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Mouse models for melanoma: a personal perspective

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Abstract: Complex biological processes often require *in vivo* analysis, and many important research advances have been made using mice as a model for the study of various biological systems. Cutaneous melanomas are tumors originating from skin melanocytes, which are present in hair follicles, and interfollicular epidermal and dermal layers. Until recently, mouse melanoma models were largely based on transplantation models, i.e. transplantation of either syngeneic or xenogeneic melanoma cells into wild type or genetically modified animals. More recently, however, the use of novel technologies specifically modifying the genome allows for the generation of mouse strains, which may develop spontaneous melanoma. Nevertheless, it should be kept in mind that animal models provide only an approximation of reality in humans. In this review, we will discuss a representative selection of currently available transplantation and transgenic melanoma models; despite the fact that this selection will be biased by personal experience, we are confident to demonstrate how the use of mouse melanoma models facilitates translational research in several biomedical disciplines.

Key words: knock out mice – syngeneic transplantation – trangenic animals – xeno-transplantation

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Introduction

Although cell culture experiments as well as *in vitro* biochemical studies have contributed many recent advances in molecular physiology and pathology, the complexity of biological processes often requires *in vivo* analysis; however, the study of human biology *in vivo* is severely limited by ethical and technical constraints. Thus, there is a growing need for animal models to improve our understanding of human disease without putting individuals at risk (1–3). This notion holds true for neoplasms particularly, e.g. melanoma, which will be the focus of this review. Indeed, several mouse melanoma models have been developed and are used: (i) to determine the function of particular proteins in melanoma progression; (ii) to approximate certain biological aspects of human melanomas; and (iii) to critically evaluate novel drugs/therapies.

As a result of space limitations and the large number of published melanoma models, we can discuss only an arbitrary selection of the currently available models in this review which include: (i) xeno-transplantation models; (ii) syngeneic transplantation models; and (iii) models involving genetically modified animals. The relevance of each particular model depends on how closely it represents the genetic and epigenetic aberrations, histology, physiological effects and metastatic pattern observed in human melanoma. Although genetically modified mice have been instrumental in leading to a better understanding of the molecular mechanisms involved in tumor initiation and are currently receiving most of the attention, they have been less successful in modelling advanced cancer (4–6). This notion is particularly puzzling as metastases are the main determinants of the clinical course of melanoma and patient survival (7); moreover, metastases are the targets of systemic therapy. In this regard, xeno-transplantation models were particularly successful in mimicking advanced, metastatic melanoma (2,8). However, in these models, the role of the immune system – both to fight or to promote cancer – is not considered at all (9). Thus, each model has its characteristic advantages, which may render it more suitable for answering a respective scientific question. For example, genetically modified animals are largely used to address melanomagenesis, while melanoma immunology is studied in syngeneic transplantation models, and xenogeneic transplantation models are employed to critically analyse the behaviour of melanoma cells *per se*, e.g. invasiveness, metastatic potential or the role of tumor stem cells.

The selected examples discussed below demonstrate how the use of mouse melanoma models facilitates translational research in several biomedical disciplines and how a suitable choice can help to overcome any remaining limitations of mouse melanoma models.

As the necessity of animal experiments is frequently questioned in public discussions, most governments aim to control both the number and frequency of individual animal use as well as the degree of pain that may be inflicted without anaesthetics (10,11). In our view, although the value of animal models for melanoma research is beyond doubt, every scientist should carefully consider possible alternative approaches to answer specific questions.

Xeno-transplantation models

Cancer metastasis is the end product of a micro-evolutionary process in which diverse interactions between cancer cells and their environment yield alterations that allow these cells to transcend their programmed behaviour (7,12). While generation of metastatic clones requires genetic alterations in cancer cells, subsequent selection of such clones is heavily influenced by interactions with the surrounding tissue microenvironment. Tumor cells thus populate and flourish in new tissue habitats and ultimately cause organ dysfunction and death. It is well established that metastasis involves dynamic and multistep *in vivo* processes (13). However, reproductions of the complex cellular interactions that occur in human patients have not been accomplished in currently available *in vitro* systems yet.

Metastases arising from certain primary tumors frequently exhibit specific organ preference. In this regard, human melanoma metastasizes preferentially into lymph nodes, lung, liver or brain (14–17). This pattern can be reproduced in animal models which rely on the use of immunodeficient mice. As a result of their diminished ability to mount an effective immune response, these animals allow the growth of human melanomas and the expression of malignant properties (such as preferential colonization of certain organs), which are intrinsic to transplanted melanoma cells. The first immunodeficient mouse model of cancer to be developed was based on athymic *nude/nude* mice, which support the growth of solid human tumors. In the following, CB17-*scid* mice were shown to support the engraftment of some transplantable human melanoma cell lines, but tumor growth was limited by high levels of host natural killer (NK) cell activity (18). Thus, NK-deficient NOD-*scid* mice allowed even the growth of melanoma cell lines that grew poorly or not at all in CB17-*scid* mice (19,20).

Metastasis formation starts with dissemination of tumor cells from the primary tumor (13). Following their detachment, tumor cells invade surrounding tissue and basement membranes, intravasate into the lymphatic or blood circulation and finally, adhere and extravasate into distant organs. Degradation and remodelling of the extracellular matrix and basement membranes by proteolytic enzymes are essential steps in these processes (21). Different proteolytic enzyme systems, including the plasminogen activator system, aspartyl and cysteine proteinases as well as matrix metalloproteinases (MMPs), have been identified in cutaneous melanoma progression (Table 1). MMPs are not only expressed and activated by tumor cells but also by stromal cells. By means of murine tumor models for either experimental metastases induced by transplanting the melanoma cells directly into the blood stream or spontaneous metastases derived from s.c. transplanted primary tumors, we could characterize the differential expression of MMPs and tissue inhibitors of MMPs (TIMPs) in relation to the microenvironment and the induction of metastasis (22–24). This analysis demonstrated that MMP-2, MMP-9 and MT1-MMP were predominantly expressed at the tumor-stroma border of s.c. tumors, while functionally active MMP-2 was restricted to this invasive front (24,25). However in spontaneous lymph node or lung metastases, MMP-9 was expressed both in the centre and periphery of tumors; these tumor areas were largely negative for MMP-2 and MT1-MMP. Notably, tumor cells of experimental lung metastases did not express MMP-9 at all (22,25,26). These results indicate that expression of MMPs in melanoma metastases is not only influenced by their localization but also by the nature of tumor induction, suggesting that individual MMPs play specific roles during different stages of metastasis formation.

It has been recently hypothesized that tumors arise from a tumor stem-cell population (27,28). This hypothesis is based on the observation that for several cancers a rare, small fraction of cells can be prospectively identified, which can initiate tumor growth in xeno-transplantation models (e.g. NOD-*scid* mice), while the remaining marker negative cells cannot (29,30). However, this concept is challenged by the use of an even more immunodeficient murine model, i.e. NOD-*scid* strains which also incorporate mutation of the interleukin-2 (IL-2) receptor γ-chain locus (*Il2rg*$^{-/-}$) that lack host NK-cell activity and are deficient in innate immune function, at least for melanoma (31). Thus, Quintana et al. transplanted single human melanoma cells into NOD-*scid Il2rg*$^{-/-}$ mice and used rigorous procedures to measure the frequency of tumorigenic cells. They demonstrated that as many as one in four melanoma cells can initiate a tumor. Moreover, melanoma cells capable of producing a new tumor can have many different features, most of which are common to some, but not all, of the tumorigenic cells, and none of which shows a particular association with tumorigenic potential. However, these authors caution that the frequency of tumorigenic cells in

Table 1. Expression and function of matrix metalloproteinases in melanoma

	Expression pattern	Function
MMP-1	Invasive melanoma cells and stroma cells (25)	Knockdown of MMP-1 has no effect on primary tumor growth but decreases the metastatic potential by inhibiting collagenase activity and angiogenesis (99)
MMP-2	Heterogeneous expression either only on stromal cells such as fibroblasts and macrophages or also on tumor cells with tumor cells at the growth or invasive front demonstrating the highest expression (25)	Expression correlates with progression (25). Downregulation of MMP-2 leads to decreased invasion, migration and angiogenesis (100). Upregulation promotes experimental pulmonary metastases (101)
MMP-3	Melanoma cell lines may express MMP-3; *in situ*, however, MMP-3 expression is restricted to macrophages surrounding the tumor or adjacent to blood vessels (25,102)	Associated with tumor growth (102)
MMP-9	Expression is restricted to stromal cells (25). In a syngeneic melanoma model, MMP-9 is expressed either (i) at the tumor-stroma border; (ii) throughout the tumor; or (iii) not at all dependent on tumor induction and localization	Secreted MMP-9 promotes angiogenesis (103). Upregulation promotes experimental pulmonary metastases (101)
MT1-MMP	Heterogeneous expression either only on stromal cells or both on stroma and tumor cells (24)	Particularly for lung colonization MT1-MMP promotes invasion and dissemination (104)
TIMP-1	Expression on blood vessels adjacent to the tumor, but not on tumor cells (25)	TIMP-1 overexpression reduces tumor growth and metastatic potential (105) (106)
TIMP-2	Heterogeneous expression on tumor cells (24)	Overexpression of TIMP-2 reduces s.c. tumor growth but does not prevent spontaneous metastasis to the lung or lymph nodes (107)
TIMP-3	Heterogeneous expression (108)	TIMP-3 expression promotes apoptosis in melanoma cells through stabilization of three distinct death receptors and activation of their apoptotic signalling cascade via caspase-8 (108)

human melanoma is much higher than reported for any cancer previously suggested to follow a cancer stem-cell model, and that they could not identify phenotypic differences between melanoma cells that form tumors and those that do not (31).

Syngeneic transplantation models

While xeno-transplantation melanoma models could only be adopted once immunodeficient mice were available, syngeneic transplantation models could be established earlier. Indeed, models such as the Harding-Passey melanoma in BALB/c × DBA/2F1 mice (32), the Cloudman S91 melanoma in DBA/2 mice (33) or the B16 melanoma in C57BL/6 mice (34,35) have been used for approximately half a century. Most importantly, such models still are useful, especially for experiments designed to study or modulate immune responses to melanoma which require an intact immune system. Anticancer immune therapy has been extensively studied in animal models and in clinical trials. While immune therapy can lead to tumor protection in numerous murine models, objective tumor regressions after anticancer vaccination in clinical trials have been rare (36). However, even in the murine model most strategies had only limited success when therapy was used for well-established tumors.

Notably, the immunophenotypes, i.e. the susceptibility to develop certain immunological responses, of inbred mouse strains may differ and are usually well established. For example, C57BL/6 mice are more likely to develop a Th1 responses, while BALB/c mice predominately display a Th2 response (37,38). Similarly, the cell lines and sublines thereof display a wide degree of heterogeneity with respect to tumor growth rate, tumor take and metastasis formation (35,39,40). However, due to their frequent use, the behaviour of these respective sublines has been well characterized.

The most frequently used syngeneic murine melanoma model is B16 derived from a spontaneously arising melanoma of C57BL/6J origin (35). B16 melanoma expresses rather low levels of major histocompatibility complex class I molecules impeding the recognition by $CD8^+$ T cells (41,42). Therefore, the B16 model was regarded as poorly immunogenic. Nevertheless, B16 melanoma cells express several melanoma-associated antigens such as Tyrosinase-related protein-2 [TRP-2] or gp100 that may serve as targets for autologous T cells (43). Indeed, tumor regression can be induced by means of immunotherapeutic intervention which demonstrates the immunogenicity of B16 tumors. To study the *in vivo* T-cell response against B16 melanoma, with particular emphasis on diversity and systemic involvement, we examined the spectra of T-cell clonotypes in coexisting B16 melanoma lesions in C57BL/6J mice (44). Three tumors obtained from individual animals were examined for the presence of clonotypic T cells using the highly sensitive T-cell receptor (TCR) clonotype mapping technology. Systematic analysis of the TCRB variable regions revealed up to 30 clonotypic TCR transcripts in

each tumor demonstrating its immunogenicity. To scrutinize intra- and inter-individual variations in T-cell responses, more than 600 clonotypic TCR transcripts were compared for sequence identity. Overall, approximately 2% of the T-cell clonotypes was detected in more than one tumor from the same animal (44). Furthermore, none of the clonotypes detected was present in more than one animal, arguing against recurrent or 'public' T-cell responses against B16 melanoma.

As mentioned above, the B16 melanoma model has been used to test a multitude of immunotherapeutic intervention including cytokines, immune-modulating antibodies, vaccines or combinations thereof (45). These experiments are still performed as it is generally assumed that the efficacy of immune therapies against B16 might be a reasonable predictor of the effectiveness of immune therapies against human tumors. Indeed, there are several lines of evidence supporting this notion: (i) The efficacy of vaccine regimens tested against established human cancers in clinical trials has been very limited (46,47); similarly, it has never been reported that any class of vaccine alone can consistently eradicate established, palpable B16 tumors (48,49) and; (ii) lymphodepleting preparative regimen followed by adoptive transfer of tumor-reactive T cells plus administration of high-dose IL-2 is an effective therapy for melanoma in humans and one of the most effective therapies for established B16 tumors (45,47). The vaccination regimens used to treat murine B16 melanoma included recombinant viral, DNA, dendritic cell, whole-tumor and peptide vaccines. One general observation from these studies which unfortunately cannot be brought forward to the human system is that prophylaxis against tumor implantation can be achieved by many types of vaccines (41,43,50,51). By contrast, eradication of established, palpable B16 tumors can only be consistently accomplished by the combination of vaccination with other treatment modalities (45,47,49).

In this regard, we have recently demonstrated that dendritic cell-based peptide vaccination in mice required IL-2 to mount an effective immune response against established melanoma metastases (48). This effect can be further improved by using tumor-targeted IL-2, which was shown to enhance pre-existing T-cell responses to tumors more efficiently than systemic IL-2 (52,53). This measure actually boosted the therapeutic effect of a TRP-2 vaccine, resulting in the partial or complete regression of established tumors. However, it also interfered severely with the development of a protective, systemic memory (54). Indeed, high local concentrations of IL-2 at the dermal/s.c. tumor site favoured the development of non-lymphoid memory cells homing to the dermal/s.c. compartment, whereas lymphoid memory cells either did not develop at all or were depleted from lymph nodes by this measure. Thus, mice treated by vaccination and targeted IL-2 demonstrated subsequent to the excision of the initial s.c. tumor, an effective protection against s.c. tumor challenges, whereas pulmonary challenges proved to be fatal.

These observations illustrate that a syngeneic mouse melanoma model in combination with naturally processed peptides derived from melanoma-associated antigens (Ag) provides a valuable tool to further optimize therapeutic vaccinations to treat this cancer. Furthermore, these results confirm earlier studies demonstrating that induction of autoreactivity to a non-mutated melanocyte differentiation Ag can lead to tumor destruction associated with an autoimmune disease, i.e. vitiligo (46,55). Approximately, one-third of successfully treated animals with vaccination in combination with targeted IL-2 exhibited a loss of pigmentation (56). Cutaneous lesions, similar to those, were described in a series of studies reporting on the successful immune therapy of murine melanoma (49,57). All of these patterns resembled vitiligo observable in some melanoma patients who responded to IL-2 treatment (36,58,59). Thus, T-cell populations with a similar reactivity in mouse and man seem to be responsible for the destruction of normal and neoplastic melanocytic cells. In this regard, we were able to demonstrate the presence of identical T-cell clonotypes in melanoma and melanoma-associated vitiligo (59). However, the observation that only some animals developed vitiligo indicates that its initiation is dependent on the coincidence of at least two different events: the presence of specific lymphocyte populations as well as specific features of the skin presenting a target for these cells.

Murine models have also been used to test other antibody–cytokine fusion proteins, including GM-CSF, IL-12, TNF and lymphotoxin-α (LTα), although not as thoroughly applied as the antibody–IL-2 fusion proteins (60). Interestingly, not until an antibody–LTα fusion protein was tested in melanoma models, was the mechanism of its *in vivo* therapeutic effect revealed: Originally designed to induce apoptosis of tumor cells directly, this therapeutic effect *in vivo* was found to depend on the presence of immune-competent cells. In a xenograft melanoma model, these were B- and NK-cells, whereas in a syngeneic melanoma model, the anti-tumor effect was mediated by T cells (61). Detailed analyses of effects of LT fusion protein in the syngeneic melanoma model revealed that such effects depended on the induction of tertiary lymphoid tissue next to the tumor (62,63). This proximity provided all the requirements necessary for T-cell priming. Indeed, this antibody–LT fusion protein demonstrated comparable anti-tumor efficiency that was accompanied by the induction of tertiary lymphoid tissue even in splenectomized LT knock-out mice, i.e. in the absence of any preformed major secondary lymphoid tissue (63). Therefore, syngeneic murine melanoma models could demonstrate the different mechanisms of antibody–cytokine fusion proteins, i.e. boosting of

pre-existing immune responses by antibody–IL-2 and induction of new anti-tumor immune responses by antibody–LTα fusion proteins.

In conclusion, many different immune therapeutic strategies have been tested in murine melanoma models. These studies demonstrated that it is possible to generate potent anti-tumor immune responses even against poorly immunogenic tumors such as B16. To be effective at treating tumors, therapeutic regimens should generate large T-cell responses, which can be measured *ex vivo*. To this end, cytokines such as IL-2 or IL-15 are able to enhance the efficacy of multiple therapeutic regimens. Moreover, the avidity of the TCR of these anti-tumor T cells is a critical factor. Design of future anticancer vaccination regimens should take into account these factors that were associated with effective treatment of murine melanoma.

Genetically modified animals

A series of genetic and epigenetic changes are considered to be the main causes for turning a normal cell into a cancer cell. These include the evasion of apoptosis, self-sufficiency in growth signals, insensitivity to growth-inhibitory signals, development of a limitless replicative potential and boosts of angiogenesis; all of which allow tissue invasion and metastasis (64). These changes are mediated by activated oncogenes as well as inactivated tumor suppressor genes. Recent evidence revealed that different cancer types have different characteristic patterns of such aberrations. Nevertheless, a distinct set of proteins and pathways was identified, which are repeatedly involved in the carcinogenesis of many different tumor types (65). Several of these genes have been analysed for their relevance in the molecular pathogenesis of melanoma and for their potential impact on the clinical course of this malicious disease.

Numerous genetic and epigenetic abnormalities have been detected in human melanomas (66,67). These aberrations include the inactivation of tumor suppressor genes (e.g. tumor suppressors of the INK4a/ARF locus), activation of oncogenes (such as N-Ras or B-Raf), increased expression of anti-apoptotic molecules (e.g. MCL-1 or Survivin), inactivation of pro-apoptotic molecules (e.g. APAF-1) and modification of DNA repair enzyme activities. The identification of molecular pathways involved in melanomagenesis and the molecular cloning of the respective oncogenes, which allowed the demonstration of their causal implication in melanoma, facilitated the development of novel models for human melanoma (68). In the early 1980s, a technology for generating lines of mice carrying cloned genes integrated into the mouse genome was introduced as a tractable and reproducible method (69,70). The liaison of these two areas of research resulted in genetically engineered mice, characterized by heritable predispositions to the development of melanoma. Currently, mouse modelling of melanoma is possible through the ectopic expression of oncogenes, introduction of specific oncogenic mutations or the inactivation of tumor suppressor genes; these experiments had a deep impact on our understanding of the molecular pathways involved in melanoma initiation and progression.

It should be noted, however, that mouse skin is not identical to human skin (71,72); most important, the localization of melanocytes differs in human and murine skin. In fact, most melanocytes in humans are found at the epidermal–dermal junctions and within hair follicles; in contrast, melanocytes in the hairy parts of adult mice are mostly in hair follicles, sometimes in the dermis and occur only rarely or at specific periods (e.g. during embryogenesis or postnatal) at the dermal–epidermal junction. Most melanocytes in mouse tails and toes are in the epidermis and those in the pinnae of the ears are located in the dermis. Moreover, it should be kept in mind that, with respect to genetic, histopathological and clinical characteristics, human melanomas are quite diverse, implicating several different patterns of altered genes and signalling pathways in melanoma progression (73). Consequently, one single murine melanoma model can never reflect all types of the human disease.

Consequently, various different transgenic mouse models have been reported (2,4–6,66,68,74,75). For example, mouse lines expressing known oncogenes such as Ret or mutant forms of Ras and Raf under the regulation of ubiquitous or tissue-specific promoters develop melanocytic hyperplasia, retinal pigmented epithelial tumors and melanoma, which in some cases can metastasize to a variety of organs (76–83). Ectopic expression of hepatocyte growth factor/scatter factor, a melanocyte mitogen which stimulates the receptor tyrosine kinase Met, did also lead to a mouse line that develops melanomas, besides tumors in the mammary glands and further locations (75). Moreover, transgenic mice with melanocyte-specific expression of activated Ras bred into a p16INK4A/Arf deficient background also develop cutaneous and ocular melanomas (84–86). While the latter melanomas evolve spontaneously with an incidence of 33%, some of the other transgenic animals require different combinations of chemical carcinogens and UV irradiation to develop melanoma. A number of excellent, recent reviews have addressed these genetically modified mice and their use as melanoma models (2,4–6,66,68,74,75); thus, we will review and discuss here only a genetic melanoma model which was not adequately covered in these prior reviews; i.e. mice characterized by an ectopic expression of the G-protein-coupled receptor GRM-1 (metabotropic glutamate receptor-1) (87,88).

G-protein-coupled receptors (GPCR) are the largest family of receptors with more than 500 members. Evaluation

of GPCR gene expression in primary human tumors identified over-expression of GPCR in several tumor types (89–91). Analysis of cancer samples in different disease stages also suggests that some GPCR may be involved in early tumor progression while others may play a critical role in tumor invasion and metastasis. The glutamate receptor family is divided into two major groups: ionotropic glutamate receptors and metabotropic glutamate receptors (mGluR) (92). mGluRs are seven-transmembrane domain GPCRs and are further subdivided into three groups based on sequence homology and downstream signalling (90,91). GRM-1 is normally expressed and functional in the mammalian central nervous system and is implicated in learning and memory formation.

The GRM-1 has the ability to couple to multiple second messenger systems such as IP3, DAG and cAMP in the presence of GRM-1 agonists (93). Signalling cascades mediated by GRM-1 have been extensively studied in the central nervous system demonstrating that GRM-1 can activate extracellular-signal regulated kinases (ERK1/2) upon stimulation with its natural ligand, glutamate or other agonists (89,92). It is well established that the constitutive activation of the MAPK signalling cascade contributes to melanomagenesis (94). In this regard, it should be noted that aberrant GRM-1 expression also seems to contribute to some cases of human melanoma. Thus, testing of more than 120 human melanoma tissues and 25 human melanoma cell lines for GRM-1 expression revealed that approximately 40% of these samples expressed GRM-1 at both mRNA and protein levels while normal skin biopsies were negative. The GRM1 gene is located at 6q24 in humans. Interestingly, in a recent genetic association study to elucidate whether the GRM1 gene contributes to human melanoma, it was demonstrated that the single nucleotide polymorphism rs362962 was significantly associated with melanoma susceptibility in patients who reported a low level of sun exposure and whose tumors were located on skin zones that are not usually exposed to the sun (95).

The laboratory of Suzie Chen has developed two mouse models for melanoma, which are based on the aberrant expression of GRM-1 (88,90). The first model was set up accidentally when a transgenic mouse was found to develop multiple melanomas due to the insertion of the transgene into intron 3 of the GRM-1 gene (87). The tumor tissues displayed expression of GRM-1. To confirm the causal role of GRM-1, a second mouse model was established, which expressed GRM-1 under the control of the melanocyte-specific dopachrome tautomerase promoter. Again, these mice developed spontaneous melanoma. The lesions in GRM-1 transgenic animals are located in skin, eyes, lymph nodes, lung, inner ear, brain and muscle. Additional studies clearly demonstrated that primary tumors initiated in tissues in which normal neural-crest derived melanocytes reside, and that additional lesions in other tissues were rather metastatic (88,90). The appearance of initial melanomas and tumor progression depends on the zygosity of the inserted transgene. Homozygosity results in the onset of the tumor at 2–4 months of age; if heterozygous, the lesions are first detectable at 6–8 months. Notably, histo-morphological characteristics of these melanomas are independent of age at which the tumor occurs. Chen et al. could demonstrate that the aberrantly expressed GRM-1 is functional in melanoma cell lines derived from these mice (93); they responded to stimulation or suppression by agonist or antagonist of GRM-1. Significantly, GRM-1 stimulation induced ERK1/2 activation in these mouse tumor derived cells via PKCε (93). Consequently, it was not surprising that both tumor cell lines and tumor biopsies derived from GRM-1-transgenic mice were wild type for both N-RAS and B-RAF, which are the ERK activating oncogenes that are typically activated by mutation in human melanoma.

Notably, in a xenograft melanoma model riluzole, an oral GRM1 blocking agent inhibited tumor growth compared with the untreated controls (91). This study was followed by phase 0 trial of riluzole in patients with melanoma who received 200 mg of oral riluzole per day for 14 days, which revealed that glutamate blockade with riluzole inhibited signalling through the mitogen-activated protein kinase and phosphatidylinositol 3-kinase/AKT pathways and suppresses the metabolic activity of melanoma (96). Thus, the ectopic expression of mGluRs seems to be important in the pathogenesis of human melanoma, and targeting this pathway may be an effective therapy.

Conclusions

It is not uncommon for murine melanoma models to demonstrate that new anti-cancer drugs or therapies produce highly effective, and sometimes even spectacular anti-cancer treatment results (1,2,45). Unfortunately, such preclinical results are often followed by failure of this same drug/therapy in clinical trials, or reveal at best results of only the modest efficacy by comparison. Not surprisingly, this has provoked considerable scepticism about the value of using such preclinical models for early stage *in vivo* preclinical drug testing. However, close inspection of retrospective and prospective studies in the literature reveals that these murine melanoma models can be remarkably predictive of cytotoxic chemotherapeutic drugs that have activity in humans. This is the case when the drugs are tested in mice with established tumors using pharmacokinetically clinically equivalent or 'rational' drug doses. What may be at variance with clinical activity, however, is the magnitude of the benefits observed in mice, both in terms of the degree of tumor responses and overall survival. It is argued that this disparity can be significantly minimized by the use of

metastatic tumor models in which treatment is initiated after the primary tumor has been removed and the distant metastases are well established and macroscopic, i.e. the bar is raised and treatment is undertaken on advanced, high volume, metastatic disease. Under such circumstances, survival should be used as an endpoint; in addition, changes in tumor burden using surrogate markers or micro-imaging techniques can be used as well to monitor effects of therapies; however, these parameters should be used with the same precautions as they are used in the human setting (97,98). Adoption of such procedures would more accurately recapitulate the phase I/II/III clinical trial situation in which treatment is initiated on patients with advanced, high-volume metastatic disease. Nevertheless, it should be kept in mind that mice are not men, i.e. mouse melanoma models can only serve as an approximation of this disease situation in humans.

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