

ARTICLE

New *BAGE* (*B melanoma antigen*) genes mapping to the juxtacentromeric regions of human chromosomes 13 and 21 have a cancer/testis expression profile

Myriam Ruault¹, Pierre van der Bruggen², Marie-Elisabeth Brun¹, Shelagh Boyle³, Gérard Roizès¹ and Albertina De Sario^{*1}

¹Institut de Génétique Humaine, CNRS UPR 1142, 141, rue de la Cardonille, 34396 Montpellier, France; ²Ludwig Institute for Cancer Research, Brussels Branch, 74 av Hippocrate, B1200 Brussels, Belgium; ³MRC Human Genetics Unit, Western General Hospital, Crewe Road, Edinburgh, UK

A first *BAGE* (*B melanoma antigen*) gene, *BAGE1*, was identified because it encodes a human tumour antigen recognised by a cytolytic T lymphocyte. Here, we characterised five new *BAGE* genes mapping to the juxtacentromeric regions of human chromosomes 13 and 21 and nine *BAGE* gene fragments mapping to the juxtacentromeric regions of chromosomes 9, 13, 18, and 21. Genes and gene fragments share extensive regions of 90–99% nucleotide identity. We analysed the expression of *BAGE* genes on 215 tumours of various histological types and on nine normal tissues. Similar to *BAGE1*, the new *BAGE* genes are expressed in melanomas, bladder and lung carcinomas and in a few tumours of other histological types. All the normal tissues were negative, with the exception of testis. Our results show that human juxtacentromeric regions harbour genes, which are transcribed and translated, in addition to gene fragments that are generally not expressed. We suggest that the pattern of expression restricted to cancer/testis is a feature of the few genes mapping to juxtacentromeric regions.

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By screening a human melanoma cDNA library with an anti-tumour cytolytic T lymphocyte (CTL), we isolated a 1004 bp-long transcript coding for the tumour antigen recognised by the CTL.¹ It was named *BAGE*, for human *B melanoma antigen* (gb U19180) and, hereafter, will be referred to as *BAGE1e*. *BAGE* encodes a putative protein of 43 amino acids and the antigen recognised by the CTL consists of *BAGE1*-encoded peptide AARAVFLAL bound to HLA-Cw16 molecules.¹ *BAGE1a* was found to be expressed in tumours of different histological types. It is silent in normal and foetal tissues except for testis.

Several other unrelated families of genes with a pattern of expression similar to that of *BAGE1* have been

identified, notably the *MAGE*, *GAGE* and *LAGE/NY-ESO-1* families.^{2–7} These genes are located on the X chromosome and their function is still unknown. The *MAGE* family contains 18 related genes divided into three clusters (*MAGE-A*, *B*, and *C*) located on the X chromosome.^{3,8,9}

A subset of these male germline-specific genes are activated in a wide variety of tumours, in which they code for tumour-specific antigens recognised by autologous T lymphocytes.¹⁰ Male germline cells do not express molecules of the major histocompatibility complex (MHC), which are surface molecules required to present antigenic peptides to T lymphocytes.¹¹ The antigens recognised by T lymphocytes that are encoded by *BAGE*, *MAGE*, *GAGE* and *LAGE/NY-ESO-1* families are, therefore, strictly tumour-specific and may prove useful for cancer immunotherapy. Clinical trials involving defined *MAGE* and *LAGE* tumour antigens are proceeding.^{12,13} Long-lasting

*Correspondence: A De Sario; Institut de Génétique Humaine, CNRS UPR 1142, 141 rue de la Cardonille, 34396 Montpellier, France.
Tel +334 99 61 99 77; Fax: +334 99 61 99 01; E-mail: desario@infobiogen.fr
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tumour regressions have been observed in a minority of patients.

During annotation of genomic sequences of human chromosome 21,¹⁴ we identified three DNA stretches having 92–99% nucleotide identity with *BAGE1a* mRNA. The *BAGE*-related sequences are located less than 1 Mb away from the centromere: two in 21p and one in 21q. We searched for new *BAGE* transcripts and show here that *BAGE* is a gene family composed of expressed genes that map to the juxtacentromeric regions of chromosomes 13 and 21, and of unexpressed gene fragments that are scattered in the juxtacentromeric regions of several chromosomes. We have analysed the expression of the new *BAGE* genes in tumours and normal tissues.

Materials and methods

Primer sequences and detailed protocols are available online (<http://www.igh.cnrs.fr/equip/centromere/Bage.html>).

Screening of a melanoma library

The MZ2.MEL melanoma cell line cDNA library was screened using probes derived from the 5' and 3' regions of *BAGE1a*. Hybridisations were done at stringent conditions according to standard procedures.¹⁵

5' and 3' RACE and cDNA cloning

5' and 3' RACE experiments were done using the Marathon Ready human testis cDNA (#7414-1, Clontech) and the Expand PCR kit (Boehringer) according to the manufacturer protocols. 5' and 3' RACE products were purified using the Wizard kit (Promega) and cloned using the pGem-T PCR cloning kit (Promega). Afterwards, specific primers were designed to conserved regions of the 5' and 3' end sequences and were used to reamplify three cDNA libraries: two testis and melanoma (MZ2-Mel43) cDNA libraries¹ and a purchased testis cDNA library (Clontech #7414-1). The obtained 2 kb-products were gel purified and cloned using the pGem-T PCR cloning kit (Promega).

BAGE genomic structure

The genomic structure of *BAGE* genes was determined through alignment of transcripts with genomic sequences. The genomic organisation of *BAGE1* was obtained through alignment of the six transcripts (*BAGE1a*, *BAGE1b*, *BAGE1c*, *BAGE1d*, *BAGE1e*, and *BAGE1f*) with the genomic sequences AF499647 containing (exon 1 and 2) and AC064811 containing (exons 3 to 7). The genomic organisation of *BAGE2* and *BAGE5* was obtained through alignment of the transcripts with the genomic sequences AL163201 and AL161418, respectively.

Sequencing

Sequencing was done with an Applied Biosystems 373XL sequencer.

Computer analysis

Nucleotide sequences and predicted amino acid sequences were analysed by BLAST¹⁶ and by CLUSTALW at <http://www2.ebi.ac.uk/clustalw>

Chromosome mapping

In situ hybridisation A 1.8-kb genomic fragment spanning from exon 1 to exon 2 was labelled by nick translation with Bio-16-dUTP (Boehringer) and hybridised to normal human metaphase chromosomes as described in Fantes *et al.*¹⁷ Hybridisation signals were visualised using a Zeiss Axioplan epifluorescence microscope. Images were captured using Digital Scientific Smartcapture software.

Somatic hybrid cell lines Primer pairs specific for *BAGE* were used to amplify DNA from the monochromosome somatic hybrid cell lines (NIGMS mapping panel 2).¹⁸ PCR products were cloned in pGem-T vector (Promega). To map *BAGE1*, 10 colonies per cloning reaction were sequenced with universal primers SP6 and T7.

Expression analysis

Expression analysis was done on nine normal tissues (heart, brain, placenta, lung, liver, skeletal muscle, kidney, pancreas, and testis; Clontech #1420-1; #7414-1) and on 215 tumour samples. Total RNA was extracted from tumour samples by the guanidine-isothiocyanate procedure as described.¹⁹ Reverse transcription was done on 2 µg of total RNA with 2 mM oligo(dT)₁₅ primer and 200 U of MoMLV reverse transcriptase (GIBCO-BRL). The quality of the RNA preparation was tested by PCR amplification of human *β-actin*. Primers used for the expression analysis were located in different exons. A first set of primers, *bage14* and *bage19*, was used to amplify a PCR product of 490 bp corresponding to *BAGE1* and *BAGE5* transcripts. A second set of primers was used to amplify a 368-bp PCR product corresponding to *BAGE2*, *BAGE3*, and *BAGE5* transcripts. To identify individual genes, 15 µl of the PCR product was digested with restriction enzymes. Digestion of the 495 bp PCR product with either *AluI* or *BstNI* allowed us to identify *BAGE2* and *BAGE3* cDNAs, respectively. Digestion of the 368 bp PCR product with *BssKI* allowed us to identify *BAGE1* (presence of the restriction site) and *BAGE5* (absence of the restriction site).

To analyse the expression of *BAGE* gene fragments we amplified a testis cDNA library (Clontech #7417-1) with primers *bage4* and *bage5*. The PCR product was cloned in pGEM-T vector (Promega). Twenty colonies were sequenced and compared with genomic sequences in databases.

Results

BAGE transcripts and genes

We renamed *BAGE1* the gene corresponding to the transcript (gbU19180) previously isolated from a human melanoma cDNA library.¹ The same library was hybridised

with probes corresponding to the 5' and 3' regions of *BAGE1* and six alternatively spliced mRNAs, whose sizes range from 1 to 2.3 kb, were isolated: *BAGE1a* (gbU19180, ref. 1), *BAGE1b* (AF527550), *BAGE1c* (AF527551), *BAGE1d* (AF527552), *BAGE1e* (AF527553), *BAGE1f* (AF527554) (Figure 1A).

Afterwards, 5' and 3' RACE amplifications were done on a testis Marathon cDNA library (Clontech), using primers

specific for *BAGE1* (Figure 1B). We failed to amplify sequences upstream of the 5' region of *BAGE1* and concluded that the 5' untranslated region is complete. Interestingly, 3' RACE amplifications yielded fragments that differed in sequence and length from the *BAGE1* mRNAs. PCR primers were chosen in the conserved 5' and 3' regions and full length cDNAs were amplified from two testis and one melanoma cDNA libraries. Full length cDNAs were

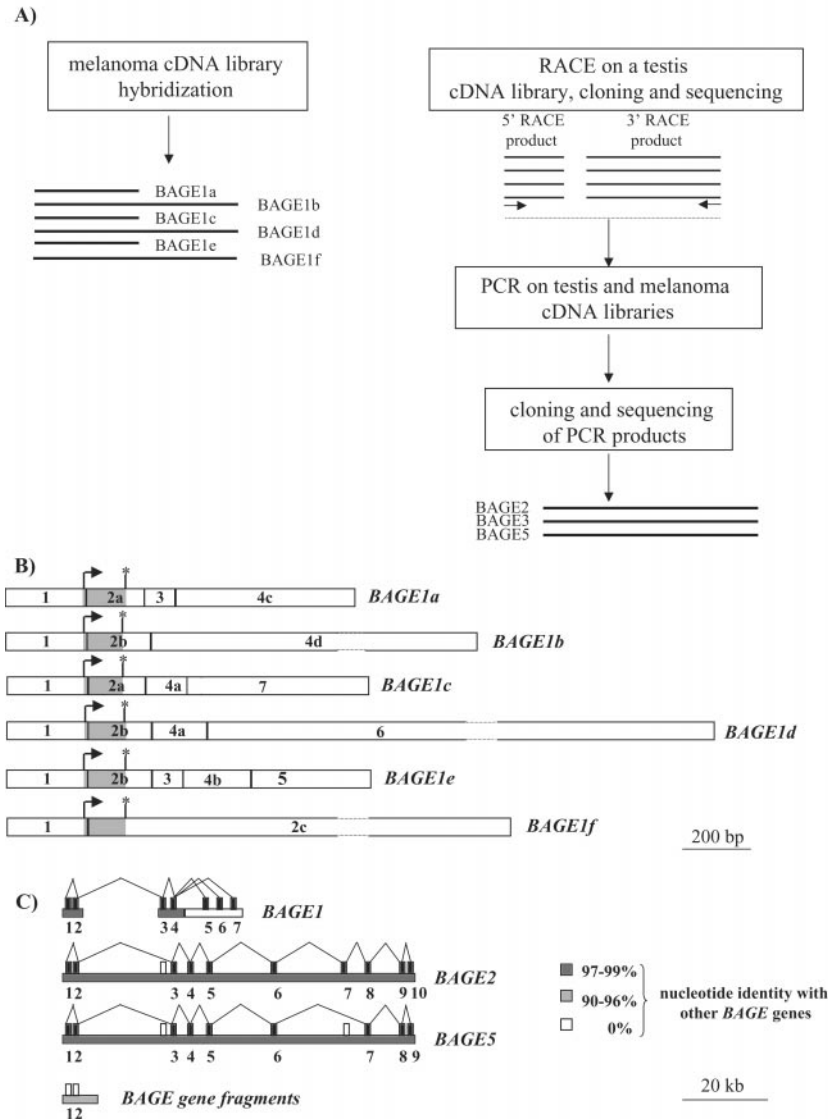


Figure 1 (A) Strategy used to isolate new *BAGE* transcripts: (left side) Hybridisation of a melanoma cDNA library with probes specific for *BAGE1* allowed us to isolate six alternatively spliced *BAGE1* isoforms; (right side) Through RACE experiments on a testis cDNA library we cloned the 5' and 3' ends of different *BAGE* transcripts. Then using primers designed in the conserved sequence of different *BAGE* ends, we amplified full length cDNAs on three different (melanoma and testis) cDNA libraries. These transcripts correspond to new *BAGE* genes. (B) Exonic structure of the *BAGE1* transcripts. Open rectangles are exons, filled (grey) rectangles correspond to open reading frames (arrows indicate the initiation and stars the stop codons). Exons 2c, 4d, and 6 are not drawn to scale. (C) Nucleotide sequence identity among different members of the *BAGE* family. Black rectangles are transcribed exons. White rectangles are predicted exons, i.e. exon 3 of *BAGE1* (an Alu sequence included in the 3' untranslated region of *BAGE1a* and *BAGE1e* mRNA variants) was also present in *BAGE2* and *BAGE5* genomic sequences, but not in the corresponding transcripts. Likewise, exon 7 of *BAGE2* was also present in the *BAGE5* genomic sequence, but not in the *BAGE5* transcript.

cloned in a plasmid and, for each PCR product, 10 colonies were sequenced. We isolated three 1.9 kb-long transcripts, which were named *BAGE2* (gbAF218570), *BAGE3* (gbAF339514), and *BAGE5* (gbAF339516).

BAGE2 and *BAGE3* have putative open reading frames (ORFs) of 330 bp encoding predicted proteins of 109 amino acids. *BAGE5*, like *BAGE1*, has an ORF of 132 bp and encodes a predicted protein of 43 amino acids.

We determined the exon/intron structure of the *BAGE* genes (Figure 1B and Table 1). *BAGE1* comprises seven exons. We could not measure the gene length because the corresponding genomic sequence is unordered and uncompleted. *BAGE2* and *BAGE5* span 76 kb and comprise 9 and 10 exons, respectively. All the splicing sites conform the consensus gt/ag sequence. The exon/

intron structure of *BAGE3* could not be determined because the corresponding genomic sequence is not available in databases.

BAGE genomic sequences

BAGE genomic sequences were searched in databases and we retrieved 14 sequences with a significant nucleotide identity (90–100% $P < 10^{-4}$) with the *BAGE* transcripts described above (Table 2). *BAGE* genomic sequences were classified as follows: gene, when a transcript could be assigned to the genomic sequence; predicted gene, when no transcript could be assigned to the genomic sequence, but the predicted ORF was intact; gene fragment, when the gene was truncated and the predicted ORF was disrupted by deleterious mutations (deletions and nucleo-

Table 1 Exon/intron structure of *BAGE* genes
BAGE1

Genbank AC064811	Exons ^b	Size (bp)	5' Splice site	3' Splice site	Introns	Size (kb)
a	1	≥237		GGAGCG/gtaaga	I	1.5
a	2a	170	ttgtag/GTTTTT	AGGAGG/gtaacc	II	nd
a	2b	190	ttgtag/GTTTTT	CAAGTG/gtagga		
a	2c	1856	ttgtag/GTTTTT	t		
133 676–133 586	3	91	cagttag/AGATGG	TTACAG/gtgtga	III	1.4
132 178–132 016	4a	163	tttcag/CGATGT	AAAGAT/gtaagt		
132 215–132 016	4b	200	ttcaag/GTTTTA	AAAGAT/gtaagt		
132 178–131 651	4c	528	tttcag/CGATGT	t		
132 178–131 099	4d	1080	tttcag/CGATGT	t		
120 733–120 383	5 ^c	351	aaaaag/GATGAG	t	IV	nd
120 345–118 665	6 ^c	1680	ggtag/TAATAG	t	V	0.03
5420–4887	7 ^c	533	tgacag/GGTCTC	t	VI	nd

BAGE2

Genbank HS21C001	Exons	Size (bp)	5' Splice site	3' Splice site	Introns	Size (kb)
–191 680	1	≥223		GGAGTG/gtaaga	I	1.1
190 623–190 523	2	101	ttgtag/GTTTTT	AGCTCT/gtgagt	II	38.8
151 755–151 593	3	163	tttcag/CGATGT	AAAGAT/gtaagt	III	8.5
143 054–142 883	4	172	ttatag/CGAAGG	CTGCAA/gtaagt	IV	1.8
141 028–140 914	5	115	ttttag/ACAATC	TGCAAA/gtaagt	V	8.1
132 862–132 161	6	702	atacag/TGAATA	AAACAG/gtaggg	VI	9.3
123 134–123 015	7	120	tcttag/GAGGCT	AAAGTG/gtacct	VII	2.8
120 252–120 136	8	117	acatag/GGCCAT	CCTGGG/gtgagg	VIII	5.5
114 624–114 523	9	102	ttatag/GTGTCT	AATCAG/gtttga	IX	0.2
114 330–	10	≥76	cttcag/GATATG			

BAGE5

Genbank AL161418	Exons	Size (bp)	5' Splice site	3' Splice site	Introns	Size (kb)
–76 589	1	≥203		GGAGCG/gtaaga	I	1.5
78 261–78 450	2	190	ttgtag/GTTTTT	CAAGTG/gtagga	II	≥38.6
132 037–132 199	3	163	tttcag/CGATGT	AAAGAT/gtaagt	III	8.5
140 734–140 905	4	172	ttatag/CGAAGG	CTGCAA/gtaagt	IV	1.8
142 760–142 874	5	115	ttttag/ACAATC	TGCAAA/gtaagt	V	8
150 921–151 622	6	702	atacag/TGAATA	AAACAG/gtaggg	VI	11.9
163 514–163 630	7	117	acatag/GGCCGT	CCTGGG/gtgagg	VII	≥5.6
169 281–169 382	8	102	ttatag/GTGTCT	AATCAG/gtttga	VIII	0.2
169 575–169 650	9	≥76	cttcag/GATATG		IX	

^aThe gene structure was determined using the AF499647 genomic sequence. ^ba, b, c are alternative exons of different length. ^cOrder of exons 5–7 is not known because they are alternative terminal exons and the genomic sequence AC064811 is unordered. t=terminal exon and nd=not determined. The ag/gt intron boundaries are in bold type.

tide changes) that introduced stop codons and/or erase the initiation codon.

Overall, we identified three genes (*BAGE2*, *BAGE3*, and *BAGE5*), two predicted genes (*BAGE6* and *BAGE7*), and nine gene fragments (Table 2). *BAGE* gene fragments are 2–8 kb

Table 2 List of *BAGE* genomic sequences^a

Genbank	Gene type	Chromosome	Positions
AC064811	<i>BAGE1</i> gene	13	^b
AL163201	<i>BAGE2</i> gene	21p	191 902–114 255
AL161418	<i>BAGE5</i> gene	13	76 589–169 650
AL158811	<i>BAGE6</i> predicted gene	13	4 541–147 162
AL049849	<i>BAGE7</i> predicted gene	21p	23 819–27 258
AL163203	gene fragment	21q	66 6624–68 522
AC008443	gene fragment	nd ^c	18 576–16 465
AC008454	gene fragment	nd ^c	32 862–22 256
AL161615	gene fragment	13	210 981–213 643
AL163539	gene fragment	9	99 935–101 853
AL356136	gene fragment	9	6 126–4 209
AL359312	gene fragment	9	22 315–20 417
AC068255	gene fragment	18	76 105–78 004
AP001896	gene fragment	18q22	^b

^aTranscripts were tentatively assigned to a specific locus if they had >99.5% nucleotide identity with a given genomic sequence. This threshold was chosen in order to distinguish allelic variations from nucleotide variations corresponding to independent loci, the frequency of which being estimated at 0.1–0.3% in the human genome (ref.14). ^bUnordered genomic sequence. ^cGenomic clone misassigned to chromosome 5. nd=not determined.

long and correspond to the 5' region of *BAGE* genes (they span from exon 1 to truncated intron 2).

BAGE sequences (genes and gene fragments) share extensive regions of high nucleotide identity: nucleotide identity is higher among genes (97–99%) than between genes and gene fragments (90–96%) (Figure 1C).

Chromosome mapping

To map *BAGE* genomic sequences, we hybridised a 1.8 kb genomic probe to human metaphase chromosomes. The probe was amplified by PCR from the 5' region that is common to all the *BAGE* sequences. *BAGE* sequences mapped to the juxtacentromeric regions of chromosomes 9, 13, 14, 15, 18, 21, and 22 (Figure 2). Hybridisation to chromosomes 14 and 15 was observed only in some metaphases suggesting that these *BAGE* sequences were more divergent.

We then amplified exon 1, which is common to all the *BAGE* sequences, on a panel of monochromosome somatic cell lines. Specific amplifications were obtained with chromosomes 9, 13, 15, 18, 21, and 22 (data not shown). No amplification was obtained with chromosome 14: this result can be due to nucleotide divergence between the primers used and the target sequence.

To map individual *BAGE* loci, we analysed the localisation of the genomic sequences retrieved from databases (Table 2). Assignment of genomic sequences to chromo-

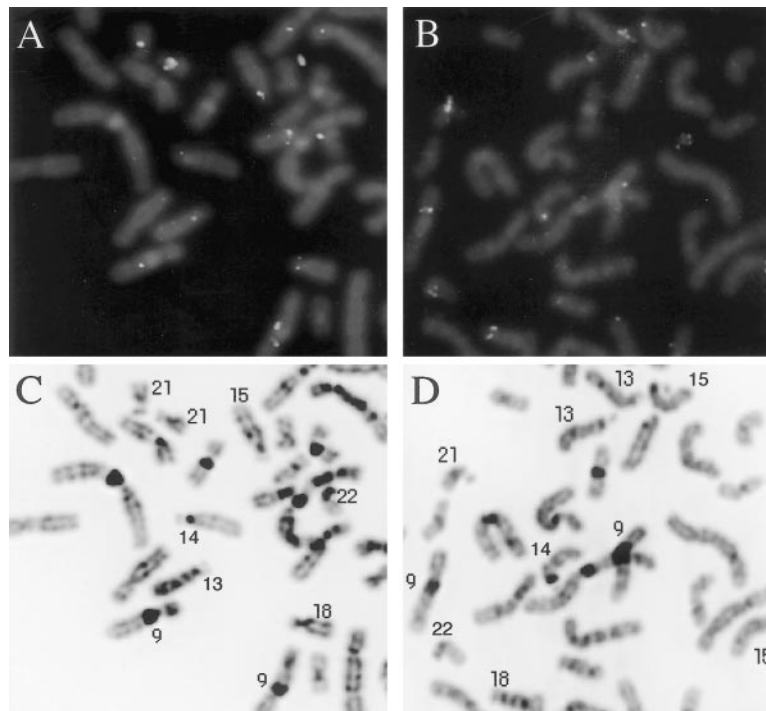


Figure 2 (A) and (B) *In situ* hybridization of the 5' region of *BAGE* to two partial human metaphases. (C) and (D) DAPI-stained G banding of the partial metaphases.

somes 9, 13, 18, and 21 was consistent with our mapping results; by contrast, assignment to chromosomes 4 and 5 was at variance with both *in situ* hybridisation and somatic hybrid analysis. To ascertain the actual localisation of *BAGE1*, which has 100% nucleotide identity with the genomic clone AC064811 assigned to chromosome 4, we did FISH experiments: the genomic clone AC064811 hybridises to chromosomes 13, 14, 21 and 22 (data not shown). We confirmed this result by PCR on somatic hybrids: a genomic sequence matching (100% nucleotide identity) the *BAGE1* transcripts was amplified from chromosome 13 and from no other chromosome (data not shown).

In conclusion, *BAGE* sequences map to the juxtacentromeric regions of different human chromosomes, each one containing more than one locus: *BAGE* genes map to chromosomes 13 and 21, whereas *BAGE* gene fragments map to chromosomes 9, 13, 18, and 21. *BAGE*-related sequences that are not yet characterised map to chromosomes 14, 15 and 22.

Predicted *BAGE* proteins

Multialignment of *BAGE* predicted proteins shows that amino acid sequence identity ranges from 88 to 98% (Figure 3). Although *BAGE2* and *BAGE3* differ only in two amino acids, transcripts encoding these proteins are unlikely to be allelic because they have 1.2% nucleotide divergence.

The nonapeptide AARAVFLAL (amino acids 2 to 10 of the *BAGE1* predicted protein; boxed in Figure 3) is the sequence of the antigen recognised by a CTL.¹ *BAGE* predicted proteins have two amino acid changes with respect to the sequence of the *BAGE1* antigenic peptide: (R→G)₃ and (A→V)₄. Synthetic peptides containing these amino acid variations are not recognised by the CTL that recognises *BAGE1* (data not shown).

After database searches, we concluded that *BAGE* predicted proteins have no significant identity/similarity to any known protein.

Expression analysis

We analysed the expression of *BAGE* genes in 215 tumour samples of various histological types. To distinguish individual genes, we took advantage of the few nucleotide variations that characterise different transcripts and we designed a strategy based upon PCR followed by restriction enzyme digestion.

A first set of primers (bage14/bage19) amplified a PCR product of 490 bp corresponding to *BAGE1* and *BAGE5*

transcripts. Fifteen per cent (15 out of 103) of melanomas scored positive, whereas all the normal tissues were negative, with the exception of testis (Table 3). In a previous study with a different set of primers that amplified the same transcripts, the percentage of positive melanomas was comparable (22%).¹

A second set of primers (bage20/bage21) was also used to amplify a PCR product of 368 bp corresponding to *BAGE2*, *BAGE3*, and *BAGE5* transcripts. Thirty-four per cent (35 out of 103) of melanomas scored positive, indicating that a significant number of tumours expressed *BAGE2* or *BAGE3* without expressing the other *BAGE* genes, in particular in primary melanomas (Table 3). Here again, all the normal tissues were negative, with the exception of testis.

PCR products were then digested with restriction enzymes *Bss*KI, *Alu*I, *Bst*NI, to identify individual transcripts for *BAGE1/BAGE5*, *BAGE2* or *BAGE3*, respectively. In melanomas, *BAGE2* (23%) was more frequently expressed than *BAGE1* (14%), *BAGE3* (14%) and *BAGE5* (9%) (Table 3). Individual tumours generally expressed more than one *BAGE* gene simultaneously (data not shown).

In accordance to a previous analysis,¹ our results confirmed that *BAGE* genes are expressed in melanoma, bladder and lung carcinomas and in a few tumours of other histological types. Leukemias, colorectal carcinomas, and renal carcinomas scored negative. Here, no head and neck tumours expressed *BAGE*, whereas 8% were found positive in the previous study. This could be explained by the small number of samples analysed.

To analyse transcription of *BAGE* truncated genes we amplified a testis and a melanoma cDNA libraries with primers derived from the 5' region that is common to genes and gene fragments. After having cloned the obtained PCR product, we sequenced 20 colonies and no transcript corresponding to a truncated gene was obtained. We tentatively concluded, therefore, that *BAGE* gene fragments are not expressed.

Discussion

In this paper, we show that the *BAGE* gene family comprises genes that are transcribed and translated (as indicated by the antigenic properties of *BAGE1*) and gene fragments that are not expressed. Genes and predicted genes map to the juxtacentromeric regions of human chromosomes 13 and 21, whereas gene fragments map to the juxtacentromeric regions of chromosomes 9, 13, 18, and 21. The list of *BAGE* genes and gene fragments is incom-

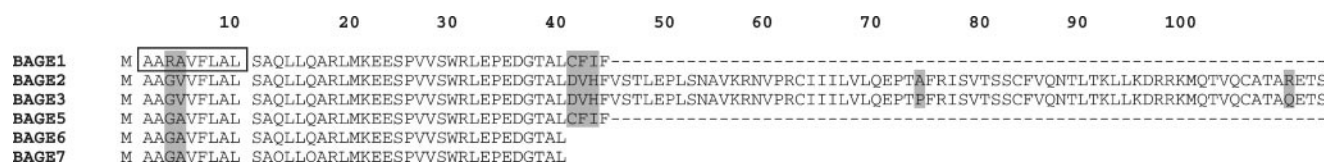


Figure 3 Multialignment of *BAGE* predicted proteins. *BAGE6* and *BAGE7* are translations of predicted genes. Non-conserved amino acids are shaded. The frame encloses the sequence of the *BAGE1* antigenic nonapeptide.

Table 3 Expression of *BAGE* genes in normal tissues and tumour samples

Histological type	Number tested	Number of <i>BAGE</i> -positive samples					
		PCR		PCR-restriction enzyme			
		a <i>BAGE1</i>	b <i>BAGE2</i> <i>BAGE3</i> <i>BAGE5</i>	BssKI+ <i>BAGE1</i>	AluI+ <i>BAGE2</i>	BstNI+ <i>BAGE3</i>	BssKI- <i>BAGE5</i>
<i>Tumour samples</i>							
Melanoma	103	15	35	14	24	14	9
primary lesion	26	3	10	2	4	4	2
metastatic lesion	77	12	25	12	20	10	7
Bladder carcinoma	20	4	6				
Lung carcinoma NSCLC	13	0	3				
Mammary carcinoma	16	0	1				
Sarcoma	10	0	1				
Prostatic carcinoma	6	0	1				
Neuroblastoma	1	1	1				
Head & neck tumour	11	0	0				
Leukemia	10	0	0				
Renal carcinoma	10	0	0				
Colorectal carcinoma	9	0	0				
Uterus	2	0	0				
Brain tumour	2	0	0				
<i>Normal tissues</i>							
Testis	1	1	1				
Placenta	1	0	0				
Heart	1	0	0				
Brain	1	0	0				
Lung	1	0	0				
Liver	1	0	0				
Skeletal muscle	1	0	0				
Kidney	1	0	0				
Pancreas	1	0	0				

^aRT-PCR with primers bage14/bage19. ^bRT-PCR with primers bage20/bage21.

plete: (i) *BAGE*-related sequences that are not yet characterised map to chromosomes 14, 15, and 22; and (ii) most of human juxtacentromeric regions are still unsequenced.²⁰

The sizes of the new *BAGE* transcripts isolated in this work are consistent with those of the two mRNA species (1 and 2.4 kb) previously identified in Northern blot experiments.¹ *BAGE1* transcripts have a great variety of alternative terminal exons. Eleven per cent of genes undergoing alternative splicing have alternative terminal exons.²¹

BAGE genes and gene fragments share extensive regions of 97–99% nucleotide identity which may be accounted for by concerted evolution. Concerted evolution is supposed to be the molecular mechanism responsible for sequence conservation among human ribosomal genes. Ribosomal genes map to the short arms of the five acrocentric chromosomes and participate in the formation of a common nucleolus. In the human germline cells, acrocentrics undergo frequent interchromosome DNA exchanges.^{22,23} *BAGE* sequences may therefore undergo similar interchromosome exchanges that promote sequence homogeneity.

In addition, we observed that *BAGE* genes have a higher nucleotide identity than *BAGE* gene fragments. Interestingly, we have found that *BAGE* genes, but not the *BAGE* gene fragments, are under selective pressure and that the *BAGE* gene family was generated by chromosome rearrangements during the evolution of hominoids (De Sario, personal communication).

Similar to *BAGE1*, the new *BAGE* genes are expressed in different cancer cells but silent in normal tissues other than testis. Chromatin compaction and/or DNA methylation may account for gene silencing. DNA methylation was already shown to be the primary mechanism responsible for the silencing of the genes encoding the MAGE antigens.^{24,25}

Given their expression profile restricted to cancer cells, the *BAGE* genes isolated during this work may encode new tumour antigens useful for cancer immunotherapy. *BAGE2*, which is expressed in 22% of melanomas and has a putative coding region of 309 residues, is the most promising.

Other genes located in the regions flanking human centromeres have a cancer/testis expression profile: *TPTE*

(*Transmembrane Phosphatase with Tensin homology*)²⁶ and *CT2* (*Creatin Transporter2*)²⁷ mapping to the juxtacentromeric regions of chromosomes 21 and 16, respectively, are exclusively expressed in testis; an *NF1*-related gene mapping next to the centromere of chromosome 15 is only expressed in neuroblastoma.²⁸ These results lead us to suggest that the restricted pattern of expression is a feature of the few genes mapping to juxtacentromeric regions and that these are candidate genes encoding tumour antigens.

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