation database (searchable).
Http://bioscience.org/knockout/alphabet.htm	Alphabetical listing of gene-targeted mice.
Http://research.bmn.com/mkmd	Mouse knockout and mutation database.
http://immunology.tch.harvard.edu/knockouts	Mouse mutants with immunological phenotypes
http://www.mshri.on.ca/nagy/cre.htm	This page contains links to Cre recombinase and floxed gene databases.

backcrossed to obtained homozygous-deficient mice. By following the incidence and kinetics of tumor development, investigators have assessed the role of the deleted gene in tumor resistance (see [43] for an example of this strategy). Table 3 lists websites containing databases describing mice deficient for various immunologically relevant genes.

Conclusions

Although most investigators believe that animal models can provide useful pre-clinical information about novel immunotherapies and cancer vaccines, others have argued that animal studies are uninformative because they are not predictive of results with humans. If poor prognostic results from animal studies are due to inadequate models, then better models must be developed. As tumor immunologists select the models they use, they should ensure that they mimic as closely as possible the human cancer for which the therapy or vaccine is designed. Is tumor onset comparable to that in humans? Are tumor progression and staging similar? Is the pathology of the animal tumor similar to that of its human counterpart? Is the extent of tumor burden comparable? Is hormone responsiveness similar? If the therapy being tested is designed for the treatment of metastatic disease in a post-surgery setting, is the animal model appropriate? If the targeted patients have tumor-induced immune suppression, is the animal model comparably immune suppressed? If the targeted patients are immunocompromised because of age, does the animal model show a similar immune deficit? Consideration of these issues when selecting the appropriate animal model may yield pre-clinical results that more closely predict clinical outcomes.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Lelekakis M, Moseley J, Martin T, Hards D, Williams E, Ho P, Lowen D, Javni J, Miller F, Slavin J *et al.*: **A novel orthotopic model of breast cancer metastasis to bone**. *Clin Exp Metastasis* 1999, **17**:163-170.

2. Pulaski B, Ostrand-Rosenberg S: **Mouse 4T1 breast tumor model**. In *Current Protocols in Immunology*. Edited by Coligan J, Margulies D, Shevach E, Strober W, Kruisbeek A. John Wiley & Sons; 2000:20.22.21-20.22.11.

3. Pulaski B, Terman D, Khan S, Muller E, Ostrand-Rosenberg S: **Cooperativity of SEB superantigen, MHC class II, and CD80 in immunotherapy of advanced metastases in a clinically relevant post-operative breast cancer model**. *Cancer Res* 2000, **60**:2710-2715.

4. • Provinciali M, Smorlesi A, Donnini A, Bartozzi B, Amici A: **Low effectiveness of DNA vaccination against HER-2/neu in ageing**. *Vaccine* 2003, **21**:843-848.

This paper uses a transplantable tumor derived from a spontaneous tumor of BALB/c–neuT mice to demonstrate that aging dramatically effects the induction of cellular and humoral immunity following vaccination with a DNA vaccine. The paper points out that age of the recipient must be considered when selecting animal models for testing therapeutic efficacy.

5. • Wolpoe ME, Lutz ER, Ercolini AM, Murata S, Ivie SE, Garrett ES, Emens LA, Jaffee EM, Reilly RT: **HER-2/neu-specific monoclonal antibodies collaborate with HER-2/neu-targeted granulocyte macrophage colony-stimulating factor secreting whole cell vaccination to augment CD8+ T cell effector function and tumor-free survival in Her-2/neu-transgenic mice**. *J Immunol* 2003, **171**:2161-2169.

This paper describes a combination immunotherapy for the treatment of transplanted mammary tumors derived from neuN transgenic mice. The administration of a whole cell vaccine (3T3/neu/GM-CSF cells) plus her2/neu-specific antibodies stimulates heightened anti-tumor immunity that results in increased survival, demonstrating the improved therapeutic efficacy of therapies that combine humoral and cellular immunity. These studies are an example of how transplantable tumors derived from spontaneous tumors of transgenic mice are used as therapy models.

6. Masumori N, Thomas TZ, Chaurand P, Case T, Paul M, Kasper S, Caprioli RM, Tsukamoto T, Shappell SB, Matusik RJ: **A probasin-large T antigen transgenic mouse line develops prostate adenocarcinoma and neuroendocrine carcinoma with metastatic potential**. *Cancer Res* 2001, **61**:2239-2249.

7. Greenberg NM, DeMayo F, Finegold MJ, Medina D, Tilley WD, Aspinall JO, Cunha GR, Donjacour AA, Matusik RJ, Rosen JM: **Prostate cancer in a transgenic mouse**. *Proc Natl Acad Sci USA* 1995, **92**:3439-3443.

8. Xia J, Tanaka Y, Koido S, Liu C, Mukherjee P, Gendler SJ, Gong J: **Prevention of spontaneous breast carcinoma by prophylactic vaccination with dendritic/tumor fusion cells**. *J Immunol* 2003, **170**:1980-1986.

9. Guy CT, Cardiff RD, Muller WJ: **Induction of mammary tumors by expression of polyomavirus middle T oncogene: a transgenic mouse model for metastatic disease**. *Mol Cell Biol* 1992, **12**:954-961.

10. Chen D, Xia J, Tanaka Y, Chen H, Koido S, Wernet O, Mukherjee P, Gendler SJ, Kufe D, Gong J: **Immunotherapy of spontaneous mammary carcinoma with fusions of dendritic cells and mucin 1-positive carcinoma cells**. *Immunology* 2003, **109**:300-307.

11. Mukherjee P, Ginardi A, Madsen C, Tinder T, Jacobs F, Parker J, Agrawal B, Longenecker B, Gendler S: **MUC1-specific CTLs are non-functional within a pancreatic tumor microenvironment**. *Glycoconj J* 2001, **18**:931-942.

12. Mukherjee P, Ginardi AR, Madsen CS, Sterner CJ, Adriance MC, Tevethia MJ, Gendler SJ: **Mice with spontaneous pancreatic cancer naturally develop MUC-1-specific CTLs that eradicate tumors when adoptively transferred**. *J Immunol* 2000, **165**:3451-3460.

13. Connolly DC, Bao R, Nikitin AY, Stephens KC, Poole TW, Hua X, Harris SS, Vanderhyden BC, Hamilton TC: **Female mice chimeric for expression of the simian virus 40 TAg under control of the MISIIR promoter develop epithelial ovarian cancer**. *Cancer Res* 2003, **63**:1389-1397.

14. Bradl M, Klein-Szanto A, Porter S, Mintz B: **Malignant melanoma in transgenic mice**. *Proc Natl Acad Sci USA* 1991, **88**:164-168.

15. Powell WC, Cardiff RD, Cohen MB, Miller GJ, Roy-Burman P: **Mouse strains for prostate tumorigenesis based on genes altered in human prostate cancer**. *Curr Drug Targets* 2003, **4**:263-279.

16. Guy CT, Webster MA, Schaller M, Parsons TJ, Cardiff RD, Muller WJ: **Expression of the neu protooncogene in the mammary epithelium of transgenic mice induces metastatic disease**. *Proc Natl Acad Sci USA* 1992, **89**:10578-10582.

17. Boggio K, Nicoletti G, Di Carlo E, Cavallo F, Landuzzi L, Melani C, Giovarelli M, Rossi I, Nanni P, De Giovanni C *et al.*: **Interleukin 12-mediated prevention of spontaneous mammary adenocarcinomas in two lines of Her-2/neu transgenic mice**. *J Exp Med* 1998, **188**:589-596.

18. Andrechek ER, Hardy WR, Siegel PM, Rudnicki MA, Cardiff RD, Muller WJ: **Amplification of the neu/erbB-2 oncogene in a mouse model of mammary tumorigenesis**. *Proc Natl Acad Sci USA* 2000, **97**:3444-3449.

19. Nanni P, Nicoletti G, De Giovanni C, Landuzzi L, Di Carlo E, Cavallo F, Pupa SM, Rossi I, Colombo MP, Ricci C *et al.*: **Combined allogeneic tumor cell vaccination and systemic interleukin 12 prevents mammary carcinogenesis in HER-2/neu transgenic mice**. *J Exp Med* 2001, **194**:1195-1205.

20. Reilly RT, Machiels JP, Emens LA, Ercolini AM, Okoye FI, Lei RY, Weintraub D, Jaffee EM: **The collaboration of both humoral and cellular HER-2/neu-targeted immune responses is required for the complete eradication of HER-2/neu-expressing tumors**. *Cancer Res* 2001, **61**:880-883.

21. Lollini PL, Forni G: **Antitumor vaccines: is it possible to prevent a tumor?** *Cancer Immunol Immunother* 2002, **51**:409-416.

22. • Cappello P, Triebel F, Iezzi M, Caorsi C, Quaglino E, Lollini PL, Amici A, Di Carlo E, Musiani P, Giovarelli M *et al.*: **LAG-3 enables DNA vaccination to persistently prevent mammary carcinogenesis in HER-2/neu transgenic BALB/c mice**. *Cancer Res* 2003, **63**:2518-2525.

This paper describes a novel cancer vaccine consisting of lymphocyte-activating gene-3/CD223. The prophylactic and therapeutic treatment is highly effective in neuT mice, which die from massive multi-focal breast tumors if untreated.

23. Ercolini AM, Machiels JP, Chen YC, Slansky JE, Giedlen M, Reilly RT, Jaffee EM: **Identification and characterization of the immunodominant rat HER-2/neu MHC class I epitope presented by spontaneous mammary tumors from HER-2/neu-transgenic mice**. *J Immunol* 2003, **170**:4273-4280.

24. Ellwood-Yen K, Graeber TG, Wongvipat J, Iruela-Arispe ML, Zhang J, Matusik R, Thomas GV, Sawyers CL: **Myc-driven murine prostate cancer shares molecular features with human prostate tumors**. *Cancer Cell* 2003, **4**:223-238.

25. Fodde R, Edelmann W, Yang K, van Leeuwen C, Carlson C, Renault B, Breukel C, Alt E, Lipkin M, Khan PM *et al.*: **A targeted chain-termination mutation in the mouse Apc gene results in multiple intestinal tumors**. *Proc Natl Acad Sci USA* 1994, **91**:8969-8973.

26. •• Melani C, Chiodoni C, Forni G, Colombo MP: **Myeloid cell expansion elicited by the progression of spontaneous mammary carcinomas in c-erbB-2 transgenic BALB/c mice suppresses immune reactivity**. *Blood* 2003, **102**:2138-2145.

This paper demonstrates that spontaneous tumor development in transgenic mice that contain the transforming ErbB-2 gene under the control of the MMTV promoter (BALB/c-neuT mice) is directly correlated with the development of myeloid-induced immune suppression. As many patients with malignancies are also profoundly immune suppressed, this spontaneous tumor model is particularly appropriate for studying tumor-induced immune suppression.

27. • Andrechek ER, Laing MA, Girgis-Gabardo AA, Siegel PM, Cardiff RD, Muller WJ: **Gene expression profiling of neu-induced mammary tumors from transgenic mice reveals genetic and morphological similarities to ErbB2-expressing human breast cancers**. *Cancer Res* 2003, **63**:4920-4926.

This paper reports gene expression profiling of ErbB-2-expressing human mammary carcinomas and two mammary carcinomas from transgenic mice in which the her2/neu gene is regulated by its endogenous promoter or by the MMTV promoter. Although the transgenic tumors have similar histology, they are very different in time of onset and metastatic characteristics. Gene profiling results demonstrate that, although expression of some genes is restricted to each type of tumor, there is considerable similarity between the endogenously regulated transgenic tumor and ErbB-2 positive human mammary cancers.

28. Freeman DJ, Li AG, Wei G, Li HH, Kertesz N, Lesche R, Whale AD, Martinez-Diaz H, Rozengurt N, Cardiff RD *et al.*: **PTEN tumor suppressor regulates p53 protein levels and activity through phosphatase-dependent and -independent mechanisms**. *Cancer Cell* 2003, **3**:117-130.

29. • Wang S, Gao J, Lei Q, Rozengurt N, Pritchard C, Jiao J, Thomas GV, Li G, Roy-Burman P, Nelson PS *et al.*: **Prostate-specific deletion of the murine Pten tumor suppressor gene leads to metastatic prostate cancer**. *Cancer Cell* 2003, **4**:209-221.

This paper describes a Pten$^{-/-}$ knockout mouse that spontaneously and rapidly develops prostate cancer. Unlike the TRAMP and other prostate cancer models that develop neuroendocrine tumors, homozygous deletion of the Pten gene produces epithelial tumors which are the type of tumors seen in human prostate cancer.

30. Abate-Shen C, Banach-Petrosky WA, Sun X, Economides KD, Desai N, Gregg JP, Borowsky AD, Cardiff RD, Shen MM: **Nkx3.1; Pten mutant mice develop invasive prostate adenocarcinoma and lymph node metastases**. *Cancer Res* 2003, **63**:3886-3890.

31. Nagy A: **Cre recombinase: the universal reagent for genome tailoring**. *Genesis* 2000, **26**:99-109.

32. • Flesken-Nikitin A, Choi KC, Eng JP, Shmidt EN, Nikitin AY: **Induction of carcinogenesis by concurrent inactivation of p53 and Rb1 in the mouse ovarian surface epithelium**. *Cancer Res* 2003, **63**:3459-3463.

This paper describes a novel approach for initiating ovarian cancer development by inoculating the ovarian bursa of p53 and Rb1 floxed mice with a recombinant adenovirus containing the bacterial recombinase Cre. A high percentage of mice with homozygous inactivation of p53 and Rb1 develop progressive and metastatic ovarian tumors.

33. Wei C, Willis RA, Tilton BR, Looney RJ, Lord EM, Barth RK, Frelinger JG: **Tissue-specific expression of the human prostate-specific antigen gene in transgenic mice: implications for tolerance and immunotherapy**. *Proc Natl Acad Sci USA* 1997, **94**:6369-6374.

34. Greiner JW, Zeytin H, Anver MR, Schlom J: **Vaccine-based therapy directed against carcinoembryonic antigen demonstrates antitumor activity on spontaneous intestinal tumors in the absence of autoimmunity**. *Cancer Res* 2002, **62**:6944-6951.

35. Guy CT, Cardiff RD, Muller WJ: **Activated neu induces rapid tumor progression**. *J Biol Chem* 1996, **271**:7673-7678.

36. Rowse GJ, Tempero RM, VanLith ML, Hollingsworth MA, Gendler SJ: **Tolerance and immunity to MUC1 in a human MUC1 transgenic murine model**. *Cancer Res* 1998, **58**:315-321.

37. Reilly RT, Gottlieb MB, Ercolini AM, Machiels JP, Kane CE, Okoye FI, Muller WJ, Dixon KH, Jaffee EM: **HER-2/neu is a tumor rejection target in tolerized HER-2/neu transgenic mice**. *Cancer Res* 2000, **60**:3569-3576.

38. Rovero S, Amici A, Carlo ED, Bei R, Nanni P, Quaglino E, Porcedda P, Boggio K, Smorlesi A, Lollini PL *et al.*: **DNA vaccination against rat her-2/Neu p185 more effectively inhibits carcinogenesis than transplantable carcinomas in transgenic BALB/c mice**. *J Immunol* 2000, **165**:5133-5142.

39. Soares MM, Mehta V, Finn OJ: **Three different vaccines based on the 140-amino acid MUC1 peptide with seven tandemly repeated tumor-specific epitopes elicit distinct immune effector mechanisms in wild-type versus MUC1-transgenic mice with different potential for tumor rejection**. *J Immunol* 2001, **166**:6555-6563.

40. Pupa SM, Invernizzi AM, Forti S, Di Carlo E, Musiani P, Nanni P, Lollini PL, Meazza R, Ferrini S, Menard S: **Prevention of spontaneous neu-expressing mammary tumor development in mice transgenic for rat proto-neu by DNA vaccination**. *Gene Ther* 2001, **8**:75-79.

41. Smyth MJ, Thia KY, Street SE, MacGregor D, Godfrey DI, Trapani JA: **Perforin-mediated cytotoxicity is critical for surveillance of spontaneous lymphoma**. *J Exp Med* 2000, **192**:755-760.

42. Pulaski BA, Smyth MJ, Ostrand-Rosenberg S: **Interferon-gamma-dependent phagocytic cells are a critical component of innate immunity against metastatic mammary carcinoma**. *Cancer Res* 2002, **62**:4406-4412.

43. Ostrand-Rosenberg S, Sinha P, Clements V, Dissanayake S, Miller S, Davis C, Danna E: **Signal transducer and activator of transcirption 6 (Stat6) and CD1: inhibitors of immunosurveillance against primary tumors and metastatic disease**. *Cancer Immunol Immunother* 2004, **53**:86-91.

44. Muller WJ, Sinn E, Pattengale PK, Wallace R, Leder P: **Single-step induction of mammary adenocarcinoma in transgenic mice bearing the activated c-neu oncogene**. *Cell* 1988, **54**:105-115.

45. Gingrich JR, Barrios RJ, Morton RA, Boyce BF, DeMayo FJ, Finegold MJ, Angelopoulou R, Rosen JM, Greenberg NM: **Metastatic prostate cancer in a transgenic mouse**. *Cancer Res* 1996, **56**:4096-4102.

46. Clarke P, Mann J, Simpson JF, Rickard-Dickson K, Primus FJ: **Mice transgenic for human carcinoembryonic antigen as a model for immunotherapy**. *Cancer Res* 1998, **58**:1469-1477.

47. Wei C, Callahan BP, Turner MJ, Willis RA, Lord EM, Barth RK, Frelinger JG: **Regulation of human prostate-specific antigen gene expression in transgenic mice: evidence for an enhancer between the PSA and human glandular kallikrein-1 genes**. *Int J Mol Med* 1998, **2**:487-496.

48. Piechocki M, Ho Y, Pilon S, Wei W: **Human ErbB-2 (Her-2)**
•• **transgenic mice: a model system for testing Her-2 based vaccines**. *J Immunol* 2003, **171**:5787-5794.
This paper describes the generation of a transgenic mouse model in which the human ErbB-2 gene is expressed under the control of the whey acidic promoter. This model will be very useful for studying the immunogenicity of mammary cancer vaccines because, unlike other her2/neu transgenic models, it contains the human ErbB-2 gene, which is only overexpressed in the mammary gland during pregnancy and lactation, similar to her2/neu expression in humans.