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Review

Murine models of melanoma

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ABSTRACT

Melanoma is an aggressive and highly metastatic skin cancer, carrying a poor prognosis with a median survival time of 5.3–10 months depending on the stage of disease. Research has advanced our understanding of the underlying pathology of melanoma and strategies to prevent and treat melanoma. Mouse models have been developed to elucidate the molecular, immunological, and cellular mechanisms contributing to proliferation and metastasis of melanoma. This review article aims to provide an overview of various types of murine melanoma models, including xenograft and syngeneic transplantation models, genetically modified models, ultraviolent radiation models, and chemically induced models, and discuss the advantages and limitations of each model.

1. Introduction

Melanoma is a type of skin cancer that originates in the pigment-producing melanocytes and characterized by its aggressive and highly metastatic nature [1]. According to the Centers for Disease Control and Prevention, there were an estimated 76,665 cases and 9324 deaths in the United States in 2014, and the incidence continues to rise every year. Metastatic malignant melanoma carries a poor prognosis with a median survival time of 5.3–10.0 months depending on the stage of disease [2,3]. In the past few decades, research has advanced our understanding of the underlying pathology of melanoma and treatment strategies. Mouse models have been developed to elucidate the molecular and cellular mechanisms contributing to proliferation and metastasis of melanoma. They have also been used to examine responses to therapeutic agents (Singh, 2017). In this article we will describe the various types of murine models used for investigating melanoma, which are summarized in Table 1.

2. Current murine models of melanoma

2.1. Xenograft transplantation models

Xenograft mouse model has been developed to study the metastasis of melanoma. In this model, human melanoma cells are cultured and implanted subcutaneously into the skin or organ of immunocompromised mice [1]. These immunocompromised mice may be nude athymic and thus deficient of T-cell function or severe combined immunodeficient (SCID), which is characterized by both B-cell and T-cell immunodeficiencies [1]. The advantage of the xenograft model is that it uses the human derived melanoma cells and therefore mimics the complex genetic heterogeneity of cancer [4]. Xenograft models would also allow us to predict the human tumor response to therapeutic drugs [1]. Other advantages of these models are that they can be easily developed and that the results can be collected within a few weeks [4]. However, because most of the melanoma cell lines have been produced under non-physiological conditions instead of in natural tissue, they no longer represent the original tumor [5]. In addition, xenografts do not provide adequate tumor microenvironment as they lack an immune system [5]. A recent study has shown that the host's immune system contributes to both inhibition and metastasis of melanoma [6]. This is known as concomitant immunity; the primary tumor can induce an immune response and immunosuppressive mechanisms that suppress the growth of a secondary tumor [6]. Hence, the results obtained with xenograft models have poorly predicted clinical outcomes, including the efficacy of therapeutic agents [4]. However, although xenograft models lack functional B and/or T lymphocytes, they can accept human immune cells [7,8]. Sabzevari and Reisfeld [8] found that human cytotoxic T-cell line in SCID mice maintained their ability to suppress the growth of metastatizing melanoma cells. In addition, Basel et al. [9] recently developed a xenograft model in immunocompetent mice by exposing mice fetuses (day E14) to human tumor cells. These findings would optimize the use of xenograft tumor models in future melanoma studies.

The limitations of xenograft models can be addressed by allowing serial passage and proliferation of a single melanoma sample through several generations of mice [4,5]. As a result, the tumor grows in

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Table 1
A summary of advantages and disadvantages associated with each murine model of melanoma.

Model Type	Advantages	Disadvantages
Cell-line xenograft models	• Mimic the complex genetic heterogeneity of cancer • Allow us to predict the human tumor response to therapeutic drugs • Are easily developed • The results can be obtained within a few weeks.	• Melanoma cells are not produced under physiological conditions • Lack an immune system • Results can poorly predict clinical outcomes
Patient-derived xenograft models	• More closely reflect human tumor microenvironment compared to cell-line xenograft models	• Expensive • Technically complicated • Time-consuming to develop
Syngeneic models	• Tumor cells and the recipient share same genetic background • Have a functional immune system • The recipient's immune system would not reject the transplanted tissue	• May not represent the genetic diversity of human melanoma • Mice may be sacrificed within a couple of weeks because of short time to tumor and tumor burden
Genetically modified models	• Are immunocompetent • Specific mutations can be reproduced and mimic those in human melanomas	• Cannot fully reproduce the complex genetic heterogeneity of melanoma cells • Expensive, time-consuming, and labor intensive • Tumor development and progression can be variable
UV radiated models	• Useful for studying the risk factors and pathogenesis of human melanoma • Useful for studying UV-induced changes in the skin microenvironment during the development and progression of melanoma	• Mice are relatively less responsive to UV radiation • Melanoma arising in mice often do not histologically resemble human melanoma
Chemically induced models	• Chemical carcinogens can be easily applied to mice • Can be used in combination with other modeling techniques	• Application of chemical agents may not be homogeneous • Not clinically relevant to the human melanoma

physiological conditions instead of cell cultures [5]. Even though their immune system cannot be fully restored, these patient-derived xenograft mice are more closely reflective of human tumor microenvironment and thus predict the therapeutic response of tumor in a human patient [1]. Studies have demonstrated similar histology and molecular characteristics between tumors in patient-derived xenograft mice and parent tumors [10]. However, these xenograft models are expensive, technically complicated, and time-consuming to develop [4].

2.2. Syngeneic transplantation models

In syngeneic transplantation models, melanoma cells derived from a certain mouse strain are inducted and transplanted into the same species [1]. Because the tumor cells and the recipient share the same genetic background, the transplanted tissue will not be rejected by the recipient's immune system. Hence, the advantage of syngeneic transplantation models is that they have a functional immune system, representing the tumor's microenvironment more accurately compared to xenograft models [7]. They are therefore useful for evaluating the efficacy of immunotherapies, such as cytokines, immune-modulating antibodies, and vaccines [1,11]. For instance, Zhao et al. [12] found that B16F10 vaccines in mice increased the cytotoxic activity and IFN-y, resulting in tumor shrinkage and mouse lifespan extension. Syngeneic transplantation models are also valuable for studying interactions of melanoma cells with immune cells such as T-cells, B-cells, and dendritic cells [1].

Although there are a few syngeneic models, including Harding-Passey melanoma in ICR mice and S91 melanoma in DBAmice, B16 melanoma in C57BL/6 mice is the most commonly used model [1]. The sample protocol for its implementation is described in Overwijk and Restifo [35]. There are two subclones of the B16 melanoma cell lines, which are B16F1 and B16F10 [1]. B16F1 has low metastatic potential and is used for studying primary tumor growth, whereas B16F10 has high metastatic potential to lungs [1]. B16F10 appeals to researchers because of its rapid growth resulting in tumor-induced mortality within 2 to 4 weeks [1,13]. For these models, time to tumor is both a benefit and a limitation, as mice typically have to be sacrificed within a couple of weeks because of tumor burden. As a result, syngeneic models do not exhibit the chronic inflammatory environment present in human melanoma [5]. Another potential limitation is the differences in immunophenotypes of transplantable tumor cell lines [11]. For instance, C57B/6 and BALB/c mice are more likely to display Th1 and Th2 responses, respectively [11]. Nevertheless, syngeneic models are useful in studies examining immune responses to melanoma and identifying immunotherapies [11]. While B16 melanoma cells express low levels of major histocompatibility complex class I (MHC I) molecules and are poorly immunogenic, they possess high levels of melanoma-associated antigens, including gp100 and tyrosinase related protein 2 (TRP2), which are the targets of immunotherapies [1]. A recent study demonstrated that TRP2 peptide vaccination in tumor-bearing mice increased the secretion of T cell IFN-y and inhibited tumor growth in B16F10 mice, preventing lung metastasis [14]. Transplanted mouse tissue may also not represent the genetic diversity of human melanoma as it originates from a single mouse strain [1,5].

2.3. Genetically modified models

The development of genetically engineered mouse models has advanced our knowledge of molecular pathogenesis of melanoma. They have been very useful in identifying novel genes/pathways and possible therapeutic targets for melanoma. Genetically engineered models include transgenic mice with engineered gene overexpression and knockout mice with selectively deleted regions of genome [15]. Recent studies have utilized models involving deletion of CDKN2A locus, TP53, PTEN and BRAF; and activation of GRM1, GNAQ, and RAS [1]. Further details and findings of these models are discussed in Kuzu et al. [1], Chin [16], McKinney and Holmen [7], Walker and Hayward [17]

Genetically modified models have several advantages. They are immunocompetent and provide tumor microenvironment similar to human melanoma [4]. Specific mutations can be reproduced and mimic those in human melanoma, enabling us to evaluate the effects of genetic alternations on melanoma development and progression [4]. Robinson et al. [18] established a murine model with suppressed NRAS expression and found an overexpression and activation of receptor tyrosine kinases, demonstrating the importance of receptor tyrosine kinases in melanoma. The authors recommended that RAS and receptor tyrosine kinases should be targeted for therapies. Genetically modified models also allow us to investigate various therapeutic approaches at different stages of melanoma [4]. For instance, Liu et al. [19] demonstrated that BRAFV600E peptide vaccine elicited a robust cytotoxic T cell response and resulted in potent tumor growth inhibition in a murine BRAF-mutant model. However, there are a few limitations of genetically modified models. Since studies often target one or two genes in their subjects, genetically modified models cannot fully reproduce the complex genetic heterogeneity of melanoma cells [4]. Melanoma may contain multiple genetic abnormalities that result in its initiation,