Melanoma as a model tumour for immuno-oncology

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Melanoma is one of the most aggressive forms of skin cancer. Furthermore, incidence rates are increasing. Until recently, no agent had been shown to improve survival over supportive care and treatment guidelines recommended that patients with metastatic disease were entered into clinical trials. With so few treatment options available, there was a clear need for new, more effective treatments in this setting. Melanoma serves as a ‘model’ tumour for understanding immunity to cancer. Melanoma tumour-associated antigens were among the first cancer antigens to be identified and classified, with further studies showing that many of these are also expressed by other tumour types. In addition, melanoma regression has been associated with vitiligo, visibly confirming an active role of the immune system in this type of cancer, and spontaneous regression of primary melanomas has also been observed in some cases. These observations, relating to the activity of the immune system in melanoma, provided strong evidence that this tumour would be amenable to immunotherapy, with immunotherapies such as cytokines, adoptive cell transfer and T-cell modulators shown to be an effective therapeutic approach. Against this background, melanoma has long been at the cutting edge of immuno-oncology research and will likely continue to be used as a model tumour to increase our understanding of immuno-oncology and to inform development in other types of cancer.

Key words: immuno-oncology, immunotherapy, melanoma, model, tumour-associated antigens, vitiligo

introduction

Although melanoma accounts for only 4% of all skin cancers, it causes the greatest number of skin cancer-related deaths worldwide. In 2008, there were 84 000 new cases of cutaneous melanoma and 20 100 deaths in Europe and 62 480 new cases and 8420 deaths in the United States [1, 2]. Despite prevention campaigns aimed at reducing the excessive sun exposure, the incidence of melanoma is increasing at a faster rate than most other cancers, particularly in young Caucasian women.

Early detection and excision of superficial cutaneous melanoma is the best means of reducing mortality. Once a patient develops metastatic disease, however, the prognosis is dismal. In a recent meta-analysis of phase II trials, 1- and 2-year overall survival rates in patients with metastatic melanoma were ~25% and 10%, respectively, and median survival time was 6.2 months (Figure 1) [3].

Before 2011, approved treatment options for patients with metastatic melanoma were limited to just chemotherapy and interleukin-2 (IL-2). Although chemotherapy with dacarbazine is the best established treatment, it has never been shown to improve survival over supportive care [4]. In addition, treatment with IL-2 is restricted to treatment centres with intensive care facilities and specialists, skilled in cardiopulmonary or intensive care medicine, to deal with the prevention and management of side effects [5].

Metastatic melanoma has therefore been a focus for the development and application of novel approaches, particularly as treatment guidelines recommend that patients with metastatic melanoma be preferentially enrolled on clinical trials for new therapies [4]. In the past two years, two agents have been shown to significantly improve the survival of patients with metastatic melanoma in phase III trials: ipilimumab, an antibody against cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4), and vemurafenib, an inhibitor of mutated BRAF protein kinase [6–8]. These improvements in treatment have been made possible due to an increased understanding of melanoma as a model tumour.

why use immunotherapy in metastatic melanoma?

Studies of melanoma have played a central role in understanding the immune response to cancer for a number of reasons, many of which are more practical than immunological. Investigations have largely been facilitated by the relative accessibility of melanoma lesions and the fact that melanoma is one of the easiest cancers to adapt to tissue culture, meaning tissue samples and cell lines are readily available for research [9].

However, a number of clinical observations relating to the activity of the immune system in melanoma also provide strong evidence that the immune system can naturally react to and destroy or control melanoma, justifying the use of immunotherapies to manipulate or enhance the immune response [10].

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tumour-associated antigens are expressed by melanoma cells

Tumour-associated antigens (TAAs), which are recognised by autologous antibodies and T cells and are capable of inducing tumour-directed immune responses, were identified and classified in melanoma earlier than in many other tumours of different histotypes [9, 11]. Melanoma-associated cancer antigens can be broadly categorised as differentiation antigens such as gp100, tyrosinase, Melan-A and the retained intron in tyrosinase-related protein (TRP-2-INT2) that are expressed by normal and malignant cells derived from melanocytes but not other cell types; cancer testis antigens (CTA) such as melanoma antigen-1 (MAGE-A1), the highly immunogenic tumour antigen NY-ESO-1 and preferentially expressed antigen of melanoma (PRAME) that are expressed in various tumour types, but not in normal tissue except testis and placenta, and mutated or aberrantly expressed molecules such as CDK4 and MUM-1 [12–16]. All of these TAAs are potential targets for immunotherapies. Therefore, because CTAs and mutated or aberrantly expressed molecules have been identified in the range of malignant histotypes, including solid tumours and haematological malignancies, the melanoma model is potentially transferable to a variety of cancers.

spontaneous tumour regression in patients with primary melanoma

Between 13.8% and 50% of primary melanomas undergo spontaneous regression, defined as the partial or complete disappearance of a malignant tumour in the absence of treatment or in the presence of therapy which is considered inadequate to exert a significant influence on neoplastic disease [17]. Regression occurs more frequently in melanoma than other tumours; however, this may simply be because melanomas are pigmented and most frequently develop on the skin and are therefore easier to visualise than internal cancers, such as lung or breast cancer (Figure 2A) [9, 18]. Nevertheless, there is indirect evidence that spontaneous regression is the result of an efficient immune response against melanoma cells [17], with cellular inflammation, primarily comprised of tumour infiltrating CD4+ and CD8+ T lymphocytes, having been observed in regressing melanomas (Figure 2B) [19].

As melanoma progresses, however, the immune system can alter the phenotype of the tumour in a process known as immunosculpting, resulting in the evolution of melanoma cells that can avoid immune destruction. This might explain why complete spontaneous or therapy-induced regression in metastatic disease is a very rare phenomenon [20, 21].

melanoma regression is associated with autoimmune skin depigmentation and vitiligo

Vitiligo is a disorder characterised by hypopigmented skin lesions that is thought to have a predominantly autoimmune aetiology, as evidenced by the presence of antibodies reactive against melanocyte differentiation antigens, including tyrosinase, gp100 and TRP, in the serum of vitiligo patients [22]. Vitiligo has been observed in melanoma patients, and several lines of evidence suggest that it may be associated with an antitumour response. For example, histological analyses of vitiligo biopsy specimens always show a lymphocytic infiltrate, which are almost exclusively T cells, and mostly CD8+. Furthermore, vitiligo-infiltrating lymphocytes have a clonal or oligoclonal T-cell receptor profile, possibly reflecting specific antigenic stimulation, and recognise differentiation antigens shared by normal melanocytes and melanoma cells. In addition, a higher frequency of melanoma-associated antigen-specific CD8+ reactivity has been observed in the peripheral blood of melanoma patients with vitiligo compared with melanoma patients without vitiligo [23].

This is in accordance with the frequent occurrence of vitiligo in melanoma patients receiving immunotherapy (Figure 3), which is generally considered a sign of good prognosis. For example, in a retrospective analysis of 374 patients with metastatic melanoma treated with high-dose IL-2, among 84 patients with vitiligo, one-third (28 patients) responded to therapy compared with 30 of 290 patients (10%) without vitiligo ($P < 0.0001$). These 28 patients comprised almost half of all patients with a response to IL-2 ($n = 58$) [24]. Similarly, in a study of 49 patients with metastatic melanoma treated with a maintenance regimen of IL-2 and granulocyte-macrophage colony-stimulating factor following induction with dacarbazine, cisplatin, vinblastine, IL-2 and interferon α-2b, 21 patients (43%) developed vitiligo and had a median overall survival from the start of maintenance therapy of 18.2
months compared with 8.5 months for the 28 patients without vitiligo \((P = 0.027)\) [25].

All these observations suggest that vitiligo in melanoma patients could be the visible consequence of a spontaneous antitumour immune response, which in turn leads to tumour regression.

**Clinical evidence of activity with immunotherapies**

Over the years, many immunotherapeutic approaches have been investigated in patients with melanoma, with varying clinical success. Immunotherapies used in the treatment of melanoma can be broadly categorised as adoptive or active and as specific or nonspecific (Figure 4). Adoptive therapies use components of the immune response that are generated ex vivo, whereas active therapies require the endogenous development of an immune response. Specific therapies use defined antigen sources, whereas in nonspecific therapies the antigen source is defined by the host [10].

Cytokines, for example, are nonspecific, active agents that induce T-cell activation and proliferation [26]. High-dose IL-2 has been shown to induce durable responses in a minority of patients with metastatic melanoma [27] and was approved by the Food and Drug Administration for the treatment of patients with this disease in 1998 [24].

Adoptive cell therapy (ACT) with tumour-infiltrating lymphocytes (TIL) is an example of a specific, adoptive approach to therapy that has proven to be an effective treatment of metastatic melanoma patients. ACT involves the identification and ex vivo growth of antitumour lymphocytes that are then infused into the cancer patient [28]. Lymphodepletion before ACT-based immunotherapy enhances antitumour responses by augmenting innate immunity, increasing access to homeostatic cytokines and depressing the numbers of lymphocytes or myeloid cells with immunosuppressive activity [28, 29]. ACT has been shown to result in the durable complete regression of bulky metastatic melanoma in patients refractory to approved treatments. Among 93 patients with refractory melanoma treated with TIL selected for tumour recognition following lymphodepletion, objective responses were seen in 50%–70% patients, including 15 patients who had complete responses, all but one of which were ongoing at 31–89 months. Despite promising clinical results, however, the extensive effort, cost and time required generating individual TIL cultures limit the use of ACT to only a few institutions [30].
Another active approach to treatment is to use monoclonal antibodies that target T cells. Ipilimumab and tremelimumab, for example, are monoclonal antibodies against CTLA-4 that augment T-cell activation and proliferation to enhance the immune response against tumours. In two recent phase III trials, ipilimumab significantly improved overall survival in pretreated and previously untreated patients, respectively, with hazard ratios for death of 0.68 for ipilimumab plus gp100 vaccine compared with gp100 alone (P < 0.001), 0.66 for ipilimumab alone compared with gp100 alone (P = 0.003) in pretreated patients and 0.72 for ipilimumab plus dacarbazine compared with dacarbazine alone (P < 0.001) in previously untreated patients [7, 8]. Based on the results in pretreated patients, in 2011 ipilimumab at a dose of 3 mg/kg became the first agent since IL-2 in 1998 in the United States, and fotemustine in 1989 in selected EU countries, to be approved for the treatment of patients with metastatic melanoma. Monoclonal antibodies against programmed death 1 (PD1) or its ligand (PD-L1) are also in development. The PD1/PD-L1 pathway is a negative regulator of T-cell proliferation and cytokine production; therefore, the blockade of PD-L1/PD1 increases the T-cell activation and the elimination of tumour cells [31].

Although immunotherapy holds much promise for the treatment of melanoma, it is important to note that it can be associated with novel mechanism-based toxic effects. For example, most TAAs are expressed by some cells of normal tissues; therefore, the transfer of TAA-specific T cells can lead to the induction of endogenous immunity against self-antigens and subsequent destruction of normal tissue. Alternatively, boosting the natural immune response against cancer may result in the expansion of self-reactive T cells [32, 33]. Finding a balance between the effectiveness of immunotherapies and the incidence of immune-related adverse events is therefore an important consideration in the field of immuno-oncology.

biomolecular characteristics of melanoma cells that may influence response to therapy

Because of the relatively low response rates and potential toxic effects associated with immunotherapies, it is increasingly important to characterise prognostic factors or biomarkers that can effectively be used to identify which patients would be most likely to respond to treatment [24]. At present, the best predictor of survival in patients with melanoma is the stage of disease. Patients with stage I melanoma, for example, have an overall survival rate of 95% compared with 7% for stage IV patients [34]. However, even within the same stage category, patients can behave very differently, and identifying those patients with highly aggressive or more indolent courses of disease is difficult [35]. The identification of prognostic biomarkers is therefore an important focus of research.

Transposable elements (TEs), for example, are discrete pieces of DNA that can move from site to site within genomes. Retrotransposons are one class of TE, characterised by the presence or the absence of long terminal repeats (LTRs). Non-LTR retrotransposons are typified by various elements, including long interspersed nucleotide element-1 (LINE-1) which constitutes ~17% of the human genome [36]. It has been postulated that LINE-1 could potentially act as a surrogate marker for genomic DNA as a whole. Genomic DNA hypomethylation is a frequent molecular event in cancer development and progression and mainly reflects the hypomethylation of repetitive genomic sequences, such as LINE-1. In a recent study of tumour cell cultures grown from patients with stage IIIC melanoma, LINE-1 hypomethylation identified melanoma patients with a significantly better prognosis compared with those with hypermethylated LINE-1 sequences, suggesting that evaluation of LINE-1 methylation levels may be useful in guiding the clinical management of patients with melanoma [35].

summary

Melanoma serves as a ‘model’ tumour for understanding immunity to cancer for many reasons. First, melanoma TAAs were among the first cancer antigens to be identified and classified; therefore, much is known about the antigenic profile of melanoma tumours. Secondly, there is clinical evidence that the immune system is capable of mounting an immune response against melanoma, which has been visibly confirmed with spontaneous or treatment-related vitiligo and the complete regression of primary melanomas in some patients. Thirdly, studies in melanoma have provided proof-of-principle that the immune system can be manipulated to fight cancer with immunotherapy clearly an effective therapeutic approach. As a result, immunotherapies for this cancer have been at the forefront of immuno-oncology research.

However, many questions remain unanswered regarding the optimisation and use of immunotherapy. For example, might immunotherapies be more effective if used when the disease burden is lower and the immune system less compromised by the tumour, what is the optimal combination and sequencing of treatment and how can surrogate or predictive biomarkers be used to determine the patient population that will most benefit from each type of therapy. It is likely, therefore, that melanoma will remain at the cutting edge of research, helping to improve our knowledge of immuno-oncology and informing development in other types of cancer.

disclosure

The author declares no conflicts of interest.

references


