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REVIEW

Modeling metastasis *in vivo*

Chand Khanna[1,3] and Kent Hunter[2,3]

[1]Comparative Oncology Program and [2]Laboratory of Population Genetics, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, Maryland, USA

[3]To whom correspondence should be addressed
Email: khannac@mail.nih.gov or hunterk@mail.nih.gov

Metastasis, the spread of a tumor from its primary site to other parts of the body, continues to be the most significant problem in the field of cancer. Patients who present with metastatic disease or those who develop metastases after successful management of the primary tumor carry a universally grave prognosis. To improve treatment outcomes for these patients a broader understanding of the biology of metastases is necessary. The biological complexity that characterizes metastasis requires complex experimental systems for its study. To a large extent the modeling of this biological complexity is only possible using animal models. The following review will summarize the strengths and weaknesses of available *in vivo* models of metastasis including transplantable syngeneic mouse and human–mouse xenografts, genetically engineered mice and naturally occurring cancers of companion animals (pet dogs and cats). No single metastasis model is sufficient to answer all questions. As such, the selection of the optimal model(s) for each biological or translational question is necessary.

Introduction

Based on the work of several groups it has long been understood that metastatic cancer cells are the 'decathelete' cells of the tumor (1–3). They are able to leave the site of the primary tumor and pass through the tumor basement membrane, either through or between endothelial cells to enter the circulation (intravasation) (4). While in the circulation tumor cells are able to resist anoikis (programmed cell death associated with loss of cellular contact) (5), evade immune recognition and physical stress and eventually arrest at distant organs (6–8). At this distant site the cell either leaves the circulation or proliferates within the vessel, survives in the novel microenvironment of a foreign tissue site (9), proliferates, creates new blood vessels (angiogenesis), co-opts existing blood vessels or grows within an existing vessel and then successfully grows into a clinically relevant metastatic lesion (10,11). It is generally believed that metastases can settle within the same organ or inhabit distinct tertiary organs; as such the steps outlined above continue even after the successful management of the primary tumor. A more detailed understanding of each of the steps associated with the metastatic process has emerged from the recent interest and investment in a diversity of disciplines not previously active in this area of cancer biology. The application of mathematic models, bioinformatics, genomic screening, physics and physical chemistry has provided the field with unique 'systems' perspectives and investigative tools (12–15). The validation of these perspectives and the use of novel tools within the field of metastasis biology have and will require the appropriate use of *in vivo* models. In the field of cancer, there is a tendency to characterize the value of a model in terms of the similarities shared between the animal and the human cancer that is being modeled. This approach leads to value assignments and an attempt to define the best model of a cancer. It is unlikely, however, that the complexity of cancer in human patients can be entirely modeled by one system alone. By understanding the strengths and weaknesses of a set of models, it becomes possible to choose the appropriate model(s) for study of individual problems or questions. This is true in the use of animal models in pre-clinical drug development and for studying a complex problem like cancer metastasis. The following review will summarize currently available *in vivo* models of metastasis, define the limitations and advantages of each modeling option and suggest the basis with which particular models should be used to answer questions relating to metastasis biology and ultimately, therapeutic interventions. These *in vivo* models include transplantable cancer models commonly characterized in rodents, genetically engineered mice that develop metastatic cancers and cancers that naturally develop in outbred large animals, primarily pet dogs (often referred to as comparative models).

Abbreviations: GEM, genetically engineered mice.

Transplantable cancers that metastasize in small animals

A foundation of cancer research over the last 30 years has been the use of cancer cell lines or tissues that can be grown in mice or rats. Such transplantable models can be divided into two broad groups, syngenic models or xenograft models. Syngeneic transplantable models most often refer to mouse or rat (murine) cancer cell lines or tissues that result in tumors in inbred animals of the same genetic background as the derived cell line or tissue. Until recently syngeneic cell lines have either been derived from carcinogen-induced tumors or tumors that spontaneously develop in a particular mouse or rat (16–18). The advantage of syngeneic models is that the transplanted tissues, the tumor microenvironment, and the host are from the same species. This is particularly important when considering the close interaction between tumor and host characterized by the process of metastasis. However, these model systems lack many of the important features of human tumors. For example, they usually are derived from homozygously inbred mice and therefore lack the genetic complexity of human tumors. In addition, due to species-specific differences in oncogenesis, for example differences in carcinogenic xenobiotic metabolism, they may not bear the same constellation of mutations observed in human patients (19). Thus, care must be

taken to validate observations and conclusions drawn from these models and to confirm their relevance to human cancers.

Human–mouse xenograft models define the other major category of transplantable cancer models used to study metastasis. Such xenograft models refer to human cancer cell lines or tissues that can be transplanted into immunocompromised animals and effectively grow tumors. The resultant tumors that emerge from xenograft transplantations are a mosaic of human cancer cells and murine stromal cells. Several lines of evidence suggest the importance of cancer cell–stromal cell interactions in the biology of cancer progression and metastasis. For some pathways, species specificity does not allow this interaction to occur across species boundaries. The impact of this more limited tumor–stromal interaction must be considered in the use of xenograft models (20–22). Furthermore, in order for human tumors to grow in mice the murine host must be immunocompromised to prevent immune rejection. This eliminates the ability to examine the role of the immune system in tumor progression in xenografts. A number of immunocompromised murine hosts are used in the development of xenografts, including nude mice, SCID (severe combined immunodeficiency) and mice strains with more significant immunosuppression (i.e. SCID–Beige mice, where the beige mutation eliminates NK cell function) (23). In addition to immunosuppression, there are specific features of each immunocompromised mouse strain that can influence the biology and study of metastasis (24,25). Nude mice that have marked depletion of T cells and impaired T cell and B cell function, have been described as having impaired angiogenesis (26); SCID mice have combined deficits in number and function of both B and T cells; however, a high resting NK cell activity and an age-associated 'leakiness' in the SCID mutation that can lead to immune-mediated elimination of metastatic cells, especially in longer term metastasis models. Finally, there have been reports that significant differences exist for angiogenesis between transplanted and autochthonous tumors, which may reduce the predictive power of xenografts to clinical tumors (27,28). These characteristics of the mouse host need to be kept firmly in mind when interpreting data and attempting to make clinical correlations and may potentially be important factors that have reduced the predictive power of xenograft models in pre-clinical testing (29).

Transplantation model approaches

The use of transplantation models, either syngeneic or xenogeneic, can include one of two (or both) experimental approaches. These approaches, or assays, are referred to as experimental metastasis assays and spontaneous metastasis assays. These assays differ in the way that cells are delivered to the recipient animals. The types of questions that can be asked of each assay are distinct and in many cases complimentary.

Experimental metastases models

The most widespread use of transplantable cancers as models of metastasis is based on the strategy of experimental metastasis. Experimental metastasis refers to the injection of tumor cells directly to the systemic circulation. Depending on the site of injection and tropism of the tumor cell, distant metastases may (or may not) develop at a number of anatomic locations throughout the body. The site of injection largely defines the site to which metastases develop in these experimental systems. The most common site of tumor cell injection employed for experimental metastasis models is the lateral tail vein in mice. Tail vein injection results primarily in pulmonary metastases. In contrast, intrasplenic or portal vein injection of tumor cells is the most common site employed for developing metastasis in the liver. Intracardiac injection of cells may result in metastases to several sites, including bone. The influence of injection site on end-organ target is in part explained by the first capillary bed that tumor cells face following injection of cells. This first capillary bed principle readily explains the development of lung metastases following lateral tail vein injection and the development of hepatic metastasis following portal vein injection; however, the development of bone metastasis following introduction of cells to the arterial circulation (intracardiac injection) requires additional consideration of tumor–host interactions. These tumor–host determinants have been described in the seed-and-soil hypothesis, first described by Paget. This hypothesis suggests that the eventual outgrowth of a tumor, in this case at a metastatic site, is defined by determinants of the tumor cell (seed) and the ability of the tumor cell to receive appropriate growth and survival signals from its microenvironment (soil) (3). Support for the seed-and-soil effect is provided by several experimental metastasis models. Following intracardiac (left ventricle) injection of tumor cells, single tumor cells or emboli can be found in most organs in the body; however, for many breast and prostate cancer cell systems, successful metastases are found in the bone (30). More support follows from cell lines that metastasize to non-lung sites following tail vein injection, even though the lung is the first and largest capillary bed seen by tumor cells during metastasis (31–34).

An important result of experimental metastasis models employing direct injection of cells into the circulation has been the development of clonally related variants that differ in metastatic potential. Based in the hypothesis of tumor heterogeneity, Fidler *et al*. developed cell lines from the same parent tumor that were characterized by progressively higher metastatic potential through successive collection of metastases from the lung and re-injection in the tail vein (35,36). The best characterized of these models is the B16 melanoma model. The B16F1 parental cells are capable of forming experimental metastases at a rate of ~1.3×10^{-5} per cell per generation while B16F10 cells, generated by successive tail vein metastasis had an effective metastasis rate of 5×10^{-5} per cell per generation (37,38). Comparative investigations of paired cell line clones that differ in metastatic potential have been particularly helpful in defining both metastasis-associated and metastasis-suppressing genes in several cancer histologies. Table I lists pairs of clonally related cells that differ in experimental metastatic potential.

Experimental metastasis models provide several advantages for investigation. The time course for model maturity is generally rapid, the biology of metastasis is reproducible and consistent, and the user has control of the number and type of cells that are introduced to the circulation. This control of 'input' has been used by Chambers *et al*. to define important features of the steps involved in the cascade of metastasis and the process of cellular extravasation and survival at distant metastatic sites (39). However, the fact that the early steps in the metastatic cascade are eliminated through experimental metastasis modeling is a potential disadvantage. It is believed that many tumor cells metastasize within emboli consisting of tumor cells that break free from a primary tumor and become

Table I. Examples of murine cancer cell lines useful in transplantable metastasis assays

Histology	Name	Syngeneic strain	Experimental metastasis	Spontaneous metastasis	Variants with distinct metastasis biology	Select references
Mammary	4T1/4T07	BALB/c	Yes: TV	Yes: Orth - MFP	Yes: 4T1; 4T07; 67NR	80
Mammary	D20R/D2A1		Yes: TV			81
Mammary	Pei-1 Cmyc/VEGF bitransgenic	FVB	Yes: TV	Yes: Orth - MFP, SQ		
Mammary	EMT-6	BALB/c	Yes: TV		Yes: EMT-6J, EMT-6H	82
Mammary	LM3	BALB/c	Yes: TV	Yes: Orth - MFP		83
Mammary	M6C	FVB		Yes: SQ	Yes M6; M28	84
Melanoma	B16	C57BL/6	Yes: TV	Yes: Orth - FP, ear	Yes: B16F10, B16bl6, others	37,85
Osteosarcoma	K7M2	BALB/c	Yes: TV	Yes: Orth - Limb	Yes: K12, K7, K8, others	48
Rhabdomyosarcoma	INK4a KO/HGF TG	Mixed	Yes: TV	Yes: Orth - Limb	Yes	86
Colon	CT26	C57BL/6	Yes	Yes, Orth - caecum		87,88
Colon	MCA38	C57BL/6	Yes			89
Prostate	Pr14C	FVB	Yes	Yes	Yes: Pr111, Pr117, Pr14	90
Lung	Lewis Lung	BALB/c	Yes: TV	Yes: Orth - lung	Yes	91–93
Kidney	RENCA	BALB/c	Yes: TV	Yes: Orth - renal	No	94
Lung	TC-1	C57BL/6	Yes: TV	No	No	95
Hepatic	Hca/163-F(F)	C3H		Yes: SQ	Hca/A2-P(P) and Hca/163-F(F)	96

TV, tail vein; s.c., subcutaneous; Orth, Orthotopic; MFP, mammary fat pat; FP, foot pad.

associated with platelets and other host cells before arresting at distant sites. Following tail vein injection, cells circulate, often in high numbers, as single cells or small clusters of platelets. These single cells may not arrest or interact with target tissues at distant sites in the same ways as tumor cells that spontaneously metastasize (as emboli). Furthermore, the process of spontaneous metastasis from a primary site may be associated with selection events that yield a distinct profile of successful metastasis. As a result, experimental metastases have often been described as multiple primary tumors developing in the lung. Yamamoto *et al*. demonstrated differences in the expression of matrix metalloproteinase enzymes in the metastasis that result from experimental metastases and those that develop from spontaneous metastases (40). Related to concern of the relevance of experimental metastasis is the fact that many experimental metastasis models have been selected for metastatic propensity. It is important to also consider features of metastatic biology that are selected against through this same process. These critical features include metastatic dormancy and metastatic inefficiency. Furthermore, the compressed time course of metastasis seen in these experimental metastasis models often precludes their use in defining agents active against established metastatic cancers.

Spontaneous metastasis

Historically, transplantable tumor models were characterized by and selected for rapid primary tumor growth at subcutaneous (s.c.) (heterotopic) sites. In this setting, it was uncommon to observe spontaneous metastasis to distant sites. As such transplantable models were often labeled as 'non-metastatic'. Application of the 'seed and soil' hypothesis to transplantation modeling resulted in the use of orthotopic transplantation of tumor cells into mice (41–47). Orthotopic transplantation refers to the delivery of cancer cells to the anatomic location or tissue from which a tumor was derived. The use of orthotopic injection transplantation has resulted in tumor models that may more closely resemble human cancers including tumor histology, vascularity, gene expression, responsiveness to chemotherapy and metastatic biology (47,48). As more has been learned about the importance of host–microenvironment interactions it is understandable why orthotopic tumors are preferred over more conventional flank (s.c.) models. That orthotopic models are more frequently associated with metastasis than s.c. tumor injection of cells, lends support to the value of providing more relevant host–tumor interactions. Similarly, the fact that spontaneous metastases arise from a primary transplanted tumor provides an opportunity to study the metastatic process and many aspects of the metastatic cascade that are bypassed using experimental metastasis models. Orthotopic transplantation of cancer cells may come from direct injection of tumor cells or the surgical implantation of intact fragments of tumor. For many orthotopic models the use of surgical implantation of fragments improves the reproducibility and metastatic outcome within the model (46,48). For those orthotopic models where primary tumor growth is rapid, the use of surgery is often necessary to control morbidity associated with excess primary tumor burden (47,48). In some cases, it may be argued that the removal of the primary tumor contributes to the metastatic phenotype of the model; however, primary tumor removal has been shown to both enhance and suppress metastasis depending on the model studied. Examples of commonly used transplantable tumor models characterized by spontaneous metastasis are presented in Table I. Examples include spontaneous metastasis from the favored orthotopic and heterotopic implantation sites.

Genetically engineered mouse models of metastasis

While *in vitro* and *in vivo* experimental or 'spontaneous' transplantable models have yielded many important insights into the potential molecular mechanisms of metastasis, a number of important caveats remain. Introduction of cells into the circulatory system bypasses a number of important events thought to be major roadblocks in metastatic dissemination, including escape from the primary tumor, invasion into adjacent tissue and extravasation into the hematopoetic system. Ectopic or orthotopic implantation, while potentially reintroducing a more natural setting for the process, still suffers from potential problems. The past decades have revealed that tumorigenesis is not simply the result of proliferative activation of a mutated cell, but rather is a complex interaction between neoplastic tissue and the stromal and organismal environment in which it arises (49). Implantation models do

not necessarily recapitulate all of the interactions and microenvironmental components that may play important roles in tumor dissemination. In addition, mechanical disruption of the target tissue during the implantation process may permit escape of tumor cells into the circulatory system at the time of implantation, thus seeding distant sites at the onset of the experiment rather than subsequent to tumor growth, as is the intent. Since the work of Folkman *et al*. has shown that transplanted tumors can suppress the growth of secondary tumors via diffusible factors (50), it is possible that ectopic or orthotopic implantation, through suppression of secondary lesions, may not always permit the analysis of the earliest phases of the metastatic process.

A further common caveat of both systems is their reliance on cultured cells. Since the pioneering work of Fidler, most metastatic cell lines and variants have been generated by serial passage and selection (36,51). During the selection process and/or subsequent cell culture, the resulting cell lines and clones have been passaged *in vitro*. Thus, the cells used in experimental or spontaneous metastases assays have been adapted to growth on a two-dimensional rather than a normal three-dimensional matrix platform, in an artificial or foreign milieu. The adaptations that permit perpetual growth in tissue culture may have significant impact on the pathways and mechanisms by which autochthonously arising metastases subsist. While these experimental systems may imply some of the mechanisms and biological processes that function in tumor dissemination, they only partially represent those steps in the metastatic process that can be successfully targeted for intervention.

To complement experimental and spontaneous metastasis models, investigators need access to autochthonously arising tumors capable of completing the entire metastatic process. Ideally this would consist of naturally occurring or chemically induced tumors in animal models, more truly replicating the normal initiation and progression observed in humans. Unfortunately the majority of the spontaneously arising tumors, at least in mouse models, do not metastasize, metastasize with a very long latency or are characterized by intravascular metastases alone, precluding easy and efficient analysis. Fortunately a number of genetically engineered models have been developed that produce bona fide metastatic disease (see Table II). These encompass a variety of tissue types, with differing degrees of penetrance and different latencies. While it is possible that the constitutive activation or loss of a gene in these models may not completely replicate native metastasis, the tumors do arise in their normal context, more closely replicating the clinical setting and in a system possessing a functional immune system.

Genetically engineered animal models of metastasis also permit the investigation of important determinants of cancer that are difficult or impossible to address using cell culture and transplant systems including the influence of genetic heterogeneity on tumor phenotype. Genetic heterogeneity is known to have a profound impact on the expression of oncogenic mutations. For example, in spite of mutations in the tumor suppressor gene BRCA1, only 50–60% of carriers in the Ashkenazi population develop breast cancer, presumably due to the influence of polymorphic modifier genes segregating in the population (52). Genetic polymorphisms can have a profound impact on phenotypes for every trait tested to date, even rescuing prenatal lethal mutations (53,54). Thus, it should come as no surprise that the efficiency of metastasis might also be significantly influenced by genetic background. Introduction and evaluation of genomic polymorphism, however, is not feasible using cell culture-based systems. It requires the meiotic segregation that animal models of metastasis provide to investigate.

That genomic polymorphism plays a major role in metastasis has been demonstrated using one of these genetically engineered models. Using the polyoma middle-T mammary tumor model and a breeding scheme, metastatic efficiency was shown to vary significantly according to the strain combination (55). Since all of the metastatic tumors were induced by the same primary neoplastic event, expression of the transgene, the most likely explanation is the influence of the genetic background. This finding has several important implications. First, it suggests that individuals might be predisposed to efficient metastatic involvement before tumor induction, based on their polymorphic profile. In addition, it suggests that there may be high-risk metastasis-families in the human population, analogous to the families at risk for various tumor syndromes (56). Most importantly, if a significant portion of their metastasis risk is based on polymorphic profile, rather than on tumor initiating mutations or subsequent metastasis-promoting events, then it should be feasible to identify those individuals at high risk for disseminated disease at the time of, or preceding, primary tumor diagnosis. This would enable clinicians to identify those patients who would most benefit from aggressive neo-adjuvant therapy, reducing subsequent morbidity and mortality, and sparing low-risk patients from unnecessary treatment.

Recently, microarray technology has been used to identify metastatic predictive gene expression signatures in tumor tissue (57,58). If the metastasis-modifying role of genomic polymorphism is carried to its logical conclusion, theoretically it should be possible to obtain a predictive signature from more easily obtained normal tissues (e.g. peripheral blood or buccal cavity swab). Using microarray technology and meiotically segregating metastasis animal models it has recently been demonstrated that at least some high and low metastatic genotypes can be distinguished based on expression of a small set of signature genes, using two normal tissue types (K.Hunter, L.Lukes and M.Lancaster, submitted for publication). Whether this holds true in human populations remains to be seen.

Thus, while genetically engineered models of metastastic involvement represent only a fraction of the tumor types requiring investigation, they provide a valuable conduit into important metastasis-determining factors that cannot be easily accessed in less expensive, more reproducible transplant-based systems. A significant disadvantage of these systems, however, is the expense. While transgenic tumors metastasize at a higher frequency than spontaneously arising or chemically induced tumors, the latency for metastases for most is still measured in months. In addition, the relative penetrance for metastatic disease is often significantly lower than that of tumor incidence. As a result large numbers of animals are often required to be held for long periods of time in order to generate enough population-based data for analysis. In addition, due to the variability in tumor dissemination and penetrance of metastatic disease it is difficult if not impossible to stage animals or detect the presence of metastatic disease before death. While recently developed imaging technologies may help alleviate some of these problems they are expensive and labor intensive.

Table II. Examples of genetically engineered animal tumor models with metastatic progression

Rat

Transgene	Tumor type	% Animals with metastases	Latency	Site of metastasis	Reference
Probascin-Tag	Neuroblastoma	64	20 weeks	Lymph node Spinal cord Lung	97

Mouse

Transgene	Tumor type	Tumor incidence	% Animals with metastases	Latency	Site of metastasis	Reference
S100A4/MMTV-neu	Mammary		50%	12 months	Lung	98
Antithrombin III_Tag	Liver		Reported	8 months	Lung	99
Alpha-Amylase-Tag	Brown adipose		Reported	>12 months	Liver Lung Spleen Heart Adrenal	100
Cryptdin2-Tag	Prostate		40	6 months	Lymph node Lung Liver Bone	101
Ck19-Tag	Bladder		20	3 months	Lung	102
T7-Pkc	Squamous cell carcinoma	40%	50	6 months	Lymph node	103
Mt-Met	Mammary		Reported	10 months	Lung Lymph node Kidney Heart Cecum	104
MMTV-Wnt1	Mammary	50%	>50%	6 months	Lymph node Lung	105
Nf-2 KO	Various	50%		22.4 months	Lung Liver Spleen	106
Rip-VEGF-C/RipTag	Pancreatic	100%	37%	12–15 weeks	Lymph node	107
C3(1)Tag	Mammary	100%	10%	7 months	Lung	108
C3(1)Tag FVB/129 hybrid	Mammary	100%	62%	7 months	Lung	108
MMTV-neu	Mammary	100%	72%	3 months	Lung	109
Wap-ras	Salivary/Mammary	100%	14%	6 months	Lung	110
MT-HGF/SF	Melanoma	22%	21%	15 months	Liver Spleen Skin	111
H19-Igf2	Mammary	50–100%	38%	>9 months	Spleen Lung Liver	112
Probascin-Tag	Prostate		66–88%	6–9 months	Lymph nodes Lung Liver	113
MMTV-PyMT	Mammary	100%	>85%	3 months	Lung	114
MTB/TAN	Mammary	100%	92%		Lung	115
Kras/Ink4aKO	Pancreas	100%		7–11 weeks	Liver Lymph node	116

A second potential obstacle to the use of genetically engineered mice (GEM), particularly in drug development relates to patent law. These patent concerns focus on OncoMouse technology, which was issued to Harvard University and exclusively licensed for most purposes to DuPont Inc (US Patents 4,736,866, 5,087,571 and 5,925,803). These patents claim rights in: (i) mice or reagents developed from any non-human mammal containing any activated oncogene sequence; (ii) cell lines isolated from the above mouse model; and (iii) the use of such a mouse model for drug testing. DuPont has executed agreements with some academic institutions, the United States Public Health Service and pharmaceutical and biotechnology companies (for reference: http://ott.od.nih.gov/textonly/oncomous.htm). The potentially restrictive effect of these patents on the use of GEM has been discussed extensively in the scientific and lay press (59,60). The limited use of GEM by the pharmaceutical and biotechnology industry may reflect DuPont exercising its rights under these patents, which may include claims for 'reach through' rights to discovered therapeutic agents.

Imaging metastasis in the mouse

Several novel imaging strategies including bioluminescence, magnetic resonance imaging or positron enhanced tomography scan have been developed for use in mice (61–64). Imaging studies for metastasis have included the use of bioluminescence and fluorescent imaging techniques to evaluate the fate of single metastatic cells within the mouse. These single-cell

imaging studies have shed new light on the biology of metastasis and the interactions of metastatic cells with their microenvironment early in the process of metastasis (9). The option to detect metastatic cell clusters and gross metastases within the whole animal, using imaging, has improved our understanding of organ-specific targeting of metastases and has facilitated the inclusion of metastasis endpoints in the pre-clinical development of new drugs (65,66). In general, transplantation models of cancer offer greater flexibility for imaging strategies. Tumor cells may be transfected to express targets for imaging (e.g. green fluorescent protein, luciferase) or may be labeled immediately before delivery to mice (e.g. quantum dots, CMFDA) (9,67,68). GEM that have imaging constructs included in their design are increasingly available. The expression of 'foreign proteins' in mouse cells (e.g. green fluorescent protein, luciferase) has been shown to be immunogenic in some syngeneic mouse models and can influence the biology of cancer models by both immune and non-immune mechanisms (69). As such, validation of a model's metastatic biology is necessary following labeling modifications.

Comparative models of metastasis

A significant and at present under utilized group of cancer models are the naturally occurring cancers seen in companion animals (pet dogs and cats) (70–72). The significant anatomic and physiologic similarities that exist between dogs and humans have been the basis for their use in biomedical research for over 70 years. Dogs continue to be used to define safety profiles for novel cancer agents destined for use in human phase I clinical studies. A paradigm shift is now underway to include tumor-bearing pet dogs in efforts to understand the biology and treatment of cancer and cancer metastasis.

Cancer in the companion animal (pet) population is a spontaneous disease. In many cases these spontaneous cancers share many features with human cancers. Companion animal owners, motivated by the hope of prolonging quality of their animals' life, frequently seek out specialized care and treatment from veterinary oncologists at private referral veterinary hospitals and veterinary teaching hospitals. It has been estimated that there are ~55 million dogs and 60 million cats at risk for developing cancer in the US. Cancer is the number one cause of death from disease in dogs. Using crude estimates of cancer incidence, there are roughly 4 million new cancer diagnoses made in dogs and a similar number in cats each year (70,72). This large population of companion animals with cancer provides an opportunity to include them in studies of cancer biology and therapy. Examples of such spontaneous models are listed in Table III and include non-Hodgkin's lymphoma (NHL), prostate carcinoma, lung carcinoma, head and neck carcinoma, mammary carcinoma, melanoma, soft tissue sarcoma and osteosarcoma. Many factors contribute to the value of these spontaneous cancers as relevant models for human cancer and cancer metastasis. These animals share many environmental risk factors with their human owners suggesting their value as sentinels of disease (73,74). The strong genetic similarity between dogs and humans, as evidenced by the recent draft of the canine genome project, has allowed dogs with spontaneous cancers to be used in the identification of cancer-associated genes (75,76). These cancers share tumor biology and behavior with human cancers, including metastatic propensity. In most cases the prevalence of these cancers is sufficient for pre-clinical trials and biological studies. Furthermore, the lack of 'gold standard' treatments allows early and humane testing of novel therapies and the more rapid progression (compared with humans) and metastatic failure seen in these pet dogs allow timely completion of clinical trials.

The biology of cancer in companion animals, as is the case in human cancers, is dependent on the specific cancer and has been summarized recently (63). In general, for any given cancer histology in dogs, the progression can be expected to be slower than the same cancer in a murine model, however, more rapid than for the same human cancer. Whereas most murine models of metastasis are characterized by rapid progression of metastatic disease; the more expanded 'investigational window' provided by dog cancer models makes them particularly important for defining agents active against metastasis. Table III includes a list of naturally occurring canine cancers, a brief description of each cancer model, its natural biology and metastatic behavior. For each canine cancer model there are important similarities and differences from the human disease. An understanding of both is necessary such that appropriate questions are asked within a model system and so that rationale combinations of models are used to answer more complex problems.

Until recently a significant weakness in the study of cancer biology in canine cancer models has been the availability of reagents. The development of novel technologies for molecular reagents, antibody development, protein expression and protein-purification has lowered the hurdle for developing canine-specific reagents to study spontaneous disease. A significant advancement in our ability to characterize companion animal models has and will come from the efforts of the canine genome project (77,78). A recent report by Kirkness *et al*. suggests greater homology between dogs and humans 'by several measures' than either species and the mouse (79). This genetic similarity and the relatively outbred nature of companion animals provide a strong rationale for the use of dogs in biomedical research and more importantly dogs with spontaneous disease (including cancer). For the more commonly studied canine cancers, strong similarities with the same human cancers (e.g. canine osteosarcoma and canine NHL) have been shown. Efforts to validate reagents and further characterize models using more sophisticated techniques have been ongoing within several comparative oncology laboratories around the world. Contributing to this effort, the intramural program of the National Cancer Institute's Center for Cancer Research has recently launched the Comparative Oncology Program. The goals of this program will be to facilitate the use of companion animal cancers in the process of cancer research through the characterization of these models and the design and implementation of pre-clinical translational trials (http://ccr.nci.nih.gov/resources/cop/).

In vivo models have served as important vehicles to explore a variety of phenotypes associated with metastatic progression. They will continue to do so until the time comes when an *in vitro* system is developed that faithfully replicates all of the myriad steps and challenges that disseminating tumor cells face. Because of the complexity of the metastatic process and the changing microenvironmental cues and interactions that a disseminated cell experiences, the development of such an *in vitro* assay system is unlikely in the near future. *In vivo* models, therefore, must continue to be an important workhorse

Table III. Description of prevalence, histology and metastatic biology of selected naturally occurring cancers seen in pet dogs

Prevalence	Histology	Metastatic biology	Comments	Reference
Non-Hodgkin's lymphoma • 15–30/100 000	• Diffuse large B-cell most common (high-grade) • NCI Working Formulation is useful in grading • T-cell seen in 10–38%	• Multicentric nodal presentation most common • Characterized by systemic/disseminated disease • Median survival 1 year	• Pattern of disseminated disease consistent with human condidtion	70, 117–119
Colorectal • Rare	• Carcinoma • Most commonly rectal in origin	• Metastasis to regional lymph nodes and liver • 1–2 year mean survival	• Histological progression from polypoid disease not well characterized	70, 120
Prostate cancer • Unknown • Necropsy study: 1/150 dogs over age of 8 years have advanced prostate cancer	• High grade carcinoma • HGPIN present in 55% of elderly sexually intact dogs without prostate cancer • Potentially ductal in origin	• Advanced/invasive at diagnosis • Hormone independent at diagnosis • Generally chemo and radiation resistant • Median survival 30 days without treatment • Metastasis to regional lymph nodes, bone, lung, other	• Dog is the only animal to develop prostate cancer spontaneously with significant frequency	118, 121, 122
Breast cancer • 198.9/100 000 • Reduced prevalence following ovariohysterectomy (OHE)	• 50% mammary tumors are benign • Malignant: carcinoma most common; likely lobular in origin • 45% Estrogen receptor positive • Overexpression of ErbB-2 in 17/23 malignant mammary tumors	• Metastasis to regional lymph nodes, lung, bone, other • Median survival 22 months with surgery alone	• Early reduction in estrogen exposure (OHE) is protective • Canine sex hormone cycle is distinct from humans • No BRCA1/2 families identified	70, 71, 118, 123
Lung carcinoma • 4.17/100 000	• Adenocarcinoma most common • Mutations in K-ras identified	• Advanced disease at diagnosis • Metastasis to regional lymph nodes, pleural space, lung, bone, other	• Survival <2 months following surgery if lesion >5 cm or metastatic • Survival >1 year if lesion <5 cm and not metastatic	71, 117, 124
Head and neck carcinoma • 6% of canine cancers	• Squamous cell carcinoma • Adenocarcinoma	• Primarily oral and nasal • Locally invasive, slow to spread to distant sites • Metastasis to regional lymph nodes, lung	• Non-carcinoma head and neck cancers include fibrosarcoma melanoma (oral)	117
Liver cancer • Rare	• No association noted with viral exposure • Hepatocellular adenoma and carcinoma most common	• Locally invasive, slow to metastasize to distant sites		117
Brain • 14.5/100 000	• Glioma and meningiomas most common • Usually solitary	• Locally invasive • Metastasis within brain infrequently seen before primary tumor morbidity	• Management with radiation therapy or surgery • Survival >1 year with radiation therapy	117, 125

Table III. *Continued*

Prevalence[a]	Histology	Metastatic biology	Comments	Reference
Pancreatic cancer • Rare	• Adenocarcinoma—ductal or acinar	• Often associated with extension to local tissues resulting in biliary and intestinal obstrauction • Metastasis within abdomen at diagnosis very common	• Palliative GI bypass for obstruction • No definitive therapy	117
Osteosarcoma • 8000 new cases/year	• High grade • Complex karyotype with no consistent translocation	• Primary bone tumor most commonly appendicular • Metastases to lungs most common • Metastasis expected within 4 months without adjuvant therapy	• Occurs in middle aged to older large breed dogs • Amputation or limb spare of primary tumor • Adjuvant therapy: Doxorubicin, platinum	126
Soft tissue sarcoma • 1% of malignant tumors in dogs • 35/100 000	• Loosely associated family of cancers with several histological forms (fibrosarcoma, leiomyosarcoma, synovial sarcoma, gastrointestinal stromal tumor, histiocytic sarcomas) • Translocation status not studied	• Diverse biology dependent on specific histology • Mutated c-kit in gastrointestinal stromal tumors • Metastasis to regional lymph nodes, lung, bone, other		70, 71
Melanoma • 25/100 000	• Histological progression of lesions not defined	• Bucal, ocular, and digital are malignant • 95% of cutaneous melanomas are benign • Not likely associated with sun exposure		70

in metastasis research. Selection of an *in vivo* model must be tailored to the nature of the question being asked and with full knowledge of the caveats and inadequacies of each model system. These models, in conjunction with *in vitro* modeling and manipulation of tumor cells, have enabled and will continue to enable investigators to explore the critical questions that remain, including the true nature of metastatic dormancy, the role and identity of the microenviromental cues and the development of agents that can be used to prevent or treat overt metastatic disease.

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