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Feature Articles

Melanoma-associated Antigens

Stefan Carrel and Donata Rimoldi

INTRODUCTION

MALIGNANT MELANOMA is one of the few cancers for which the incidence and mortality is increasing every year [1]. Already in 1983, melanoma represented the second cause of death from cancer for Americans under 35 years of age. Projections suggest that melanoma will develop in one in nine Americans by the year 2000 [2]. In addition, nothing has modified the outcome of malignant melanoma in the past 70 years. Metastatic melanoma still represents one of the most difficult tumours to treat, the actual objective response rate of melanoma to chemotherapy being less than 25% [3], and the cure rate being virtually 0%. Fortunately, the cure rate of early melanoma results in a much better prognosis [4] and, therefore, an accurate early diagnosis of precursor lesions and of primary tumours is of crucial importance.

Human cutaneous malignant melanoma represents both clinically and experimentally one of the best studied human solid tumours, and studies on melanoma patients have long been at the forefront of these advances. The hope of tumour immunologists is that, in the near future it will become possible to engage the patient's immune system in the fight against his or her own tumour. This has come closer to reality with the increasing evidence that malignant cells differ from their normal counterparts in a manner which can be recognised by the immune system. In fact, many patients develop T lymphocytes which specifically recognise their own tumour cells [5]. Recently, the first gene encoding a tumour-associated antigen on human melanoma, recognised by cytolytic T lymphocytes was reported [6].

Malignant melanoma, like other types of cancer, can be considered as a disorder of cell differentiation and proliferation. Normal melanocytes arising from precursor melanoblasts usually undergo a series of differentiation events before reaching the final cell differentiation state [7]. Thus, a tumour can arise at any given stage of maturation when an arrest in the differentiation process is produced without loss in the proliferation capacity.

Early primary melanomas, characterised by radial growth and with limited vertical thickness (< 0.75 mm), show little tendency to metastasise, while conversely the potential for metastasis formation is associated with the onset of a vertical growth phase and correlates with increasing vertical thickness [8]. In addition to morphological features which characterise these stages, a series of monoclonal antibodies (MAbs) have been defined which identify surface markers on melanocytic lesions. The expression of these correlates with defined stages of tumour progression, and therefore may be of diagnostic use. With the help of MAbs, a whole series of biologically active structures have also been identified on human melanoma cells and these are discussed below.

Correspondence to S. Carrel.
The authors are at the Ludwig Institute for Cancer Research, Lausanne Branch, CH-1066 Epalinges, Switzerland.
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MELANOCYTIC LINEAGE MARKERS

Melanocytic lineage (differentiation) markers represent a group of molecules expressed almost exclusively by melanocytic cells and can, therefore, be used to distinguish melanocytes from other cell types [9]. This group of molecules further allows the characterisation of the various stages of melanocytic differentiation. As illustrated in Fig. 1a, these markers are expressed by the majority of cells in normal naevi, displastic naevi, early and advanced primary tumours and in metastatic lesions.

The standard pathological markers for melanocytic tumours include the S100 protein and HMB-45 [10, 11]. The calcium-binding S100 protein is a highly acidic cytoplasmic protein with a molecular weight of 21 kDa. In addition to neuronal crest-derived tissues, S100 proteins are also found on chondrocytes, adipocytes, dendritic cells of lymphoid tissue, Langerhans cells and T lymphocytes. They are expressed by a high proportion of melanomas and neurocytic cells. In the case of melanoma, the S100 molecule is of particular interest as a good diagnostic marker for secondary, amelanotic lesions, particularly for those which lack typical melanoma morphology [12].

Antibody HMB-45, directed against a cytoplasmic molecule, was found to react with more than 97% of melanomas and can, therefore, be added to the list of melanocytic lineage markers, especially since this antibody does not react with carcinomas or even other tumours of neuroectodermal origin such as gliomas, neuroblastomas and retinoblastomas [13]. Recent data suggest that the antigen recognised by the HMB-45 antibody family may be related to the product of the pmel 17 gene (possibly the mouse silver locus) [14].

Genetic methods have been used to identify gene products that can affect or determine the type of pigment produced by melanocytes. Tyrosinase and the tyrosinase-related genes are among the best characterised of these genes. Their products are specifically expressed in melanocytes at particular stages of differentiation [15]. Tyrosinase is a copper-binding enzyme encoded by the C or albino locus. A second member of the same family is the D, or brown locus protein, also called gp75 or TRP-1 [16, 17]. The gp75 is the most abundant glycoprotein in pigmented, melanocytic cells. Both tyrosinase and gp75 are transmembrane glycoproteins that are located in melanosomes, the organelles where melanin is synthesized. More recently, a

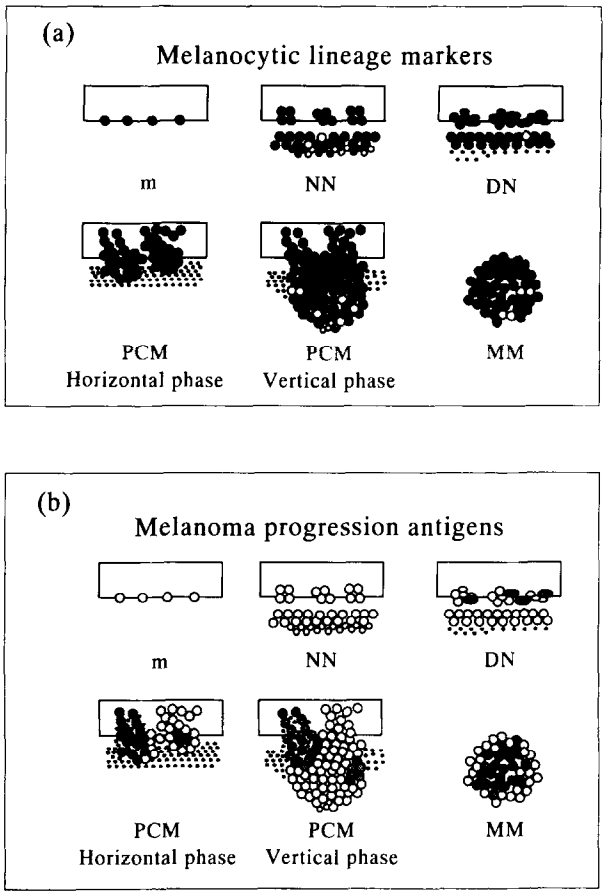

Fig. 1. (a) Schematic illustration of the expression of melanocytic lineage markers and (b) of melanoma progression antigens. Closed circles = positive cells. Open circles = negative cells. Boxes represent the epidermis; m, melanocyte; NN, naevocellular naevus; DN, dysplastic naevus; PCM, primary cutaneous melanoma; MM, metastasis.

number of other melanosomal components have been identified through MAbs, as is the case for HMSA-1 to 4, which recognise structural matrix proteins of melanosomes [14].

The high molecular weight melanoma-associated antigen (HMW-MAA), or chondroitin sulfate proteoglycan, encoded by a gene located on chromosome 15, represents one of the first lineage markers of the melanocytic lineage identified by MAbs [9]. The majority of the cells of more than 90% of melanomas cultured *in vitro* express chondroitin sulfate proteoglycan. Between 100 000 and 6 000 000 antibody binding sites are usually measured. From a multicentric MAb study it became clear that the HMW-MAA is expressed in the majority of small primary tumours (< 0.75 mm), and on the majority of cells within a tumour nodule [18]. A molecule with a molecular weight of 100 kDa, identified by MAb NKI-beteb, was found to have a similar distribution as the HMW-MAA [19].

At least four major melanoma-associated gangliosides have been identified, namely GD2, GM2, GD3 and GM3. These molecules have a remarkably restricted expression for tumours of the neural crest origin. Like oncofetal proteins, melanoma-associated gangliosides have been implicated in cell adhesion and appear to play a potentially important role in growth regulation and differentiation of melanoma cells [20, 21].

MELANOMA PROGRESSION ANTIGENS

Melanoma progression markers represent a large variety of molecules, the expression of which varies with tumour progression as well as with the differentiation state of the melanocytic cell. Progression antigens or molecules can be subdivided into two separate groups, namely those which are upregulated during tumour progression and those which are downregulated during progression. A non-exhaustive list of such antigens is presented in Table 1. Among the antigens which are upregulated, a number are expressed only on malignant melanocytes. These antigens are often almost absent in normal melanocytes or normal naevi, whereas they start to be expressed by a small percentage of cells within displastic naevi, as illustrated in Fig. 1.

Melanoma cells express a wide variety of adhesion molecules, including some members of the integrin family. The latter were found to be associated with tumour progression, since their expression increased markedly from small primary tumours to metastatic lesions. The expression of $\alpha 2\beta 1$ integrin is upregulated during melanoma tumour progression [22, 23] as is, to a lesser extent, the $\alpha 4\beta 1$ integrin VLA4 [24]. Another matrix adhesion molecule, the integrin $\alpha 5\beta 3$, was recently reported to be a specific marker for vertically invasive primary melanoma and distant metastases [25, 26]. Tumour cell lines lacking $\alpha 5$ gene expression were found to be significantly less tumorigenic in an experimental model using nude mice than $\alpha 5\beta 3$ expressing cells [26]. The intracellular adhesion molecule ICAM-1, a molecule which mediates adhesion between leukocytes and melanoma cells, was reported to be expressed on an increasing percentage of cells with tumour progression, since it was found to be preferentially present on cells of advanced primary tumours and metastasis [27, 28].

It is generally accepted that human leukocyte antigen (HLA) class II antigens can be considered as progression markers [18]. In primary melanomas with a Breslow thickness of less than 0.75 mm, HLA-DR positive cells were detected in less than 20% of the lesions, while up to 70% large primary tumours with a Breslow thickness of 3 mm or more were found to express HLA-DR molecules. Furthermore, a considerable heterogeneity exists in the percentage of positive cells even in lesions obtained from the same patient [18]. The occurrence of HLA-DR positive tumour cells was further associated with a higher metastatic potential of primary tumours, independently of the tumour thickness [13]. Expression of HLA-DR on primary melanoma cells appears to be involved in a cell-mediated immune response. Thus, tumour cells derived from early lesions which express class II antigens can stimulate autologous T cells to undergo blastogenesis when cocultured *in vitro*. On the other hand, cell lines from advanced lesions appear to lose the stimulatory capacity for autologous T cells, regardless of whether they express HLA-DR [29].

Melanoma cells express a wide variety of receptors for growth factors [30, 31]. There are at least six growth factor receptor systems active in human melanoma (some of which are related to tumour progression). For example, expression for the epidermal growth factor (EGF) receptor was found to be associated with advanced melanoma and, in agreement with this, EGF was shown to have mitogenic activity for metastatic melanoma cells [13]. The transferrin receptor can also be considered a progression marker since it is almost absent on normal or displastic naevi but is increasingly expressed in malignant lesions [18]. Receptors for the transforming growth factor (TGF)α and β are expressed constitutively by all melanoma

*Table 1. Melanoma-associated antigens**

Melanocytic lineage markers	Melanoma progression antigens	Candidate melanoma-associated antigens
S100	1. Upregulated:	Relevance for immune therapy
HMB-45	Adhesion molecules	MAGE-1/HLA-A1
Tyrosinase	α2 subunit	MAGE-3/HLA-A1
gp75	α4 subunit	Tyrosinase/HLA-A2
HMW-MAA	α5β3	
Melanin	ICAM-1	
NKI-beteb	Immunoregulatory molecules	
GD3	HLA-DR	
	Growth factor receptors	
	EGF-R, transferrin-R	
	Not yet defined	
	Muc18, Ki67	
	Matrix molecules	
	tenascin	
	2. Downregulated:	
	Adhesion molecule	
	α6 β1	
	Immunoregulatory molecules	
	HLA-A, B, C	
	Neuroglandular antigen	
	CD63	
	Growth factor receptors	
	c-Kit	

* Adapted from The International Consensus Conference on Human Melanoma Antigens held in Lausanne, Switzerland, 12–14 November 1992.

cells, but only the receptor for TGFα is present on normal melanocytes [13].

A relatively new progression marker, defined by monoclonal antibody Muc18, has recently been characterised both at the protein and at the DNA level. It was found that Muc18 represents a *N*-glycosylated glycoprotein of 113 kDa [32]. This molecule appeared to be expressed on 70–80% of all metastatic melanoma lesions examined. Although Muc18 is poorly expressed on benign lesions and on primary lesions of < 0.75 mm thickness, it is found with increasing frequency on metastatic melanomas. The cDNA encoding the core protein of Muc18 has been cloned and, similar to *N*-CAM, reveals three functional domains [33, 34].

There are few molecules that are known to be downregulated during tumour progression. It has become evident from several immunohistological studies that the number of cells within a given lesion which express HLA class I molecules decreases significantly from small primary lesions (up to 100% positive cells) to metastatic tumours (less than 50% positive cells) [18]. The assessment of HLA class I molecules on melanoma cells is crucial for an efficient application of active specific immunotherapy, since cellular immunity to melanoma antigens requires expression of HLA class I molecules by the tumour cells.

Numerous MAbs have been produced that recognise a heterogeneous glycoprotein family of 25–110 kD with a protein core of 20 kD [35]. Competition immunoprecipitation experiments confirmed that these MAbs detect different epitopes of the same molecule, which was found to be identical to CD23 [36]. The majority of primary melanomas express the CD63 glycoprotein. This antigen is located primarily in lysosomes, and because of its distribution in normal tissues, it was designated neuroglandular antigen (NGA). The CD63 glycoprotein might represent a marker for early stages of tumour progression. It is not detected on normal melanocytes but it is strongly expressed in displastic naevi and radial growth phase primary melanomas. Expression of CD63 is, however, weaker or sometimes even absent in more advanced stages of melanoma such as vertical growth phase primary melanomas and metastatic lesions [36, 37].

CANDIDATE MELANOMA-ASSOCIATED ANTIGEN

Lymphocytes specifically recognising autologous tumour cells *in vitro* can be generated from most melanoma patients. The clonal analysis of the T cell-mediated response to human melanoma has indicated that some of the cytolytic T lymphocyte clones (CTL) from a number of patients can recognise tumour-associated antigens (TAA) through a T-cell receptor and HLA restricted mechanism. The first gene encoding a melanoma-associated antigen has recently been identified [38]. This gene, named MAGE-1, directs the expression of an antigen recognised on the human melanoma cell line MZ2-Mel by autologous $CD8^+$ CTL. A nonapeptide encoded by a sequence in the third exon of the gene has been shown to bind to HLA-A1 molecules and to be recognised by MZ2-Mel specific CTLs [39]. MAGE-1 shows no significant homology to known sequences, but it does cross-hybridise with a number of other cDNAs, indicating that it is a member of a family of closely related genes. Among these MAGE-1, 2 and 3 are frequently expressed in melanoma cells.

Comparison of the MAGE-1 sequence found in melanoma with that found in normal cells has shown no difference. Expression of MAGE-1 has not yet been detected in normal adult tissues except testis [38]. MAGE-1 appears to be preferentially expressed in tumour cells, and has been detected in a variety of human tumours, including breast and lung cancer. Approximately 50% of all melanoma cell lines, 60% of glioblastoma and

30% of the neuroblastoma lines were found to express MAGE-1 [40]. As the expression of MAGE-1 is almost completely restricted to tumours, it represents a reasonable target for the immunotherapy of patients bearing the HLA-A1 allele. A pilot study has, therefore, been undertaken to determine whether vaccination increases the frequency of CTLs directed against the MAGE-1 antigen. HLA-A1 patients, whose tumours express MAGE-1 mRNA, are immunised with irradiated allogenic HLA-A1 melanoma cells which are recognised by anti-MAGE-1 CTLs. The effectiveness of the immunisation protocol can be monitored by measuring changes in the frequency of tumour- or antigen-specific CTL precursors present in the peripheral blood.

Several studies have suggested that HLA-A2, which is expressed by approximately 46% of the Caucasian population (as compared to 26% for HLA-A1), represents a major restriction element for melanoma-specific CTLs against shared as well as unique tumour antigens [41, 42]. A number of CTL lines from HLA-A2 melanoma patients have been established by culturing tumour infiltrating lymphocytes. These CTL clones were found to lyse 12 out of 18 HLA-A2 autologous and allogeneic melanomas, and none of 20 HLA-A2 negative melanomas or non-melanoma HLA-A2 positive cell lines. Lysis of autologous and allogeneic melanomas could be inhibited by antibodies against HLA-A2, indicating recognition of shared tumour antigens among the various melanoma cell lines in a HLA-A2 restricted manner [41, 43]. At present, at least two distinct antigens presented in association with HLA-A2 and common to many melanomas are being assessed and, therefore, constitute promising targets for future specific immunotherapy [43, 44]. Very recently one of them has been identified as tyrosinase (data presented at the 3rd International Conference on Melanoma, Venice 1993).

CONCLUSION

Clinical applications of melanoma antigens useful in the clinical management of patients include: (1) diagnosis of poorly differentiated melanocytic tumours, (2) differential diagnosis of benign and malignant lesions, and (3) assessment of prognosis. At present, the detection of melanoma markers in cell preparations and tissue sections has been incorporated into the common pathology practice in a number of institutions. As seen from a pathological perspective, melanoma-associated antigens can be tentatively classified into the three categories presented above, namely: (1) melanoma-associated lineage markers, (2) melanoma progression antigens and (3) candidate melanoma-associated antigens, including those melanoma-associated antigens associated with a well-defined cellular function [13].

Melanocytic lineage antigens have a preference for melanocytic lesions and are only rarely detectable in other cell types or the corresponding tumour. Presently, melanocytic lineage markers are commonly used in the diagnosis of poorly differentiated melanomas. In order to increase the efficacy of immunohistochemistry in the diagnosis of these tumours, panels of different antibodies have to be used, with each antibody expressing a different specificity and sensitivity for the different tumour types encountered in differential diagnosis.

Melanoma progression antigens have a preference for either a particular stage or only a few stages of the process of melanocytic neoplastic progression. Such antigens can be either up- or down-regulated during progression. It is important to mention that several studies have shown that the coexpression of progression markers is of a prognostic value. For example, coexpression of VLA-2 ($\alpha 2/\beta 1$) and HLA-DR indicates a risk for metastatic dissemination [22], as does HLA-DR with ICAM-1 [18]. Future studies on a large series of primary melanoma lesions have to be undertaken in order to establish the independent contribution of a given marker antigen. However, because of the complexity of this issue, it is not expected that one marker will be sufficient in this respect, but a panel of antibodies against several progression antigens may prove to be very helpful.

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Cancer Research Campaign Operation Manual for Control Recommendations for Products Derived from Recombinant DNA Technology Prepared for Investigational Administration to Patients with Cancer in Phase I Trials

R.H.J. Begent, K.A. Chester, T. Connors, D. Crowther, B. Fox, E. Griffiths, T.A. Hince, J.A. Ledermann, J.G. McVie, P. Minor, D.S. Secher, G. Schwartsmann, R. Thorpe, C. Wilbin and H. Zwierzina

1. INTRODUCTION

1.1 *Aim*

THE AIM of this document is to establish guidelines for the quality and safety of products produced in research departments by recombinant DNA technology, and to establish principles on which phase I trials of these agents will be conducted. It will also establish a basis of information which should be collected into a single document and submitted in support of phase I clinical trials to funding bodies, ethical committees and other interested parties.

Guidelines for phase I clinical trials of cytotoxic drugs, hormones and antibodies produced from hybridomas have been agreed by the Cancer Research Campaign Clinical Trials Committee. These have been used as a basis for the proposed