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Sequence and expression pattern of the human *MAGE2* gene

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Abstract. We reported previously identification of the human *MAGE1* gene, which encodes an antigen recognized on human melanoma MZ2-MEL by autologous cytolytic T lymphocytes. In addition to *MAGE1*, melanoma MZ2-MEL expresses several closely related genes, one of which has been named *MAGE2*. The complete *MAGE2* sequence was obtained and it comprises 3 exons homologous to those of *MAGE1* and an additional exon homologous to a region of the first *MAGE1* intron. Like the open reading frame of *MAGE1*, that of *MAGE2* is entirely encoded by the last exon. The *MAGE1* and *MAGE2* sequences of this exon show 82% identity and the putative proteins show 67% identity. The *MAGE2* gene is expressed in a higher proportion of melanoma tumors than *MAGE1*. It is also expressed in many small-cell lung carcinomas and other lung tumors, laryngeal tumors, and sarcomas. No *MAGE1* and *MAGE2* gene expression was found in a large panel of healthy adult tissues, with the exception of testis.

Introduction

When the blood lymphocytes of a melanoma patient are stimulated in vitro with an autologous tumor cell line, it is often possible to obtain cytolytic T cells (CTL) that lyse the tumor cells and do not lyse the healthy cells of the same patient. From these responder T-cell populations it is possible to isolate anti-tumor CTL clones (Mukerji and McAlister 1983; De Vries and Spits 1984; Knuth et al. 1984). Such a panel of CTL clones has been obtained against the human melanoma cell line MZ2-MEL (Hérin et al. 1987). These CTL clones were used to select antigen-loss variants and this led to the definition of several different antigens on the MZ2-MEL cells, one of which was named MZ2-E (Van den Eynde et al. 1989). To identify the gene directing the expression of antigen MZ2-E, a cosmid library prepared with the DNA of the tumor cells was transfected into an E$^-$ antigen-loss variant. This led to the identification of the *MAGE1* gene, which transfers the expression of this antigen (van der Bruggen et al. 1991; Traversari et al. 1992b). The gene is about 4.5 kilobases (kb) long and comprises three exons. The third exon contains an open reading frame coding for a protein of 309 amino acids. A nonapeptide encoded by this open reading frame combines to the class I major histocompatibility complex (MHC) molecule HLA-A1 to form antigen MZ2-E (Traversari et al. 1992a). The *MAGE1* gene was found to be expressed in several melanoma tumors and in other types of tumors as well. No expression was found in a panel of healthy tissues (van der Bruggen et al. 1991; Brasseur et al. 1992), but we report here that the gene is expressed in testis.

When we analyzed *MAGE1* gene expression in the MZ2-MEL cell line, we found two other species of messenger RNA that hybridized with a *MAGE1* probe. The corresponding genes were named *MAGE2* and *MAGE3*. Because these genes may also code for tumor rejection antigens recognized by cytolytic T cells, it seemed worthwhile obtaining a complete description of their structure and expression. We report here the complete *MAGE2* gene sequence and its pattern of expression in a panel of healthy and tumor tissues.

The nucleotide sequence data reported in this paper have been submitted to the GenBank nucleotide sequence database and have been assigned the accession number L18920.

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Materials and methods

Cell lines. The melanoma cell line MZ2-MEL was derived from an abdominal metastasis of patient MZ2 (Hérin et al. 1987). A number of subclones such as MZ2-MEL.43 and MZ2-MEL.3.1 were obtained. The subline MZ2-MEL.2.2, which does not express antigen MZ2-E, was obtained after repeated selection of MZ2-MEL.3.1 with an autologous anti-E CTL clone (Van den Eynde et al. 1989).

Hybridization with oligonucleotides. Oligonucleotides were labeled, using terminal deoxynucleotidyl transferase (BRL, Gaithersburg, MD) and [α-^{32}P] dCTP (3000 Ci/mmole; Amersham, Arlington Heights, IL) to a specific activity of ~2.10^8 cpm/µg. The probes were purified on Chroma Spin-10 columns (Clontech, Palo Alto, CA). Nitrocellulose filters were prehybridized for 2 h at 65° C in 10 × Denhardt's solution, 6 × standard sodium citrate (SSC) and hybridized overnight to the labeled oligonucleotides (10^6–3.10^6 cpm/ml) in 50 µl/cm^2 of 3.5 × SSC, 1 × Denhardt's solution, 25 mM sodium phosphate buffer (pH 7.0), 0.5% sodium dodecyl sulfate (SDS), 2 mM ethylenediaminotetraacetate, and 100 µg/ml denatured salmon sperm DNA at a temperature that was 5° C below the dissociation temperature of the oligonucleotide. After hybridization, the filters were washed twice at room temperature for 5 min each in 6 × SSC and then twice in 6 × SSC, 0.1% SDS at the dissociation temperature of the oligonucleotide probe for 5 min each.

Screening of the genomic library. The genomic library was derived from the MZ2-MEL.43 melanoma cell line as previously described (van der Bruggen et al. 1991). About 50 ng of DNA of each cosmid group was submitted to polymerase chain reaction (PCR) amplification in a 100 µl reaction mixture, using the GeneAmp PCR reagent kit (Perkin Elmer, Norwalk, CT) and, as primers, the previously defined oligonucleotides CHO-8 and CHO-9 (van der Bruggen et al. 1991). Each PCR reaction (18 µl) was then size-fractionated in agarose gels, blotted on nitrocellulose filters, and hybridized with oligonucleotide probe CHO-2 (van der Bruggen et al. 1991). A positive group of cosmids was then screened by colony hybridization with the CHO-2 probe (Sambrook et al. 1989).

Sequencing. Cosmid C6.3 was digested with *Bam* HI and *Pst* I, size-fractionated in agarose gels, and blotted on nitrocellulose filters. The blotted material was then hybridized with the oligonucleotides CHO-2, CDS-2, 5′-AGAACCACTGCATCTGTCGAC-3′ (*MAGE2*, positions 1375–1355 anti-sense), or CDS-1, 5′-CCAATAATCCAGCGCTGCCT-3′ (*MAGE2* positions 71–90 sense). The hybridizing fragments were subcloned in pTZ18R and single-stranded DNA was produced. The nucleotide sequence was determined, using the T7 Sequencing kit (Pharmacia Piscataway, NJ) and synthetic primers. Sequence alignments with *MAGE1* were performed with the GeneWorks computer program (Intelligenetics, Mountain View, CA). Sequence identity was estimated as the percentage of matching nucleotides contained in the aligned sequence.

Rapid amplification of the 5′ cDNA end. The rapid amplification of the 5′ cDNA end of *MAGE2* mRNA was performed essentially as described by Frohman and co-workers (1988). The primer for the synthesis of the first cDNA strand was 5′-ACTTCCACTAGAGTAG-3′ (*MAGE2* positions 2582–2567 anti-sense). For the amplification, we used 5′-GACCTGAGTCACCTTCTGAAAAC-3′ (*MAGE2* positions 1335–1313 anti-sense) as 3′ primer and the primers described by Frohman and co-workers (1988) as 5′ primers. The amplified products were cloned in pTZ18R, using the *Sal* I site of the 5′ primer and a *Pst* I site located in the *MAGE2* sequence at position 280. The resulting clones were screened with the CDS-1 oligonucleotide probe, and five positive clones were sequenced.

Southern blot analysis. DNA extraction, electrophoresis, transfer to nitrocellulose filter, labeling of the *MAGE1* 2.4 kb *Bam* HI fragment, and hybridization were performed as described (Lurquin et al. 1989), except for the last step of washing, which was performed in 0.5 × SSC and 0.5% SDS at 65° C.

mRNA expression analysis. Total RNA was extracted by the guanidine-isothiocyanate procedure (Davis et al. 1986). For cDNA synthesis, total RNA (2 µg) was diluted to a total volume of 20 µl with 4 µl 5 × HR-T buffer (BRL), 4 µl dNTPs at 2.5 mM each, 2 µl 0.1 M DTT (BRL), 1 µl of a 40 µM solution of oligo $dT_{(15)}$, 1 µl RNasin at 40 units/µl (Promega, Madison, WI), 1 µl MoMLV reverse transcriptase at 200 units/µl (BRL), and autoclaved water to 20 µl. This mixture was incubated at 42° C for 50 min and then diluted to 100 µl with water. Five microliters of this cDNA solution was used for PCR amplification performed in a volume of 50 µl containing 1 × PCR amplification buffer of the GeneAmp PCR reagent kit (Perkin Elmer), each primer at 0.5 µM, each dNTP at 200 µM (Perkin Elmer), and 1.25 units Ampli*Taq* DNA polymerase (Perkin Elmer). Primers were: 5′-CGGCCGAAGGAACCTGACCCAG-3′ (*MAGE1* positions 26–47 sense) and 5′-GCTGGAACCCTCACTGGGTTGCC-3′ (*MAGE1* positions 3331–3309 anti-sense) for *MAGE1*; 5′-AAGTAGGACCCGAGGCACTG-3′ (*MAGE2* positions 2251–2270 sense) and 5′-GAAGAGGAAGAAGCGGTCTG-3′ (*MAGE2* positions 2567–2548 anti-sense) for *MAGE2*; 5′-GGCATCGTGATGGACTCCG-3′ (human β-actin exon 3 sense), and 5′-GCTGGAAGGTGGACAGCGA-3′ (human β-actin exon 6 anti-sense) for the human β-actin gene. The cDNA was first denatured at 94° C for 5 min, amplifications were then performed for 30 cycles (1 min 94° C, 3 min 72° C for *MAGE1*; 1 min 94° C, 2 min 68° C, 2 min 72° C for *MAGE2* and the β-actin gene). Each reaction (18 µl) was size-fractionated in an agarose gel containing ethidium bromide and was visualized on an ultraviolet transilluminator.

Results

A cDNA library prepared with RNA of the melanoma line MZ2-MEL was hybridized with a 2.4 kb probe covering exons 2 and 3 of the *MAGE1* gene (Fig. 1). This led to the identification of two cDNA species whose sequences were different from that of *MAGE1* but closely homologous. The corresponding genes were named *MAGE2* and *MAGE3* (van der Bruggen et al. 1991).

A cosmid carrying gene MAGE2. To isolate the *MAGE2* gene, we screened a cosmid library prepared with the DNA of a subline of MZ2-MEL. DNA extracted from 14 groups of 50000 independent cosmids was submitted to PCR amplification with oligonucleotide primers corresponding to sequences of the third exon of *MAGE1*, which are common to the *MAGE1*, *-2* and *-3* cDNAs (van der Bruggen et al. 1991). The PCR products were then hybridized with a radioactive oligonucleotide corresponding to a region

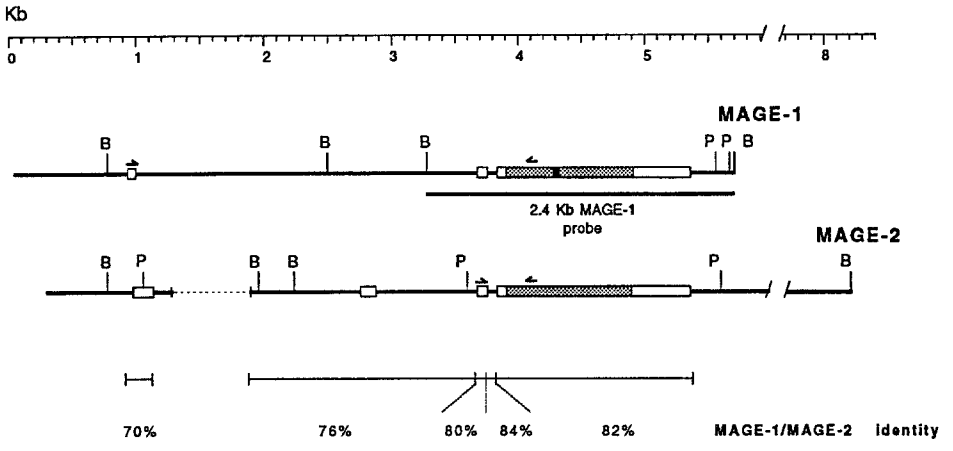

Fig. 1. Comparison of *MAGE1* and *MAGE2* gene structure. The Bam HI (*B*) and *Pst I* (*P*) restriction sites are deduced from the sequences and are consistent with Southern blot results. *White boxes* show the exons of *MAGE1* and *MAGE2*. *Filled boxes* indicate the open reading frames, and the little *black box* in *MAGE1* corresponds to the region encoding the nonapeptide of antigen MZ2-E. Gaps (.....) were introduced for best alignment of related sequences. *Arrows* (⇀↽) indicate the position of the oligonucleotides used for the RT-PCR analysis of *MAGE1* and *MAGE2* mRNA expression. Sequence identity is indicated in % of matching nucleotides for different portions of the genes.

of the *MAGE2* cDNA that contained several mismatches with the *MAGE1* and *MAGE3* sequences. The PCR products of several cosmid groups were found to hybridize, and the group presenting the most intense signal was screened by colony hybridization with the same probe. A positive cosmid, C6.3, was identified. Restriction analysis and Southern blots revealed that this cosmid contained a 38 kb insert carrying a 6 kb and an 8 kb *Bam* HI fragment, which both hybridized to the *MAGE2* oligonucleotide probe. These two fragments were subcloned in phagemid pTZ18R and their nucleotide sequence was determined. A sequence contained in the 6 kb fragment was found to contain regions that were strictly identical to the *MAGE2* cDNA. The other fragment contained a highly similar sequence (90% identity), which was named *MAGE12*.

Sequence and structure of the MAGE2 gene. The *MAGE2* gene is about 4 kb long, and the overall sequence identity with *MAGE1* amounts to 77% (Figs. 1, 2). Remarkably, the comparison of the genomic sequence with that of the *MAGE2* cDNA delineated four exons, which is one more than *MAGE1* (Fig. 1).

To determine the transcription initiation point, we carried out reverse transcription on MZ2-MEL RNA with a *MAGE2*-specific primer located near the 5′ end of the cDNA sequence. The product was tailed with a poly(A) sequence and amplified by anchored PCR (Frohman et al. 1988). Three PCR products were obtained. The largest added 55 nucleotides to the *MAGE2* cDNA, and we provisionally consider its starting point as the cap site. The two others had 5′ ends located 8 and 15 nucleotides downstream, respectively.

The first exon of *MAGE2* is longer than that of *MAGE1*. In *MAGE2*, the initiation point is located 45 nucleotides downstream of the putative initiation site of the *MAGE1* gene, and the donor splice site of the first exon is located 176 nucleotides downstream of that of *MAGE1* (Fig. 2). Except for a large deletion of 650 bp in the *MAGE2* gene, the region corresponding to the first intron of *MAGE1* is closely related to the *MAGE2* sequence (76% identity). Remarkably, however, a new splice acceptor site and a new donor site have appeared in the *MAGE2* sequence, creating a new exon that is homologous to an intron sequence of *MAGE1*. The presence of a C, instead of the G of the *MAGE1* sequence, eight nucleotides upstream of the new acceptor site of *MAGE2*, probably contributes to the creation of this acceptor site (Fig. 2).

Like *MAGE1, MAGE2* contains a large open reading frame in the last exon, and the two genes have the same translation initiation point. Twenty-nine codons downstream of this ATG codon, *MAGE1* contains a small deletion when compared to the *MAGE2* sequence. This deletion, which corresponds to exactly 7 codons, preserves the reading frame alignment between the two genes. The MAGE2 protein, which is 314 amino acids long, presents 67% identity with that of MAGE1, which is 309 amino acids long.

Hydrophobicity pattern analysis of MAGE1 and MAGE2 putative proteins indicates that they have no signal peptide. It may be worth noting the presence of two stretches of 21 and 17 amino acids that are strictly identical in both MAGE1 and MAGE2 protein sequences (Fig. 2, residues 112–132 and residues 171–187 of the MAGE1 protein). These regions may constitute parts of the protein that are essential for its function.

Fig. 2. (For legend see p. 125)

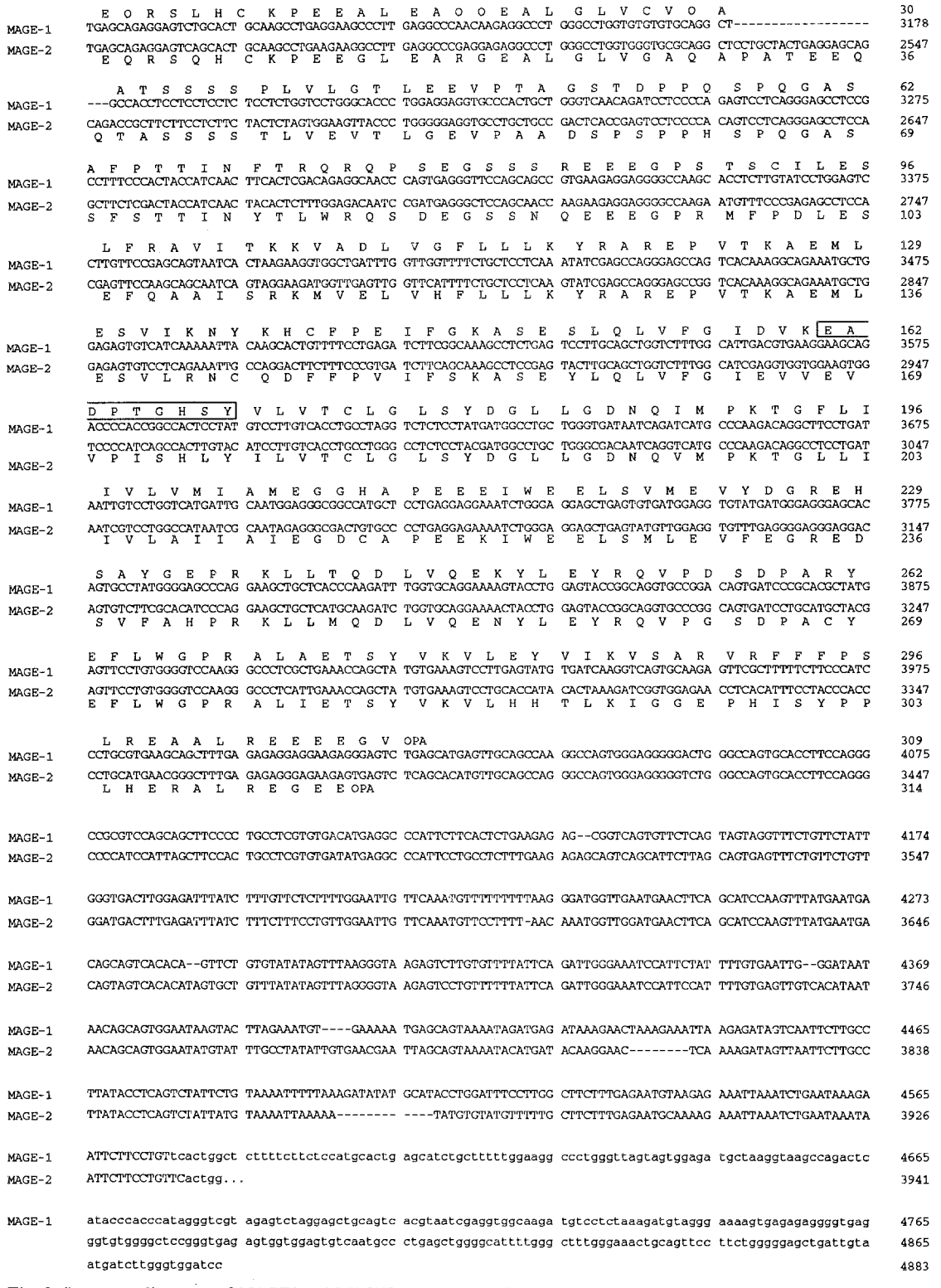

Fig. 2. Sequence alignment of *MAGE1* and *MAGE2* genes. *MAGE2* sequences corresponding to the exons with surrounding portions of intron and to the promoter region are aligned to the complete sequence of *MAGE1*. A correction to the previously described sequence of the *MAGE1* gene (van der Bruggen et al. 1991) was contributed by E. Paoletti and J. Tartaglia (Virogenetics, Troy, NY). Gaps, indicated by *dashes* (–), were introduced for optimal alignment. Exons are in *uppercase*. Untranscribed and intron sequences are in *lowercase*. *Arrows* (⟹) mark the 7 bp direct repeats in the promoter region. In this region we also indicated the potential binding sites for transcription factors AP2 (———), Ets (▬▬), Myb (▒▒), PuF (▭▭) and ATF (– – – –). The amino acid sequence of the putative protein encoded by both genes is represented. The amino acid sequence corresponding to the MZ2-E antigenic peptide is *boxed*.

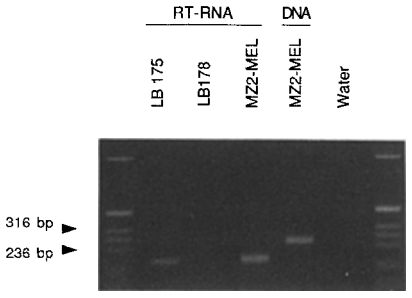

Fig. 3. Reverse transcription and PCR amplification of *MAGE2* mRNA. *MAGE2*-specific primers shown in Figure 1 were used to amplify reverse transcribed RNA (RT-RNA) from two non-small-cell lung carcinoma samples (LB175 and LB178) and from control melanoma cell line MZ2-MEL. *MAGE2* amplification with the same primers was also carried out on genomic DNA extracted from melanoma cell line MZ2-MEL. PCR products were visualized on a 1.5% agarose gel stained with ethidium bromide.

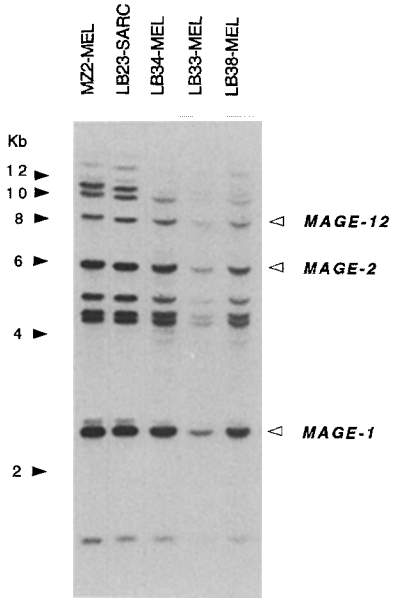

Fig. 4. Southern blot analysis of the *MAGE* genes. *Bam* HI-digested DNAs were from the melanoma cell lines of four patients (MZ2, LB34, LB33, and LB38), and from a sarcoma cell line of patient LB23. The *MAGE1* 2.4 kb *Bam* HI fragment was ^{32}P-labeled and used as a probe for hybridization. Final washings were in 0.5 × SSC, 0.1% SDS at 65° C.

The MAGE2 promoter. There are no CAAT or TATA motifs in the *MAGE2* promoter. However, by screening its sequence from –445 to –1 for the presence of upstream promoter elements (UPE) as listed by Faisst and Meyer (1992), we identified consensus sequences for the binding of Myb, Ets-1, PuF, AP2, and ATF transcription factors (Fig. 2). Consensus binding sequences for the four latter transcription factors are also present in the corresponding region of *MAGE1*. In addition, it may be interesting to note the presence of a pair of identical 7 bp direct repeat sequences located in both genes at approximatively 160 bp upstream from the first transcription start point (Fig. 2). Unlike the promoters of the housekeeping genes, which also lack CAAT and TATA motifs and typically contain GC-rich regions carrying multiple Sp1 binding sites (Melton et al. 1986), the promoter regions of *MAGE1* and *MAGE2* show a high content of C (42% and 38%, respectively) but not of G (21% and 25%, respectively), and do not contain any Sp1 consensus binding sequence.

MAGE2 gene expression. To evaluate *MAGE2* gene expression in various cells, we performed reverse transcription and PCR amplification of their RNA. Pairs of specific oligonucleotide primers were chosen, which matched sequences located in different exons, so as to distinguish the PCR products originating from mRNA from those produced by contaminating DNA (Figs. 1, 3).

A large number of tissue samples from tumors of different histological types were tested (Table 1). In order to verify the quality of the RNA preparations, we also evaluated the β-actin mRNA. A very high proportion (85%) of melanoma samples expressed *MAGE2*. This is much higher than for *MAGE1*, which is expressed in about 40% of melanomas. No melanoma sample was found where *MAGE1* was expressed and *MAGE2* was not. Laryngeal tumors expressing *MAGE2* were also more frequent (46%) than those expressing *MAGE1* (23%). For lung tumors and sarcomas the distribution was different: those expressing *MAGE1* were as numerous (35%) as those expressing *MAGE2*. Less than 10% of breast tumors were found to express the *MAGE2* gene, whereas 20% express *MAGE1*. In these types of tumor, no correlation was observed between the expression of the two genes.

We also tested a panel of healthy adult and fetal tissues (Table 1). No *MAGE1* or *MAGE2* expression was observed, with the important exception of testis. The level of expression in testis was estimated to be approximatively one-third of that observed in the MZ2-MEL cell line.

Low restriction polymorphism of MAGE genes. DNA samples from four melanoma cell lines and one sarcoma cell line were digested with *Bam* HI. Southern blots were hybridized with the *MAGE1* 2.4 kb *Bam* HI probe (Fig. 1) and washed at medium stringency. Autoradiography revealed the same pattern of 13 bands for all cell lines (Fig. 4). The two bands corresponding to the *MAGE2* and *MAGE12* genes isolated from tumor MZ2-MEL were observed with all the tumor samples. These results suggest that the *MAGE* gene family contains 10–15 members and presents a low level of polymorphism. This latter observation is relevant to the possibility of setting up for *MAGE* genes a PCR expression test that is applicable to a wide variety of cancer patients.

Table 1. Expression of *MAGE1* and *MAGE2* genes by tumoral, healthy, and fetal tissues.

Tumor samples						Healthy tissues		
Histological type	Expression of		Histological type	Expression of		Histological type	Expression of	
	MAGE1	*MAGE2*		*MAGE1*	*MAGE2*		*MAGE1*	*MAGE2*
Brain tumors			Melanomas			Adult tissues		
	0/2	0/2	LB 211	–	++++			
			MZ 2	++++	++++	Colon	–	–
Breast carcinomas			BB5	+++	++++			
LB 177	–	++	LB 40	–	+	Stomach	–	–
LB 222	++	–	LB 49	+++	++			
LB 171	++++	–	LB 124	++	++++	Liver	–	–
LB 231	++	–	LG 11	++	+++			
LB 232	+++	–	BB 1	–	+++	Medulla	–	–
LB 207	+	+	BB 2	–	++++			
LB 284	++	+++	BB 3	–	+++	Muscle	–	–
	6/34	3/34	BB 4	–	+++			
			LB 265	–	+++	Melanocytes	–	–
Colon carcinomas			LB 271	+	+++			
LB 274	–	+++	LG 7	–	++	Skin	–	–
	0/11	1/11	LG 12	++	++++			
			LB 225	+++	+++	Lung	–	–
Laryngeal tumors				8/19	16/19			
LB 266	++++	–				Breast	–	–
LB 270	++++	+++	Ovary tumors					
LB 575	++	++++		0/3	0/3	Uterus	–	–
LB 363	–	+++						
LB 430	–	++++	Sarcomas			Thymocytes	–	–
LB 512	–	+++	LB 29	–	+			
LB 516	–	++++	LB 258	++	++	Testis		
	3/13	6/13	LB 227	++	–	CLO 64027	+++	+++
				2/6	2/6	LB 451	+++	+++
Leukaemias								
	0/4	0/4	Thyroid tumors			Fetal tissues		
				0/3	0/3			
Lung carcinomas						Fibroblasts	–	–
LB 175 NSCLC	–	+++						
LB 178 NSCLC	+++	–				Liver		
LB 182 NSCLC	–	+				(20 weeks)	–	–
LB 195 NSCLC	++	++++				(22 weeks)	–	–
LB 206 NSCLC	+++	++				(35 weeks)	–	–
LB 212 NSCLC	++	+				(26 weeks)	–	–
LB 254 NSCLC	++	–						
LB 259 NSCLC	–	+				Brain (26 weeks)	–	–
LB 264 NSCLC	+++	++++						
LB 648 SCLC	+	+++				Spleen (26 weeks)	–	–
LB 444 SCLC	–	+++						
	7/19	9/19				Thymus (26 weeks)	–	–
						Lung (26 weeks)	–	–

MAGE1 and *MAGE2* gene expression was tested by specific RT-PCR amplification on total RNA. Only those tumor samples that express at least one of these genes are represented. Lung tumors noted NSCLC are non-small-cell lung carcinomas; SCLC are small-cell lung carcinomas. Absence of PCR product is indicated by –, and different levels of expression as deduced by band intensity of PCR products are represented by +, ++, +++, or ++++. All RNAs presented here showed similar amplification of the β-actin cDNA.

Discussion

Like *MAGE1*, the *MAGE2* gene is expressed in tumor cells and not in the healthy tissues that have been tested, with the exception of testis. Antigens encoded by *MAGE2* should therefore qualify as tumor rejection antigens, since a T-lymphocyte response directed against these antigens should be capable of destroying tumors, with no unacceptable side effects on healthy tissues, except possibly on testis. In the absence of

appropriate antibodies, we have not yet been able to determine which cell type expresses genes *MAGE1* and *MAGE2* in testis.

Even though no CTL recognizing a MAGE2-derived peptide has yet been identified, *MAGE2* constitutes a priori a more useful source of melanoma tumor rejection antigens than *MAGE1* because it is expressed in almost 85% of melanoma samples, whereas *MAGE1* is expressed in 40%. Like *MAGE1, MAGE2* is also expressed in many tumors other than melanomas, notably lung tumors, sarcomas, and laryngeal tumors. The *MAGE2* region, which is homologous to the *MAGE1* region coding for the MZ2-E antigenic peptide, codes for a peptide that displays five differences with the MAGE1 peptide presented by HLA-A1 (Fig. 2). It is therefore not surprising that *MAGE2* does not code for antigen MZ2-E. But we deem it likely that *MAGE2* produces other antigenic peptides that bind to other HLA molecules.

It will be interesting to determine the promoter components that ensure tumor-specific *MAGE* gene expression. Whereas the multiplicity of 5′ ends observed on *MAGE2* cDNAs could be due to incomplete reverse transcription preceding anchored-PCR amplification, it could also reflect the nature of the *MAGE2* promoter region, which contains no TATA or CAAT consensus sequence. Promoters lacking a TATA box have been divided into two classes (Smale and Baltimore 1989). The GC-rich promoters are found primarily in housekeeping genes (Melton et al. 1986), contain multiple Sp1 binding sequences, and present several, sometimes widely spread, transcription initiation sites. TATA-lacking promoters which are not GC-rich constitute the second class. They usually regulate the transcription of genes involved in differentiation or development, like the mouse terminal deoxynucleotidyl transferase gene (Smale and Baltimore 1989), or the genes coding for the δ chain of the T-cell receptor complex (Van den Elsen et al. 1986). They initiate transcription at one or a few tightly clustered start sites. The promoter of the *MAGE2* gene appears to belong to this second class.

No *MAGE12* cDNA could be isolated from a cDNA library of the melanoma cell line MZ2-MEL. However, preliminary analysis of RNA samples of different origins by reverse transcription and PCR amplification with primers located in the longest open reading frame of the *MAGE12* gene, indicated that this gene is expressed in several tumor samples but not in healthy tissues, with the exception of testis. The *MAGE12* gene may therefore also produce tumor rejection antigens. It will be analyzed further for its structure and expression pattern.

The function of the MAGE proteins is still unknown, though they may play a role in embryonal development. Our failure to observe *MAGE1* and *MAGE2* gene expression in fetal tissues may be because the analyzed tissues came from fetuses of 20–35 weeks. Since a number of genes have their expression restricted to an earlier phase of embryonal development (Pfeifer-Ohlsson et al. 1984), it will be important to examine earlier embryos. The absence of a signal peptide implies that the MAGE1 and MAGE2 proteins are neither surface nor secreted proteins. Precise intracellular localization of these MAGE proteins could help to understand their function. For this purpose, efforts are being made to obtain antibodies directed against these proteins.

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