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INFREQUENT EXPRESSION OF THE *MAGE* GENE FAMILY IN UVEAL MELANOMAS

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It has previously been reported that *MAGE-1, -2, -3* and *-4* genes are expressed in human cancers including cutaneous melanoma. *MAGE-1* and *MAGE-3* represent targets for specific immunotherapy because they encode peptide antigens which are recognised by cytotoxic T lymphocytes (CTL) when presented by HLA class I molecules, and pilot clinical trials with these peptides are currently in progress. It is likely that other members of the *MAGE* gene family may also encode antigens recognised by CTL. Uveal melanomas, like cutaneous melanomas, arise from melanocytes that are derived from the neural crest. To determine if uveal melanoma patients would be suitable for MAGE-peptide immunotherapy, the expression of *MAGE-1, -2, -3* and *-4* genes was assessed by reverse transcription followed by polymerase chain reaction (RT-PCR) amplification and ethidium bromide staining. Expression of *MAGE* genes was not detected in any of 27 primary tumours. Either *MAGE-1* or *MAGE-4* was expressed in only 2 of 26 metastatic samples, but expression of *MAGE-2* or *-3* was not detected. Our data suggest that, unlike cutaneous melanomas, uveal melanomas may not be suitable candidates for MAGE-peptide immunotherapy.

The *MAGE* gene family consists of 12 closely related human genes, of which 6, *MAGE-1, -2, -3, -4, -6* and *-12,* are expressed in tumours of different histological types; with the exception of testis and placenta, they are not expressed in normal tissues (De Plaen *et al.,* 1994). Short peptides derived from the processing of *MAGE-1* and *MAGE-3* gene products are recognised by cytotoxic T lymphocytes (CTL) in the context of HLA class I molecules and therefore represent potential targets for specific immunotherapy of tumours (van der Bruggen *et al.,* 1991, 1994*a, b;* Traversari *et al.,* 1992; Gaugler *et al.,* 1994; Celis *et al.,* 1994).

The frequency of expression of *MAGE-1, -2, -3* and *-4* in cutaneous melanoma is 35, 58, 60 and 18%, respectively, with higher proportions of MAGE-positive tumours among metastatic lesions (48, 70, 76 and 22%, respectively) than among primary lesions (16, 41, 36 and 11%, respectively), suggesting a correlation between tumour progression and incidence of *MAGE* gene expression (Brasseur *et al.,* 1995). Members of the *MAGE* gene family are also expressed in breast carcinoma (Brasseur *et al.,* 1992; Russo *et al.,* 1995), non-small cell lung carcinoma (Weynants *et al.,* 1994; Shichijo *et al.,* 1995), bladder carcinoma (Patard *et al.,* 1995) and gastric carcinoma (Inoue *et al.,* 1995).

The expression of *MAGE* genes in uveal melanoma tissue has not previously been reported. Uveal melanomas occur with an overall incidence of 5–7/million/year (Shields, 1983); they constitute over 80% of all eye malignancies and are the most common fatal intraocular disease in adults (Egan *et al.,* 1988). Our study has determined the frequency of expression of *MAGE-1, -2, -3* and *-4* genes in both primary and metastatic uveal melanoma tissue.

Peptides processed from the melanocyte lineage-specific proteins tyrosinase, gp100/Pmel17 and Melan-A/MART-1, which are present in cutaneous melanoma, have been shown to induce CTLs (Brichard *et al.,* 1993; Bakker *et al.,* 1994; Coulie *et al.,* 1994; Kawakami *et al.,* 1994). Since these antigens may represent additional candidates for peptide immunotherapy of melanoma, the expression of the genes encoding these antigens in uveal melanoma was also assessed in our study.

MATERIAL AND METHODS

Sample collection

Primary uveal melanoma tissue was frozen in the vapour phase of liquid nitrogen immediately following enucleation. Metastatic tumour biopsies were frozen in liquid nitrogen immediately after removal (in Sheffield and Brussels) or immediately following pathological dissection (in Lausanne). All samples were subsequently stored at −80°C until use. Samples were collected in the UK (n = 28), Switzerland (n = 16), Belgium (n = 5) and France (n = 4).

RNA extraction and cDNA synthesis

Total RNA was isolated from tissue samples by the guanidine-isothiocyanate/caesium-chloride procedure (Davis *et al.,* 1986). To synthesise cDNA, 2 μg of total RNA were mixed with 0.5 μl of oligo-$(dT)_{12-18}$ at 0.5 μg/μl (Pharmacia Biotech, St. Albans, UK), 0.5 μl of RNAguard at 40 U/μl (Pharmacia), 4.0 μl of 5 × First Strand buffer (Life Technologies, Paisley, UK), 2.0 μl of 0.1 M DTT, 0.5 μl of each dNTP at 40 mM each, 1.0 μl of Superscript reverse transcriptase at 200 U/μl (Life Technologies) in a total volume of 20 μl and incubated for 2 hr at 44°C. The mixture was then diluted to 100 μl with water and stored at −20°C. This procedure was applied in Brussels and Lausanne with the following modifications: M-MLV reverse transcriptase (Life Technologies) was used instead of Superscript reverse transcriptase and the reaction was incubated at 42°C for 60 min, as described by De Smet *et al.* (1994).

PCR amplification

The presence of cDNA for *MAGE-1, -2, -3* and *-4, tyrosinase, Melan-A/MART-1* and *gp100/Pmel17* was determined by PCR amplification in a 50 μl reaction volume containing 5.0 μl of cDNA, 5.0 μl of 10 × PCR buffer (Perkin-Elmer Cetus, Norwalk, CT), 200 μM of each dNTP, 0.8 μM of each primer (see below), and 1.25 units of AmpliTaq DNA polymerase (Perkin-Elmer Cetus). Reaction mixtures were heated to 94°C for 4 min and subjected to the amplification programmes listed in Table I using a thermal cycler (Sheffield and Lausanne: DNA Thermal Cycler 480, Perkin-Elmer Cetus; Brussels:

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TABLE I – PCR AMPLIFICATION PROGRAMMES

Gene	Temperature and duration			Cycle number
	Denaturation	Annealing	Extension	
MAGE-1[1]	94°C for 1 min	72°C for 1 min	72°C for 2 min	30
MAGE-2	94°C for 1 min	67°C for 2 min[2]	72°C for 2 min[2]	30[3]
MAGE-3[1]	94°C for 1 min	72°C for 2 min	72°C for 2 min	30[3]
MAGE-4	94°C for 1 min	68°C for 2 min[2]	72°C for 2 min[2]	30
Tyrosinase	94°C for 1 min	65°C for 2 min	72°C for 2 min	25[4]
Melan-A/MART-1	94°C for 1 min	60°C for 2 min	72°C for 2 min	24[4]
gp100/Pmel 17	94°C for 1 min	60°C for 2 min	72°C for 3 min	22[5]
PBGD	94°C for 0.5 min	72°C for 0.5 min	72°C for 1.5 min	33
β-actin	94°C for 1 min	68°C for 2 min	72°C for 2 min	20

[1]In Lausanne, combined duration of annealing and extension steps was 1.5 min.–[2]1 min in Lausanne.–[3]33 cycles in Sheffield.–[4]28 cycles in Lausanne.–[5]25 cycles in Lausanne and Sheffield.

TABLE II – SEQUENCES OF OLIGONUCLEOTIDE PROBES USED FOR SOUTHERN BLOTTING

Gene	Probe	Sequence	Exon
MAGE-1	KAM-1	5′-CAACCCAGAGGACAGGATTCC-3′	2
MAGE-2	KAM-2	5′-TGCCCGCACTCCTGCCTGCT-3′	4
MAGE-3/6	CHO-3[1]	5′-GCCAATCCTATGAGGACTC-3′	3
MAGE-4	KAM-4	5′-CCCCATTGCCCAGCTTTTGCCTGC-3′	3

[1]CHO-3 taken from van der Bruggen *et al.* (1991). This probe cross-hybridises to the gene *MAGE-6*, whose sequence is 99% identical to that of *MAGE-3* (De Plaen *et al.*, 1994).

Trio-Thermoblock, Biometra, Göttingen, Germany). Amplification was concluded with a final extension at 72°C for 15 min.

To ensure that the sensitivity of the assays was the same in all 3 laboratories, a 1:100 dilution of positive control RNA was distributed and assayed using RT-PCR to determine the exact number of PCR cycles necessary to obtain a faint yet clearly discernable band on agarose gels when detected by ethidium bromide staining. Table I lists the PCR cycle numbers determined by this procedure. These cycle numbers were used for RT-PCR analysis of RNA from tumour samples.

RNA integrity was verified in each sample by performing PCR amplification of the cDNA with primers for β-*actin* (De Smet *et al.*, 1994) or porphobilinogen deaminase (*PBGD;* Finke *et al.*, 1993).

Detection of PCR products

Following amplification, PCR products were size-fractionated in 1–2% agarose gels containing ethidium bromide, and nucleic acids were visualised using UV irradiation.

To detect very low levels of *MAGE* gene expression (less than 1% of the levels found in the reference cell lines), the following approach was employed in Sheffield. Samples were amplified by PCR for 33–34 cycles, subjected to size fractionation in 1–2% agarose gels, Southern blotted onto Hybond-N nylon membranes, probed with digoxigenin-labelled oligonucleotides (see below), processed according to the digoxigenin luminescence detection kit for nucleic acids (Boehringer Mannheim UK, Lewes, UK) and exposed to Hyperfilm-MP autoradiography film (Amersham, Aylesbury, UK).

Oligonucleotide primers for PCR amplification

Primers were specific for each gene. To prevent false positives due to genomic DNA contamination of RNA preparations, primers corresponded to sequences located in different exons. For primer sequences see Patard *et al.* (1995; *MAGE* genes), De Smet *et al.* (1994; *β-actin*), Brasseur *et al.* (1995; *tyrosinase, gp100/Pmel17* and *Melan-A/MART-1*) and Finke *et al.* (1993; *PBGD*).

Oligonucleotide probes for Southern blotting

Sequences for oligonucleotide probes KAM-1, -2, -4 and -PB were selected and tested for specificity using the Program Manual for the Wisconsin Package (Version 8, August 1994, Genetics Computer Group, Madison, WI) and the Program Manual for the EGCG Package (P. Rice, The Sanger Centre, Hinxton Hall, Cambridge, UK), accessed via the Seqnet service of the Daresbury Laboratory (UK). Oligonucleotides were synthesised and labelled with digoxigenin by R&D Systems (Abingdon, UK). Sequences are listed in Table II.

Control RNA samples

Control RNA samples were included in each RT-PCR run. RNA prepared from melanoma cell line MZ2-MEL-3.0 which expresses *MAGE-1, -2* and *-3,* but not *MAGE-4,* and sarcoma cell line LB23-SAR which expresses *MAGE-4* only, were used as controls for the expression of *MAGE* genes (De Plaen *et al.*, 1994). For the expression of *tyrosinase, gp100/Pmel17* and *Melan-A/MART-1,* RNA prepared from melanoma cell line SK-MEL-23 (obtained from Dr. L.J. Old, Memorial Sloan-Kettering Cancer Center, New York, NY), which expresses all 3 of these genes, and LB23-SAR, which does not express these genes, were used as controls.

RESULTS

Twenty-seven primary and 26 metastatic lesions of uveal melanoma were included in our study. With the exception of Mel 115 and its related liver metastasis (OMLM-3), no primary tumour was related to any metastasis, and each metastasis was obtained from a different patient. Twenty four of 25 primary tumours had a diameter greater than 10 mm which has been shown to confer poor prognosis (Shammas and Blodi, 1977); Mel 120 had a diameter of 6.5 mm; the diameter was not determined for tumours CF4 and LB1056.

Expression of *MAGE-1, -2, -3* and *-4* in tumour samples was assessed using reverse transcription followed by polymerase chain reaction (RT-PCR) amplification using oligonucleotide primers specific for each gene (see Material and Methods). *MAGE* gene expression was not detected in any of 27 primary tumours (Table III). Of 26 metastatic lesions, only 1 (OMLM-3) expressed *MAGE-1* and 1 (CF4) expressed *MAGE-4,* but *MAGE-2* and *-3* expression was not detected (Table IV).

The level of *MAGE-1* expression in sample OMLM-3 was estimated to be 1% of the level expressed by reference cell line MZ2-MEL-3.0 (Fig. 1); in sample CF4, the level of *MAGE-4*

TABLE III – RT-PCR ANALYSIS FOR THE EXPRESSION OF GENES *MAGE-1, -2, -3* AND *-4, tyrosinase, Melan-A/MART-1* AND *gp100/Pmel17* IN PRIMARY UVEAL MELANOMAS[1]

Tumour	*MAGE* gene 1	2	3	4	*tyro-sinase*	*gp100/ Pmel17*	*Melan-A/ MART-1*
Choroid							
Mel 87	−	−	−	−	±	±	++
Mel 89	−	−	−	−	++	+++	+++
Mel 90	−	−	−	−	++	+++	+++
Mel 91	−	−	−	−	+++	+++	++
Mel 93	−(±)	−	−	−	−	−	−
Mel 94	−	−	−	−	+++	+++	+++
Mel 95	−	−	−	−	+++	+++	+++
Mel 98	−	−	−	−	+	+++	+++
Mel 102	−	−	−	−	+++	+++	+++
Mel 110	−	−	−	−	+	+++	+++
Mel 118	−	−	−	−	+	+++	++
Mel 119	−(±)	−	−	−	++	+++	+++
Mel 123	−	−	−	−	+++	+++	+++
Mel 128	−	−	−	−	+++	+++	+++
CP4	−(±)	−	−	−	+++	+++	+++
LB1056	−	−	−	−	+++	+++	+++
Ciliary body							
Mel 96	−	−	−	−	+++	+++	+++
Mel 100	−	−	−	−	+++	+++	+++
Mel 103	−	−	−	−	+++	+++	+++
Mel 115	−	−	−	−	±	±	+++
Mel 120	−	−	−	−	+	+++	+++
Mel 121	−	−	−	−	±	+++	+++
CB/Choroid[2]							
Mel 105	−	−	−	−	+++	+++	+++
Mel 109	−	−	−	−	+++	+++	+++
Mel 112	−	−	−	−	++	+	++
Mel 113	−	−	−	−	+++	+++	+++
Mel 124	−	−	−	−	++	+++	+++

[1]Gene expression levels were scored according to band intensity of PCR products fractionated in agarose gels: +++, strong; ++, moderate; +, weak; ±, very weak. For *MAGE* genes, these symbols represent expression levels that were approximately the following, in percent of those found in the reference cell lines (see Material and Methods): +++, ≥100%; ++, 50% to <100%; +, 10% to <50%; ±, 1% to <10%; −(±), <1%; −, no gene expression detected using our conditions.–[2]Tumour location is classified as CB (ciliary body)/choroid if it is unclear whether the tumour originated from the ciliary body or the choroid.

TABLE IV – RT-PCR ANALYSIS FOR THE EXPRESSION OF GENES *MAGE-1, -2, -3* AND *-4, tyrosinase, Melan-A/MART-1* AND *gp100/Pmel17* IN UVEAL MELANOMA METASTASES[1]

Tumour[2,3]	*MAGE* gene 1	2	3	4	*tyro-sinase*	*gp100/ Pmel17*	*Melan-A/ MART-1*
Choroid							
OMLM-1	−	−	−	−	+++	+++	+++
OMLM-2	−	−	−	−	+++	+++	+++
LAU46	−(±)	−	−	−(±)	+++	+	+++
LAU49	−(±)	−	−	−	+++	+++	+++
LAU73	−	−	−	−	−	±	++
LAU88	−	−	−	−(±)	+++	−	+++
LAU110	−	−	−	−	+++	++	+++
LAU114	−	−	−	−	+++	++	+++
LAU179	−	−	−	−	+++	+	+++
LAU181	−(±)	−	−	−	+	−	+++
LAU182	−	−	−	−	+++	+	+++
CF4	−	−	−	±	+	+++	+++
LB780	−	−	−	−	+++	+	+++
LB781	−	−	−	−(±)	+++	++	+++
LB1068	−	−	−	−	+++	+++	+++
Ciliary body							
OMLM-3	±	−	−	−	+++	+++	+++
LAU26	−	−	−	−	+++	+	+++
LAU95	−(±)	−	−(±)	−(±)	−	+++	+++
LAU172	−	−	−	−(±)	+++	+++	+++
LAU180	−(±)	−	−	−	+++	+	+++
CB/choroid[4]							
LAU28	−	−	−	−	+++	++	+++
LAU83	−	−	−(±)	−	+++	+++	+++
LAU113	−	−	−	−	+++	+	+++
Uvea[5]							
LB687	−	−	−	−	++	+	++
LB967	−	−	−	−(±)	++	++	+++
LB1203	−(±)	−	−	−	++	±	+++

[1]For notation of gene expression, see Table III (footnote 1).–[2]Samples were all liver metastases with the exception of LB687 and LAU26 (cutaneous tissue), LB1068 (subcutaneous tissue) and LB780 (peritoneum).–[3]Samples are listed according to site of origin of primary tumour.–[4]See Table III (footnote 2).–[5]Exact uveal site unknown.

expression was estimated to be 1% of the level expressed by the reference cell line LB23-SAR (data not shown). To determine whether, in uveal melanoma, *MAGE* genes were expressed at levels below 1% of that found in the reference cell lines, two approaches were employed. In Sheffield, PCR products were Southern blotted and probed using digoxigenin-labelled oligonucleotides (see Material and Methods). In Brussels and Lausanne, samples were amplified by PCR for 35 cycles and visualised in agarose gels using ethidium bromide fluorescence. It was found that in addition to samples OMLM-3 and CF4, 9 other tumours (3 primaries and 6 liver metastases) expressed very low levels of *MAGE-1,* 6 other liver metastases expressed very low levels of *MAGE-4,* 2 other liver metastases expressed very low levels of *MAGE-3,* but expression of *MAGE-2* was not detected (Tables III, IV, Fig. 1). Southern blotting also revealed that reference cell line LB23-SAR expresses very low levels of *MAGE-1* (Fig. 1), but expression of *MAGE-2* and *-3* was not detected (data not shown).

In contrast to cutaneous melanomas, haematogenous dissemination to the liver is a frequent form of metastatic spread by uveal melanomas. Since only 2 of 22 uveal melanoma liver metastases were found to express *MAGE* genes at levels ≥1% of that found in the reference cell lines, biopsies of cutaneous melanoma liver metastases were also analysed for *MAGE* gene expression to determine whether the low incidence of *MAGE* gene expression in metastatic uveal melanoma liver biopsies was due to their location. As shown in Table V, 4 of 6 liver metastases from cutaneous melanomas were shown to express *MAGE* genes at levels above 1% of that found in the reference cell lines.

Since *MAGE* genes were expressed infrequently by uveal melanomas when compared with their cutaneous counterparts, the expression of *tyrosinase, Melan-A/MART-1* and *gp100/Pmel17* was also assessed. With the exception of Mel 93, at least 2 of these 3 genes, which encode melanocytic lineage-specific proteins (for review, see Pardoll, 1994) were expressed at high levels in every sample of primary and metastatic uveal melanoma (Tables III, IV, Fig. 1). Mel 93 did not express any of these 3 genes even though it was confirmed histologically as a choroidal melanoma and had been found to express very low levels of *MAGE-1.*

DISCUSSION

Our data reveal that none of 27 samples of primary uveal melanoma expressed any of *MAGE-1, -2, -3* or *-4* genes. Of the 26 metastatic samples, one (3.8%) expressed *MAGE-1* and another expressed *MAGE-4,* but expression of *MAGE-2* and *-3* genes was not detected. Using more sensitive detection methods, it was found that 3 primary tumours (11.1%) and 6 metastatic lesions (23.1%) expressed very low levels of *MAGE-1,* 2 metastatic lesions (7.7%) expressed very low levels of *MAGE-3* and 6 metastatic lesions (23.1%) expressed very low levels of *MAGE-4,* but *MAGE-2* expression was not

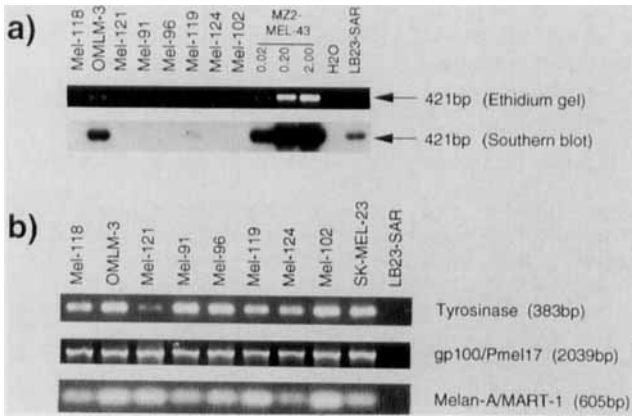

FIGURE 1 – Expression of *MAGE-1, tyrosinase, gp100/Pmel17* and *Melan-A/MART-1* in uveal melanomas. Reverse transcription and polymerase chain reaction amplification were performed as described in Material and Methods. *(a)* Expression of *MAGE-1*. Positive control cDNA from cell line MZ2-MEL-43 (a clone derived from reference cell line MZ2-MEL-3.0) was prepared from 0.02, 0.2 and 2.0 μg of RNA/20 μl cDNA reaction. PCR products were analysed by ethidium bromide fluorescence and Southern blotting. *(b)* Expression of *tyrosinase, gp100/Pmel17* and *Melan-A/MART-1*. PCR products were visualised by ethidium bromide fluorescence.

TABLE V – RT-PCR ANALYSIS FOR THE EXPRESSION OF GENES *MAGE-1, -2, -3* AND *-4, tyrosinase, Melan-A/MART-1* AND *gp100/Pmel17* IN CUTANEOUS MELANOMA LIVER METASTASES[1]

Tumour	*MAGE* gene				*tyro-sinase*	*gp100/ Pmel17*	*Melan-A/ MART-1*
	1	2	3	4			
LB998	–	++	+++	–	+++	++	++
LB1019	+	++	++	+++	+	–	±
LB1069	–	–	–	–	+	–	±
LB1219	–	–	–	–	–	+++	+++
LAU9	+	++	+	–	+++	+	+
LAU149	++	+	+++	–	+++	+	++

[1]For notation of gene expression, see Table III (footnote 1).

detected. In agreement with Russo *et al.* (1995), the reasons why some samples display very low-level expression of *MAGE* genes could be: 1) the level of *MAGE* gene expression in all the tumour cells in the lesion is low; 2) only a subpopulation of tumour cells in the lesion may express *MAGE* genes, but the level of expression in these cells may be high; or 3) the RNA of the tumour cells is significantly diluted by RNA from non-malignant cells in the sample, such as stromal cells or extraneous normal cells. This last hypothesis seems unlikely since the expression of *tyrosinase, gp100/Pmel17* and *Melan-A/MART-1*, which are only expressed by cells of melanocytic origin, was readily detectable.

In the present study, 22 liver metastases from uveal melanoma were analysed. The expression of *tyrosinase, gp100/Pmel17* or *Melan-A/MART-1* in all of these liver biopsy samples confirms that melanoma tumour material was present in the biopsy. However, only 2 of 22 (9%) uveal melanoma liver metastases expressed *MAGE* genes at levels equal to 1% of that found in the reference cell lines, 11 of 22 expressed *MAGE* genes at extremely low levels (less than 1% of that found in the reference cell lines) and no *MAGE* gene expression was detected in the remaining 9 samples. This contrasts with our observation that, in 4 of 6 (67%) liver metastases from cutaneous melanomas, high-level expression of several *MAGE* genes was detected. Thus, the low incidence of *MAGE* gene expression in uveal melanoma liver metastases does not appear to be related to their particular growth environment or to the ability of tumour cells to home to this site, but is rather a characteristic of the primary tumour. Although they derive from similar precursor melanocytes, uveal and cutaneous melanomas are often considered separate tumour entities since significantly different cytogenetic alterations are observed between the two tumour types (Trent *et al.*, 1989). However, recent data have indicated some cytogenetic similarities, suggesting the involvement of common genes (Speicher *et al.*, 1994).

Another consideration is that the anterior chamber of the eye is an unique micro-environment such that systemic tolerance to immunogenic tissues, including tumour cells, is induced if they are placed within this site (for review, see Streilein, 1994). Since these uveal melanomas are formed within an immune-privileged site they may be able to escape eradication by the immune system even though they express tumour antigens. Tumours which arise in this site may not be subjected to the same selective pressure as tumours which arise in non-privileged sites. Thus, uveal melanomas may not aberrantly express as many genes (*e.g., MAGE* genes) as other solid tumours. Indeed, the numbers of cytogenetic alterations in uveal melanomas are often less extensive than those observed in other solid tumours (Mitelman, 1994). Knowledge of the factors required for the expression of *MAGE* genes may help to explain the difference in the incidence of *MAGE* gene expression between uveal and cutaneous melanomas.

Using primary explants of uveal melanoma cells, it has previously been reported that the *MAGE-1* gene is expressed in a greater proportion of uveal melanomas (69%) compared with their cutaneous counterparts (37%) (Chen *et al.*, 1995). On the other hand, the data presented here show that only a small proportion of uveal melanomas express *MAGE* genes. The difference in the frequency of expression of the *MAGE-1* gene between primary explant cultures and freshly isolated tumour tissue suggests that culture of uveal melanoma cells *in vitro* may lead to the activation of *MAGE* genes. Thus, to identify patients suitable for MAGE-peptide immunotherapy, it would not appear to be advisable to assess the expression of *MAGE* genes from cultures of patients' tumour cells. Instead, *MAGE* gene expression should be assessed using freshly isolated tumour tissue.

The data presented here on the expression of *MAGE* genes in uveal melanoma would appear to exclude uveal melanoma patients from MAGE-peptide-based vaccine therapies since all MAGE-positive tumours expressed very low levels of *MAGE* genes ($\leq 1\%$ of that found in the reference cell lines). This level of expression is unlikely to initiate lysis of tumour cells by CTLs since it has been suggested that lysis by anti-MAGE-1 CTL occurs only in cell lines expressing this gene at levels $\geq 10\%$ of that found in the reference cell line (Brasseur *et al.*, 1995). However, other potential CTL targets may be expressed on these tumours. Our data show that uveal melanomas express high levels of the melanocyte lineage-specific genes *tyrosinase, Melan-A/MART-1,* and *gp100/Pmel17*. CTLs have been generated against cutaneous melanoma cells which recognise peptides encoded by these genes, in association with MHC class I molecules on the cell surface (Brichard *et al.*, 1993; Bakker *et al.*, 1994; Coulie *et al.*, 1994; Kawakami *et al.*, 1994). If systemic tolerance to uveal melanoma antigens can be overcome, patients with disseminated uveal melanoma may benefit from vaccines based on these antigens.

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