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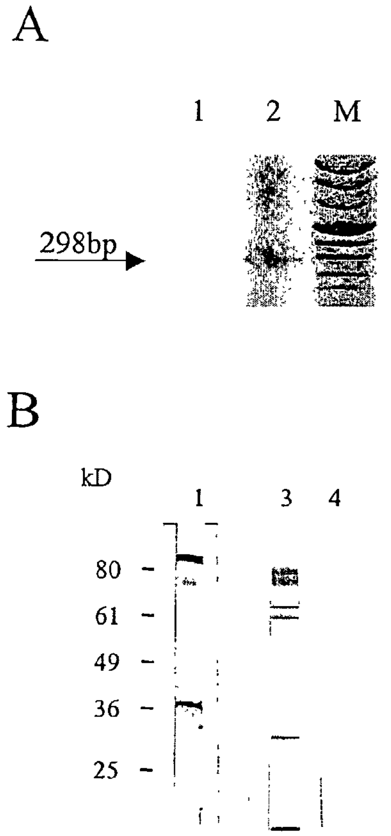
(54) Title: POLYNUCLEOTIDE FRAGMENTS OF AN INFECTIOUS HUMAN ENDOGENOUS RETROVIRUS

(57) Abstract: Described is a polynucleotide molecule being a fragment of a polynucleotide sequence of an infectious human endogenous retrovirus, said polynucleotide molecule comprising a sequence selected from the group consisting of (a) a sequence with at least 98%, preferably 99%, still preferred 99.5%, identity to a sequence according to SEQ ID No 1, (b) a sequence which hybridises under stringent conditions with a nucleotide sequence according to said SEQ ID No 1, (c) a sequence which differs from said sequences (a) or (b) due to degeneration of the genetic code and (d) a sequence comprising sequences inserted into vectors pCR4-Topo and deposited as "MERV-env", "MERV-gag", "MERV-prt" and "MERV-pol" at the DSMZ on 26 september 2001 or fragments thereof.



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POLYNUCLEOTIDE FRAGMENTS OF AN INFECTIOUS HUMAN ENDOGENOUS RETROVIRUS

The present invention relates to human endogenous retroviruses, fragments thereof, a biologically functional vector comprising sequences of said retrovirus.

Endogenous retroviruses (ERVs) are integral parts in the genomes of many, if not of all, species. ERVs most probably resulted from infection of germ line cells with exogenous retroviruses and subsequent fixation of their genetic information in the host genome.

Many types of human endogenous retroviruses (HERVs) have been characterised previously, and they have been classified into different groups, or families, partly on the basis of their sequence identity and partly according to the similarity of their primer binding sites (PBSs) to host tRNAs. Thus, members of the HERV.H family contain a PBS with a sequence similar to a region of $tRNA^{His}$, whereas the HERV.E family is primed by $tRNA^{Glu}$. Despite the large amount of data available, the classification of the many different HERV families within an overall phylogenetic framework has been hampered for several reasons: (i) some highly divergent retroviruses are primed by the same type of tRNA; (ii) many HERV families have not been fully characterised, and the sequence information that has been reported is often derived from different genomic regions, making interfamily comparisons problematic; and (iii) the relative lack of sequence information on other host taxa has made it difficult to distinguish between genuinely monophyletic HERV families and polyphyletic families that appear monophyletic only because similar viruses in other hosts have not yet been described. A pathogenic potential of non-defective endogenous retroviruses has so far only been demonstrated in mice, in which they may induce tumors and immunological disorders. In human DNA, endogenous retrovirus sequences (HERVs) are well known genetic elements. They all seem to be defective due to multiple termination codons, deletions, or the lack of a 5′ long terminal repeat (LTR). Replication competent human endogenous retrovirus genomes have not yet been isolated.

Extensive studies on animal melanomas (especially that of the hamster), which serve as model for human malignant melanoma have shown the occurrence of virus particles in the tumors. A high molecular weight (70S) RNA species as well as an RNA-directed DNA polymerase, two diagnostic features for oncorna (oncogenic RNA) viruses have been associated with these particles. Cell-free transmission experiments end to the conclusion that hamster melanomas are caused by an RNA virus.

Based on these findings the working hypothesis was proposed that human melanoma may also have a viral etiology. In experiments the occurrence of virus-like particles as well as reverse transcriptase activity in metastases of human melanoma was reported in the early 70s. (Birkmayer et al., Europ.J.Cancer 10 (1974) 419-422).

These virus-like particles are spherical or slightly ovoid and have a diameter of 90-120 nm. They have an electron-dense nucleoid measuring 50-70 nm across and are bounded by a triple-layered, 100 Å thick membrane. Sometimes fine projections extend radially from the nucleoid towards the membrane, and in this case the particles resemble those found in hamster melanoma. Their number is rather small in comparison to that found in hamster melanoma. So far, they have been seen only in the cytoplasm and no infectivity has been observed for these particles (Birkmayer et al., Die Naturwissenschaften 59 (8) (1972), 369-370).

Also Balda et al. (Proc.Nat.Acad.Sci, USA 72 (9) (1975, 3697-3700) described a high molecular weight RNA encapsulated with an RNA extracted DNA polymerase in particles possessing the identity characteristic of the RNA tumor viruses which was detected in human malignant melanomas. However, also here no infectious particles were observed.

Only many years later the expression of a family of human endogenous retrovirus sequences (HERV-K) in GH cells, a teratocarcinoma cell line producing the human teratocarcinoma-derived retrovirus (HTDV) particles was described (Löwer et al., Proc.Natl.Acad.Sci. USA 90 (1993): 4480-4484). Detailed electron microscopic surveys have revealed the existence of retrovirus-like particles in human placentas and teratocarcinoma cell lines. However, these found HERV-K/HTDV particles were also not infectious: They possess a distinct morphology: (i) they lack the electron lucent space normally seen between viral envelope and core, (ii) they seem to be predominantly arrested in budding stages, and (iii) collapsed cores, a consequence of a final proteolytic step in virus maturation, have never been observed. As long as the gag precursor remains uncleaved, resulting particles are not likely to be infectious.

A further HERV-K provirus was shown to be present in gorilla and chimpanzee genomes but not in the human genome (Barbulescu et al., Current Biology 11 (10) (2001), 779-783).

Therefore, human endogenous retroviruses have shown to be expressed, however, these virus particles found up to date were all non-infectious virus particles. Some endogenous retrovirus-like particles were found to be expressed in melanoma cells. However, the only infectious endogenous retroviruses found were some animal endogenous retroviruses, e.g. MelARV, a retrovirus capable of infecting cultured murine melanocytes which have also been shown to induce in some instances malignant transformation.

In the article by Reus et al. (Journal of Virology, October 2001, 8917-8926) it is stated that the average age of HERV-K proviruses is ca. 28 million years. It is described that at that time the HERV-Ks probably used to be infectious.

The WO 01/62937 A1 relates to retrovirus sequences and psoriasis.

In the WO 00/53789 A1 various retroviral expression vectors are listed.

The WO 00/20460 A1 relates to the detection of a HERV-K10 gag-sequence which is brought into relationship with the cancer seminoma, whereby the HERV-K10 gag-sequence is used as tumor marker as well as for theurapeutic methods.

In the WO 00/06598 A1 a HERV-AVL3-B sequence fragment is brought in connection with methods for the treatment for malign melanomas.

The WO 99/67395 A1 relates to a 600bp sequence of an env-gene of a virus family HERV-7q and relates to the use for or in connection with multiple sclerosis, and autoimmune diseases, respectively.

In the WO 98/24454 A1 the use of a retroviral ribozyme sequence HERV-PTN for the inhibition of tumor growth is described.

The JP 9252780 A relates to a method, in which a retrovirus polypeptide of the type HERV-E as a surrogate marker for cancer and other illnesses is detected.

The WO 01/70941 A2 relates to a retroviral sequence with high homology to the family HERV-H, whereby this sequence is brought into connection with multiple sclerosis.

The DE 198 11 692 A1 relates to a preparation for the protection against sun rays which is effective against herpes simplex viruses, whereby the preparation comprises organic and anorganic light filters.

The object of the present invention is therefore to provide at least a fragment of a nucleotide sequence which codes for a further human endogenous retrovirus which is infectious.

The object of the present invention is solved by a polynucleotide molecule being a fragment of a nucleotide sequence of an infectious human endogenous retrovirus which polynucleotide molecule comprises a sequence selected from the group consisting of
(a) a sequence with at least 98%, preferably 99%, still preferred 99.5%, identity to a sequence according to SEQ ID No 1,
(b) a sequence which hybridises under stringent conditions with a nucleotide sequence according to said SEQ ID No 1,
(c) a sequence which differs from said sequences (a) or (b) due to degeneration of the genetic code and
(d) a sequence comprising sequences inserted into vectors pCR4-

Topo and deposited as "MERV-env", "MERV-gag", "MERV-prt" and "MERV-pol" at the DSMZ on 26 september 2001 or fragments thereof.

It has been surprisingly found that a polynucleotide molecule as mentioned above codes for (parts of) an infectious human endogenous retrovirus. With the present invention it can be shown for the first time that not only infectious animal endogenous retroviruses exist but also infectious human endogenous retroviruses. This is particularly surprising due to the fact that human endogenous retroviruses have been known and described for more than 30 years, however, even though many groups have been carrying out research in this field over decades, infectious human endogenous retroviruses could not be isolated or detected so far.

The polynucleotide molecule according to the present invention is preferably an isolated and a purified DNA or RNA molecule.

"Infectious" in the scope of the present application relates especially to retrovirus particles which are able to bind to the receptor of a cell over the env protein and enter into the cell and carry out a reverse transcription in order to produce a cDNA which can integrate into the host cell DNA. The differences between the life cycles of different retro elements and between infectious and non-infectious retroviruses is described in the article by Löwer et al. (Proc.Natl.Acad.Sci 93 (1996), 5177-5184), which is incorporated herein by reference.

Preferably, "infectious" human endogenous retroviruses according to the present invention are able to infect bovine MDBK cells and to integrate into these cells' genome.

The sequence according to SEQ ID NO 1 comprises a region which must be present in the polynucleotide molecule in order to code for an infectious human endogenous retrovirus (HERV). However, at both ends the polynucleotide molecule according to the present invention can comprise further herein not specifically described sequence regions, in particular LTR regions common to such viruses. With the present polynucleotide molecule any here not defined sequence regions can be identified by e.g. amplifying these sequences with the help of optimally designed gene specific primers which will for example bind to the above defined sequence regions. The amplification products can then be sequenced. Such methods are well known to the person skilled in the art.

"Stringent conditions" relate to hybridisation reactions under defined hybridisation conditions which is a function of factors as concentration of salt or formamide in the hybridisation buffer, the temperature of the hybridisation and the post-hybridisation wash conditions. Such conditions for Northern Blot analysis are for example hybridisation at 68°C with a MERV-specific polynucleotide molecule labelled with dioxigenin or radioactive in a standard SSC hybridisation buffer containing 0.1%SDS followed by stringent washing in wash buffer at the same temperature. Stringent washing can be performed for example by two times washing with 2xSSC buffer followed by two wash steps with 0.5xSSC buffer. In the present case stringent hybridisation conditions will preferably involve a temperature of 15°C to 25°C below the melting temperature (Tm), whereby the Tm of a hybridisation product of a nucleic acid probe can be calculated using a formula based on the g + c contained in the nucleic acids and that takes chain lengths into account, such as the formula Tm = 81.5 to 16.6 (log $[na^{+}]$) + 0.41 (% G + C) - 600/N), wherein N = chain length (Sambrook et al. (1989), which is incorporated herein by reference). In practice an estimated Tm for an oligonucleotide probe is often confirmed and thus a person skilled in the art can calculate the Tm for any chosen probe whose nucleotide sequence is known.

Sequences which differ from a given sequence due to the degeneration of the genetic code can be defined by any person skilled in the art. This can be carried out electronically without undue burden.

In the scope of the present application the deposited vectors MERV-env, MERV-gag, MERV-prt and MERV-pol refer to plasmids deposited at the DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH) according to the Budapest. Treaty and comprise the accession numbers DSM1450, DSM14538, DSM14536 and DSM14537.

In the scope of the present invention, when sequences inserted into the deposited vectors are concerned not only identical sequences are referred to but also sequences

(a) with at least 98%, preferably 99%, still preferred 99.5%,

identity to the sequences inserted into the deposited vectors,
(b) which hybridise under stringent conditions with sequences inserted into the deposited vectors and
(c) which differ from said sequences inserted into the deposited vectors due to degeneration of the genetic code.

The above polynucleotide molecule according to the present invention may further comprise a sequence which is at least 99.7%, 99.8% or 99.9% identical to SEQ ID NO 1.

Preferably, the polynucleotide molecule comprises a sequence according to SEQ ID NO 1 and is therefore identical to SEQ ID NO 1. This polynucleotide molecule has shown to code for a complete and active infectious particle of a HERV. The ORFs of SEQ ID NO 1 are preferably as follows:

ORF	Nucleotides	genetic code
gag	1-2013	universal
prt	1993-2809	nested reading frame
pol	3032-5650	universal
env	5352-7451	universal

Still preferred a polynucleotide molecule is provided which comprises a specific fragment of said above described polynucleotide molecule with a length of at least 15 bp, preferably at least 20 bp, still preferred at least 30 bp. The term "specific" defines any fragment which comprises a sequence different to any known HERV sequence. Therefore, any fragment with a length of at least 15 bp which comprises, for example, the in Table 1 mentioned mutations with respect to the known non-infectious HERV K (GeneBank, accessionnr. 164614) will be comprised by the polynucleotide molecule according to the present invention. However, these given lengths are minimum lengths and also fragments which are longer, e.g. 100, 1000, 1500 bp, are also comprised by the term "fragment".

"Fragments" according to the present invention relate also to specific fragments of the sequences inserted into the vector pCR4-Topo and deposited as MERV-env, MERV-gag, MERV-prt and MERV-pol as mentioned above.

Table 1:

clone name	position in MERV	position in HERV K108 (Accession 164614)	nucleotide change (MERV/HERV)	aminoacid change (MERV/HERV)
MERV gag	416	1515	C/T	Ala/Val
	602	1701	C/A	Arg/Lys
	630	1729	A/G	Pro/Pro
	639	1738	T/C	Ala/Ala
	654	1753	G/T	Pro/Pro
	738	1837	G/A	Pro/Pro
	799	1898	G/A	Glu/Lys
	802	1901	C/T	Leu/leu
	866	1965	T/C	Met/Thr
	945	2044	A/G	Ser/Ser
	953	2052	T/A	Met/Lys
	972	2071	A/G	Glu/Glu
	1149	2248	A/G	Gln/Arg
	1348	2447	A/G	Lys/Gly
	1413	2512	C/T	Ala/Ala
	1642	2741	G/A	Gly/Arg
	1653	2752	C/T	Tyr/Tyr
	1668	2767	C/T	Ile/Ile
	1701	2800	C/T	Asn/Asn
	1989	3088	A/G	Pro/Pro
MERV prot	2068	3167	T/C	Ile/Thr
	2087	3186	C/A	Gly/Gly
	2090	3189	A/C	Pro/Pro
	2097	3196	G/A	Glu/Lys
	2100	3199	A/G	Arg/Gly
	2124	3223	A/C	Arg/Arg
	2163	3262	G/A	Gly/Ser
	2198	3297	G/A	Gln/Gln
	2238	3337	G/A	Gly/Arg

	2253	3352	G/C	Glu/Gln
	2273	3372	T/C	Tyr/Tyr
	2305	3404	C/T	Thr/Ile
	2313	3412	T/C	Phe/Leu
	2330	3429	G/A	Pro/Pro
	2461	3560	G/A	Arg/Gln
	2501	3600	T/A	Gly/Gly
	2502	3601	T/C	Phe/Leu
	2543	3642	T/G	Thr/Thr
	2554	3653	G/A	Arg/His
	2590	3689	G/A	Arg/Gln
	2654	3753	A/G	Ala/Ala
	2659-2661	3758-3760	---/ATC	-/Ile
	2677	3776	T/C	Leu/Ser
	2741	3840	A/G	Gly/Gly
	2763	3862	G/A	Val/Ile
	2808	3907	T/A	Tyr/Asn
	2815	3914	T/G	Phe/Cys
	2816	3915	T/C	Phe/Cys
MERV prot	2878	3977	G/A	Arg/Gln
	2938	4037	G/A	Arg/Gln
	2974	4073	A/G	His/Arg
	3052	4151	C/T	Ala/Ala
	3091	4190	T/C	Pro/Pro
	3105	4204	T/C	Leu/Pro
	3124	4223	C/T	Leu/Asp
	3178	4277	G/A	Ala/Ala
MERV pol	3363	4462	A/G	Tyr/Cys
	3664	4763	A/C	Ile/Ile
	3700	4799	A/G	Ile/Met
	4040	5139	C/A	Gln/Lys
	4115	5214	A/T	Met/Leu
	4219	5318	G/A	Gly/Gly
	4225	5324	G/A	Lys/Lys
	4255	5354	T/G	Ser/Ser
	4336	5435	C/A	Ala/Ala
	4465	5564	T/A	Thr/Thr
	4517	5616	G/C	Glu/Gln

	4771	5870	G/A	Ser/Ser
	4780	5879	T/C	His/His
MERV env	5402	6501	C/T	His/His
	5719	6818	T/C	Ile/Thr
	6456	7555	G/A	Val/Ile
	6458	7557	C/T	Val/Ile
	6464	7563	A/G	Leu/Leu
	6802	7901	G/C	Gly/Ala
	7146	8245	A/G	Lys/Glu
	7262	8361	A/G	Thr/Thr
	7340	8439	G/A	Arg/Arg
	7347	8446	A/G	Asn/Asp
	7388	8487	A/G	Ser/Ser
	7436	8535	C/T	Val/Val

According to a preferred embodiment of the present invention a polynucleotide molecule encoding for an env protein of an infectious endogeneous human retrovirus is provided, said polynucleotide molecule comprising a sequence selected from the group consisting of
(a) a sequence with at least 98%, preferably 99%, still preferred 99.5%, identity to a sequence according to SEQ ID No 2,
(b) a sequence which hybridises under stringent conditions with a nucleotide sequence according to said SEQ ID No 2 and
(c) a sequence which differs from said sequence (a) or (b) due to degeneration of the genetic code.

"Env" according to the present invention relates also to the sequence inserted into the deposited MERV-env plasmid as mentioned above or a fragment thereof.

It is known that retroviruses are "quasispecies" and sequence variation in a given virus population is given. Such quasispecies variations are, of course, included in the definition of the present viruses and of the sequences of the nucleic acid molecules according to the present invention as long as the infectivity of the virus population as a whole is given.

"Env" relates to the envelope protein which is a viral membrane protein mediating the binding of the virus particles to the cellular receptors enabling virus entry which is the first step in a new replication cycle. The env protein is particularly important for the ability to spread between cells and individuals (infectivity).

A further embodiment of the present invention relates to a polynucleotide molecule encoding for a pol protein of a HERV, whereby said polynucleotide molecule comprises a sequence selected from the group consisting of
(a) a sequence with at least 99%, preferably 99.5%, still preferred 99.8%, identity to a sequence according to SEQ ID No 3,
(b) a sequence which hybridises under stringent conditions with a nucleotide sequence according to said SEQ ID No 3 and
(c) a sequence which differs from said sequence (a) or (b) due to degeneration of the genetic code. "pol" relates to the polymerase which is an enzyme with RNA dependant reverse transcriptase activity. Also this protein is important for a correct viral replication cycle.

"Pol" according to the present invention relates also to the sequence inserted into the deposited MERV-pol plasmid as mentioned above or a fragment thereof.

A still further aspect of the present invention relates to a polynucleotide molecule encoding for a gag protein of a HERV, whereby said polynucleotide molecule comprises a sequence selected from the group consisting of
(a) a sequence with at least 98%, preferably 99%, still preferred 99.5%, identity to a sequence according to SEQ ID No 4,
(b) a sequence which hybridises under stringent conditions with a nucleotide sequence according to said SEQ ID No 4 and
(c) a sequence which differs from said sequence (a) or (b) due to degeneration of the genetic code.

The gag gene products have acquired the ability to be transported to the cell surface and abut on the cell membrane incorporating env proteins during this process. The gag gene codes for a long polyprotein which in a first step is cut from the long molecule chain and is then cut into a number of parts which are present around the RNA in form of capsomeres. Since these gag proteins are essential for the budding they are important for the infectious viral circle.

"Gag" according to the present invention relates also to the sequence inserted into the deposited MERV-gag plasmid as mentioned above or a fragment thereof.

A further embodiment relates to a polynucleotide molecule encoding for a pro protein of a HERV, whereby said polynucleotide molecule comprises a sequence selected from the group consisting of
(a) a sequence with at least 98%, preferably 99%, still preferred 99.5%, identity to a sequence according to SEQ ID No 5,
(b) a sequence which hybridises under stringent conditions with a nucleotide sequence according to said SEQ ID No 5 and
(c) a sequence which differs from said sequence (a) or (b) due to degeneration of the genetic code.

The pro gene comprises 3 regions of particular interest:
1. dUTPase, dUTPase hydrolyses dUTP to dUMP and pyrophosphate.
2. prt retroviral Aspartyl-Proteinase
3. G_patch, α-glycine rich nucleic binding domain; A predicted glycine rich nucleic binding domain found in the splicing factor 45, SON DNA binding protein and D-type Retrovirus- polyproteins.

"Pro" according to the present invention relates also to the sequence inserted into the deposited MERV-prt plasmid as mentioned above or a fragment thereof.

Advantageously, a polynucleotide molecule is provided which comprises a sequence which is complementary to one of the above mentioned sequences according to the present invention. Therefore, not only the sequence according to the RNA strand present in the retrovirus particle is provided but also a sequence according to the cDNA sequence.

Still preferred, the polynucleotide molecule comprises a detectable label. In particular if the polynucleotide molecule is a fragment of the above defined sequence, the polynucleotide molecule can be used for the detection of infectious HERV in a sample. The term "detectable label" relates to any marker well known in the art, e.g. fluorescent, radioactive, enzymatic or chemical marker.

A further aspect of the present invention relates to a polynucleotide molecule coding for a ribozyme, which comprises two sections, each of which has a length of at least 10 to 15 base pairs and which are complementary to specific sequence sections of said above identified polynucleotide molecule according to the present invention so that said ribozyme complexes and cuts the mRNA transcribed by a natural infectious human endogenous retrovirus DNA. The publication by John M. Burg "Clearing the Way for Ribozymes" (Nat.Biotechnology 15:414-415 (1997)) relates to the general mode of function of ribozymes and is included herein by reference. The ribozyme will recognise the mRNA of infectious HERV by complementary base pairing with said mRNA. The ribozyme will then cleave and destroy the RNA in a sequence specific manner before the HERV is translated. After dissociation from the cleaved substrate the ribozyme will repeatedly hybridise with specific RNA molecules and act as specific endonuclease. In general, ribozymes may specifically be produced for inactivation of a certain mRNA even if not the entire DNA sequence which codes for the infectious HERV is known.

Ribozymes are particularly efficient if the ribosomes move slowly along the mRNA. In that case it is easier for the ribozyme to find a ribosome free site on the mRNA. For this slow ribosomes mutants are also suitable as a system for ribozymes (J. Jayburg (1997), Nature Biotechnology 414-415). A further possible way is also to use a varied form of a ribozyme, i.e. a minizyme. Minizymes are efficient particularly for cleaving larger mRNA molecules. A minizyme is a hammer head ribozyme which has a short oligonucleotide linker instead of the stem/loop II. Primer minizymes are particular efficient (Kuferbara et al. (1998), Nature Biotechnology 16, 961-965).

Here again the ribozyme comprises sequence sections which are complementary to "specific" sequence sections of the above defined polynucleotide molecule according to the present invention. It is sufficient if at least one of the two sequence sections is specific, meaning that this specific sequence section will only bind to an infectious HERV according to the present invention and not to already known and described non-infectious HERV sequences. Preferably, however, both sequence sections are specific for the above defined polynucleotide molecule according to the present invention, meaning that both sequence sections bind only infectious HERV sequences.

A further aspect of the present invention refers to a primer pair whereby the primers specifically hybridise to a polynucleotide molecule according to the present invention for an amplification reaction of a fragment of said polynucleotide molecule. Here again the term "specifically" refers to primers which will only bind to the above defined polynucleotide molecule according to the present invention and not to any known already described HERV. It is sufficient, if only one of the primers is specific for the inventive polynucleotide molecule, however, preferably both primers bind specifically only to the inventive HERV polynucleotide molecule. This primer pair can be used for any detection reaction in order to detect the presence of a HERV in a sample, whereby the term "amplification reaction" relates to any reaction specifically amplifying a sequence corresponding to the above described HERV sequence according to the present invention. This may be, for example, a conventional PCR as well as RT-PCR which are both well known to a person skilled in the art. However, the primer pair can also be used to find further herein not defined parts of the infectious HERV provirus according to the present invention. The primer pair can furthermore be used to identify other infectious HERVs which comprise, at least partly, similar sequences to the sequence of the infectious HERV according to the present invention.

The skilled man in the art easily finds conditions wherein primers which are specific for the infectious HERV sequences according to the present invention (which e.g. differ from sequences of known (non-infectious) HERVs by only 1, 2 or 3 nucleotides) only hybridise or anneal to the sequences of the infectious HERVs according to the present invention, but not to e.g. the non-infectious HERV sequences known. Examples for such specific regions are e.g. the regions covering one or more of the mutations listed in Table 1. Further examples may be derived by the skilled man in the art e.g. by comparing the sequences of the present infectious HERV with the known (non-infectious) HERV-K sequences (e.g. HERV-K108).

A further aspect of the present invention relates to a biologically functional vector comprising a polynucleotide molecule according to the present invention. The biologically functional vector will comprise all elements necessary for efficiently expressing the polynucleotide molecule according to the present invention whereby these elements, e.g. the promotor, will be selected according to the host into which the functional vector will be transfected and in which the polynucleotide molecule will be expressed, e.g. to produce a protein or polypeptide encoded by the present infectious HERV sequences. A person skilled in the art can select the optimal biologically functional vector without undue burden.

Still preferred, a biologically functional vector comprising a polynucleotide molecule as defined above according to the present invention in inverse orientation with respect to the promotor is provided. This vector allows the production of antisense mRNA which is complementary to the mRNA of the infectious HERV according to the present invention. The antisense mRNA will bind to the respective region of the infectious HERV mRNA and therefore block any further translation and production of viral proteins and therefore infectious viral particles. Hereby it is sufficient, if an essential part of the infectious HERV mRNA is blocked in order to block the production of a complete infectious viral particle, e.g. part of the gag, pol, pro or env mRNA. Therefore, said biologically functional vector can be used in cell cultures to stop the viral production as well as a gene therapy against or prevention of viral replication in a patient.

A further aspect of the present invention relates to a recombinant host cell stably transfected with said biologically functional vector according to the present invention as defined above. Here, the person skilled in the art will select the vector and host cell in order to achieve an effective result, e.g. high expression of the infectious HERV or fragments thereof. Transfection methods in order to produce a recombinant host cell are well known to the person skilled in the art and may comprise any electroporation, lipofectamin transfection, salt transfection etc. Depending on the vector which is transfected into the host cell, the host cell will either express viral peptides, proteins or particles or produce antisense mRNA.

Still preferred the host cell is a mammalian cell. This will allow the production of infectious HERV proteins or peptides with correct post-translational modifications. The mammalian cell may be, for example, from any human cell line.

A further aspect of the present invention relates to a method for producing an infectious human endogenous retrovirus or at least one fragment thereof comprising the steps of
(a) transfecting a host cell with a vector as defined above according to the present invention,
(b) selecting and cultivating cells which are stably transfected with said vector and
(c) isolating and purifying said retrovirus or said fragment.

Here again the above mentioned definitions and preferred embodiments apply, in particular with respect to the transfection step and the selection of the host cell and vector. The selection of stably transfected cells may be carried out according to any method well known in the art, e.g. selection with antibiotic resistance or optical selection. The term "fragment" relates to any peptide encoded by the above defined polynucleotide molecule according to the present invention. However, the fragment will preferably refer to a peptide which can be used as an antigen. To detect fragments which can be used as antigens known homologous fragments of corresponding HERVs or ERVs can be used, however, such fragments can also be detected by providing a sample of antibodies of infectious HERV, which sample can be taken of an infected patient, incubating said antibody or antibodies with one or more fragments of the infectious HERV proteins in conditions so that a binding between corresponding antigens and antibodies occur and identifying the respective fragments which have bound to antibodies of infectious HERV.

A further aspect of the present invention relates to an infectious human endogenous retrovirus comprising as RNA a polynucleotide molecule according to the present invention. Preferably the infectious HERV according to the present invention will comprise the complete polynucleotide molecule, e.g. comprising the pol, env, gag, pro genes as well as LTR sequences. However, it is also possible that the RNA comprises only one or a few, but not all of the above mentioned genes. It is important that the HERV is able to carry out a complete retroviral replication cycle and infect further cells.

Furthermore, an infectious human endogenous retrovirus is provided being encoded by a polynucleotide molecule as defined above according to the present invention.

A further aspect of the present invention relates to a preparation comprising an infectious HERV according to the present invention. The preparation may be a pharmaceutical preparation in which case preferably a pharmaceutically acceptable carrier is provided. However, the preparation may also be designed for diagnostic purposes as well as analytical or other purposes, e.g. to produce antibodies, to study the effects in animals etc. Of course, the preparation according to the present invention will comprise any additional substances depending on the use of the preparation. The preparation may comprise further virus particles, as non-infectious HERVs. However, at least one infectious HERV according to the present invention or a significant portion of the preparation leading to the overall infectivity of this preparation must be present.

A further aspect of the present invention relates to an env protein, a pol protein, a gag, a pro protein encoded by a polynucleotide molecule as defined above according to the present invention. These proteins can be used for any analytical, diagnostic or production methods and they will be provided in any form, e.g. with additional substances, depending on the use of the protein.

Furthermore a peptide is provided which is a specific fragment of said retrovirus as defined above according to the present invention. Here again, "specific" refers to any fragment which is not identical to a non-infectious HERV fragment already known. As mentioned above already the fragment may be any fragment of said retrovirus particle, however, preferably said fragment can be used as an antigen (e.g. a T or B cell epitope) and therefore be used in diagnostic methods as well as for the induction of the production of respective antibodies in a patient or animal and/or for the induction of specific T cell responses, both TH1 and TH2. The detection of such fragments can be carried out as described above.

The following fragments are examples of such antigens:

Fragments of the gag protein may be:
LMQNEAIEQVRAICL, IPYDWEILAKSSLSP, ADQLLGIGQNWSTIS, TISQQALMQNEAIEQ, EKARKVIVELMAYEN, MAYENANPECQSAIK, PVLNKQNITIQATTT, RSKFDKNGQPLSGNE, LSGNEQRGQPQAPQQ, QPPLSQVFQGISQLP,EIIDKSRKEGDTEAW,
VSTKNLIKL, GIGQNWSTI, QYGPNSPYM, CPVLNKQNI, LTVWNDWAI, KFDKNGQPL, GKCYNCGQI, HLKKNCPVL, GRKGNIIPL, FSIKMLKDM

Fragments of the env protein may be:
PAVDSDLTESLDKHK, WNSQSSIDQKLANQI, VSMDRPWEASPSVHI, PAVQNWLVEVPTVSP, LRPRVNYLQDFSYQR, NTEVLVWEECVANSA, SAVILQNNEFGTIID, QFYHNCSGQTQSCPS, NRSKRFIFTLIAVIM,
PYMLVVGNI, IFKASKAHL, KTIGSTTII, GYHYPPICL, SYQRSLKFR, KGKPCPKEI, VEVPTVSPI, SLRPRVNYL, TFNWQHRIL

Preferably, such peptide fragments contain at least 6 amino acid residues with at least one residue being specific for the present infectious HERV sequences, especially at least one of the amino acid residues not being present in the (non-infectious) HERV-K

(HERV-K108). More preferred these peptides have a length from 7 to 15 amino acids, especially from 8 to 11. Such peptide fragments of these lengths are preferably fragments of the above mentioned gag and env fragments.

A further aspect of the present invention relates to an antibody which is directed against an antigen derived from said retrovirus as defined above according to the present invention. The production of antibodies towards any specific antigen may be carried out according to any method well known in the state of the art, e.g. by immunisation of an animal and producing the antibodies in cell culture. The term "antibody" refers to any form of antibody, e.g. monoclonal, polyclonal, humanised antibody etc. The antibodies directed against antigens from the infectious HERV according to the present invention can be used in immunising methods, diagnostic methods or therapeutic methods.

Preferably, an antibody fragment is provided which is directed against an antigen derived from said retrovirus as defined above according to the present invention. Such antibody fragments may be for example Fa, $F(ab)^2$ or Fv which are capable of binding an epitopic determinant. Furthermore, single chain antibodies are comprised by the term "antibody fragment". These antibody fragments can be used as described above for the antibodies, however, due to their smaller size they may show advantages with respect to stability, production etc. Also here, the antibody fragment is specific, meaning that the antibody fragment comprises a sequence specific to infectious HERV according to the present invention.

A further aspect of the present invention relates to a solid support which has attached to its surface said antibodies defined above according to the present invention or said antibody fragments as defined above according to the present invention. The solid support according to the present invention may be of any size or shape, e.g. in the form of a flat slide, microtiter plate, chip, filter, column, membrane etc. and may be made of any material generally used for solid supports, e.g. modified or unmodified glass, polymeric material etc. Hereby the antibody or antibody fragment can be attached to the complete surface of the solid support. However, it may also be attached in localised regions, as for example spots. Also the quantity in which the antibody is bound to said solid support may vary according to the size of the antibody or antibody fragment. Of course, for high throughput assays it will be advantageous to use micro array chips on which the antibodies or antibody fragments are bound in high density. The selection of the solid support and the mode of attachment of the antibody to its surface will depend on the use of said solid support, e.g. for preparation, purification methods, detection methods, etc. and a person skilled in the art will be able to select the most appropriate solid support and the optimum mode of attachment of said antibody or antibody fragment to said solid support.

A further aspect of the present invention relates to a method for diagnosing cancerous cells comprising the steps of
(a) providing a sample of said cells to be tested or supernatant thereof,
(b) analysing whether or not said infectious endogenous retrovirus according to the present invention as described above or fragment thereof is present in said sample whereby
(c) the presence of said retrovirus or fragment thereof in said sample diagnoses cancerous cells.

The term "cancerous cells" refers to malignant tumor cells. It has been surprisingly found that the infectious HERV according to the present invention is related to the transformation of cells into cancerous cells, especially melanomas. Therefore, by detecting the presence of said infectious HERV it is possible to diagnose cancerous cells already at a very early stage. Therefore, the term "diagnosing cancerous cells" includes also diagnosing cells which will be transformed into cancerous cells, however, with conventional methods these cells would not yet be diagnosed as transformed.

However, it is not necessary to detect the complete infectious endogenous retrovirus. It is sufficient to detect at least one specific fragment thereof. Here again, the term "specific" refers to a fragment containing a sequence which differs from the sequences of the known non-infectious HERVs. The detection method may be carried out according to any protocol known in the art whereby it is possible to detect said infectious HERV or fragment thereof on DNA, RNA or protein level. It is further possible to determine the stage of cancer by determining the amount of infectious HERV.

Preferably, said analysing step comprises an antigen-antibody reaction using an antibody or antibody fragment as defined above according to the present invention in order to detect proteins or protein fragments of said retrovirus. Here, any known antigen-antibody reaction may be carried out, e.g. on solid support, in liquid phase, by sandwich-ELISA, etc. It is, of course, possible to detect antibodies in said sample, however, since the production of antibodies may vary, it is preferred to detect an antigen in said sample by providing one or more specific antibodies whereby these antibodies may be bound to a solid support and/or comprise a label which may be detected and quantified.

According to a preferred method said analysing step comprises detecting the polynucleotide sequence of said retrovirus and a fragment of the polynucleotide sequence specific for said retrovirus, respectively, whereby a hybridisation reaction or amplification reaction is carried out using a polynucleotide as defined above according to the present invention. Hereby, the term "polynucleotide sequence" comprises RNA sequences, e.g. the polynucleotide molecule derived from the transcription of the respective DNA or the polynucleotide molecule present in the virus particle, as well as DNA, e.g. cDNA resulting from the reverse transcriptase reaction thereafter, whereby the cDNA may be still present in the virus particle or also the DNA already integrated into the host DNA. Therefor, said amplification reaction will comprise PCR as well as RT-PCR. Therefor, the above mentioned primer pair will preferably be used.

Advantageously, said polynucleotide sequence is transformed into cDNA in a reverse transcription step prior to said hybridisation step and amplification reaction, respectively. Said hybridisation step will be preferably carried out with one of the above defined fragments of said polynucleotide molecule according to the present invention as mentioned above. Hereby it is, of course, possible to use labelled fragments or labelled nucleotides, e.g.

retroactive nucleotides in order to easily detect and quantify, respectively, said polynucleotide sequence.

According to a further preferred method said analysing step comprises
(a) providing cells devoid of said endogenous retrovirus,
(b) adding said supernatant of said sample to said cells,
(c) cultivating said cells and
(d) detecting a polynucleotide molecule as defined above according to the present invention in the DNA of said cells.

Since the HERV according to the present invention is infectious, the supernatant of said sample - if it comprises an infectious HERV - will infect the cells devoid of said endogenous retrovirus. After infection the infectious HERVs will integrate into said cells and further HERV particles will be produced. After cultivating said cells, therefore, the integrated polynucleotide molecule of the infectious HERV as defined above will be detectable in the DNA of said cells. This is a very reliable and quick method for detecting infectious HERVs. Again, the detection of said polynucleotide molecule may be carried out by a hybridisation step and amplifcation reaction, respectively.

Preferably said cells are non-human cells. Since non-human cells do not comprise HERVs, the detection of a HERV polynucleotide molecule in said human cells will be a clear method of detecting infectious HERV particles. Preferably, said non-human cells are bovine cells. Infectivity of the virus particles with respect to these non-human cells is also a way of defining infectivity according to the present invention.

Still preferred said sample is a skin tissue sample from a patient. Since said infectious HERVs preferably infect skin tissue cells, a sample of a skin tissue is preferably used in order to detect said infectious HERVs.

Still preferred, said sample comprises melanocytes. Since said infectious HERVs are related to cancerous cells a sample comprising melanocytes is a preferred sample for detecting infectious HERVs according to the present invention.

According to a preferred embodiment of the present invention a method is provided for diagnosing cancer in a patient comprising the steps of
(a) providing a sample of said patient to be tested,
(b) analysing whether or not at least one antibody as defined above or antibody fragment as defined above according to the present invention is present in said sample whereby
(c) the presence of said antibody or fragment thereof in said sample diagnoses cancer.

Here again, the same definitions and preferred embodiments as mentioned above apply.

Preferably said sample is a blood sample of said patient. A blood sample is very easily and quickly taken from a patient without necessity of costly apparatuses. Furthermore, it is possible to diagnose an unlimited amount of samples in a very short time. As mentioned above for high throughput assays it is preferable to use microarrays.

According to an advantageous method said analysing step comprises an antigen-antibody reaction using an antigen derived from said retrovirus as defined above according to the present invention. Here again, the above mentioned definitions and preferred embodiments apply.

According to a further advantageous embodiment a kit is provided for diagnosing cancerous cells according to the method as mentioned above according to the present invention comprising
(a) a first reagent comprising a polynucleotide molecule as defined above according to the present invention, an antibody as defined above according to the present invention or an antibody fragment as defined above according to the present invention or a solid support as defined above according to the present invention and
(b) as a positive control a second reagent comprising a polynucleotide comprising a sequence which is complementary to the sequence of said polynucleotide in said first reagent or a retrovirus as defined above according to the present invention,

an env protein as defined above according to the present invention, a pol protein as defined above, a gag protein as defined above or a peptide as defined above according to the present invention. Here again, the same definitions and preferred embodiments as described above apply. Of course, it is also possible to provide additionally to the first reagent a further solid support without attached antibodies or antibody fragments. In this case the antibody or antibody fragment as well as the mode of attachment can be varied.

According to a further embodiment of the present invention a kit for carrying out a method for diagnosing cancer in a patient as described above is provided comprising
(a) a first reagent comprising an antigen derived from said retrovirus as defined above and
(b) as a positive control a second reagent comprising an antibody according to the present invention or an antibody fragment as defined above specific for said antigen in said first reagent. Here again the same definitions and preferred embodiments as described above apply.

Preferably, said antigen is labelled with a detectable marker. This marker may be for example radioactive, fluorescent, an enzymatically or chemically detectable marker. Such markers are well known in the state of the art and the person skilled in the art will be able to select the optimal marker.

Still preferred, said antigen is immobilised to a solid support. This allows an easy handling of said kit and the method can be carried out immediately without prior preparations.

According to a further embodiment of the present invention a PNA molecule is provided which comprises a base sequence complementary to the sequence of said polynucleotide molecule as defined above according to the present invention. PNA (peptide nucleic acid) is a DNA like sequence, the nucleic basis being bound to a pseudo peptide backbone. PNA generally hybridises with complementary DNA, RNA or DNA polymers by Watson-Creek base pair and helix formation. The peptide backbone ensures a greater resistance to enzymatic degradation. The PNA molecule thus is an improved antisense agent.

Here, the advantage lies in that neither nucleases nor proteases are capable of attacking a PNA molecule. Therefore, the PNA molecule is highly stable, if it is bound to a complementary sequence, since it comprises a sufficient steric blocking of DNA and RNA polymerases, reverse transcriptase, telemorase and ribosomes (Buga et al, "Cell Penetrating DNA Constructs Regulate Alanin Receptor Levels and Modify Transmission in vivo", Nature Biotechnology 16:857-861 (1998)).

As in the case of the antisense RNA, if the PNA molecule comprises the above defined sequence it will bind to the DNA/RNA or to a site of the DNA/RNA respectively, which codes for infectious HERV peptides. In this way, the PNA molecule will inhibit the transcription of the virus. The PNA molecule is prepared synthetically, e.g. by aid of the T-boc technique, since it is neither transcribed nor translated.

The present invention also relates to the use of anti-retroviral substances for preparing a medicament to treat melanoma. The present invention therefore provides a method for treating patients having or being at risk of developing melanoma comprising administering an effective amount of substances acting against retroviruses, e.g. substances which inhibit replication and polymerisation of retroviral (infectious HERV) genomes. Examples of such treatment are e.g. known for the treatment of HIV infections.

Preferably, a pharmaceutical composition is provided comprising a polynucleotide as defined above according to the present invention, a vector as defined above according to the present invention, an antibody as defined above, an antibody fragment as defined above and a PNA molecule as defined above, respectively, and a pharmaceutically acceptable carrier. Of course, the pharmaceutical composition may comprise any further molecules or substances useful for a therapeutical method. The pharmaceutical composition may for example be provided as a skin cream or lotion, which may for example be used as an after sun lotion in a preventive manner.

Preferably, the pharmaceutical composition further comprises at least one antiviral substance, especially anti-retroviral substances. These may be any substances well known in the art, for example the agents used in known triple anti-retroviral therapy, see for example Bartlett JA, DeMasi R, Quinn J, Moxham C, Rousseau F. Overview of the effectiveness of triple combination therapy in antiretroviral-naive HIV-1 infectedadults. AIDS. 2001 Jul 27;15(11):1369-77; Marimoutou C, Chene G, Mercie P, Neau D, Farbos S, Morlat P, Ceccaldi J, Dabis F. Prognostic factors of combined viral load and CD4+ cell count responses under triple antiretroviral therapy, Aquitaine cohort, 1996-1998. J Acquir Immune Defic Syndr. 2001 Jun 1;27(2):161-7, which are incorporated herein by reference.

According to a further embodiment of the present invention a sun cream is provided which comprises a polynucleotide as defined above according to the present invention, an antibody, an antibody fragment as defined above and a PNA molecule as defined above, respectively, and at least one UV-protection substance. Therefore, applying a sun cream with these substances will prevent or inhibit the production of or infection by infectious HERV according to the present invention.

Preferably, said sun cream further comprises at least one antiviral substance. Here, the same antiviral substance as mentioned above for the pharmaceutical composition will be preferred.

According to a further embodiment of the present invention a vaccine is provided which comprises as an antigen an attenuated retrovirus as mentioned above, an env protein, a pol protein, a gag protein or a peptide as defined above according to the present invention, respectively, and optionally an adjuvant. Such a vaccine when applied to a patient will induce the production of antibodies and/or T cells, which can inhibit infectious HERVs according to the present invention. Therefore, such a vaccine is a highly effective substance for treating infectious HERV infections. The production of vaccines against retroviruses is described in "Hyperattenuated Recombinant Influenza A Virus Nonstructural-Protein-Encoding Vectors Induce Human Immunodeficiency Virus Type 1 Nef-Specific Systemic and Mucosal Immune Responses in Mice", Boris Ferko, Jana Stasakova, Sabine Sereinig, Julia Romanova, Dietmar Katinger, Brigitte Niebler, Hermann Katinger, and Andrej Egorov. Journal of Virology, October 2001, p. 8899-8908, Vol. 75, No. 19, which is incorporated herein by reference.

The present invention is described in more detail with the help of the following examples and figures to which it should, however, not be limited.

In the drawings:

Fig. 1 shows the characterisation of particle preparations from melanoma cell supernatants.

Fig. 2 shows an electron microscopy of melanoma cell-derived supernatants.

Fig. 3 shows the expression of the retroviral pol gene in melanomas.

Fig. 4 shows an immunofluorescence analysis with HERV-K-specific antibodies against gag, cORF, and env.

Fig. 5 shows cells infected with melanoma-associated particles.

Fig. 6 shows the detection of particle associated RNA and supernatants from infected MDBK cells.

Fig. 7 shows the transfer of supernatants from melanoma particle exposed MDBK cells to uninfected MDBK cells.

Fig. 8 shows the vector pCR4Topo as well as the gag, prot, pol and env primers.

EXAMPLES

Example 1

Human melanoma cells contain RT activity in their supernatants

To detect extracellular virions, supernatants from the melanoma cell lines SKMel-28, SKMel-1, 518A2 were analysed, as well as primary melanoma cells for pelletable RT. Supernatants from cultured human neonatal melanocytes (NHEM) served as controls. Cell-free supernatants from approximately 10^6 cells were concentrated by centrifugation. The RT activity of the resulting pellets was analysed by F-PERT assay.

Supernatants from melanoma cell lines, and normal human melanocytes were centrifuged at 3000xg at 4°C and sterile-filtered through a 0.22 µm low-protein membrane (Nunc) to remove cells and cellular debris. The clarified supernatants were centrifuged for 20 min at 250000xg at 4°C in a Beckman SW50.1 rotor. Pellets were rinsed with phosphate-buffered saline (PBS) and centrifuged for 15 min at 250000xg and resuspended. The resulting particle suspensions were used as an enzyme source for RT assays.

Pelleted particles derived from 4 ml cell-free supernatants of approximately 10^6 cells were analysed. RT activity was determined by fluorescent probe-based product enhanced RT (F-PERT) assay as described, with modifications. First, pellets were suspended in lysisbuffer (Roche). Then RT was performed, using the suspended pellet as source of enzyme, the primer 3´A10 (5'-CACAGGT-CAAACCGCCTAGGAATG-3'), and 0.3µg MS2-RNA as a template (Roche # 165948). To limit unspecific reverse transcription, 0.5µg calf-thymus DNA (Sigma # D4522) was added. After incubation at 42°C for 1 h, a 5µl aliquot of this reaction was amplified by real-time PCR, by adding 25µl TaqMan Universal PCR Master Mix (Applied Biosystems # 4304437), 1 µl of the primers 3´A10 and 5'A 11 (5'-TCCTGCTCAACTTCCTGTCGAG-3') at a concentration of 10µM each, 1 µl F-PERT probe (genXpress, 10 µM, 5'(FAM)-TCTTTAGCGAGACGCTAC-CATGGCTA-(TAMRA)3'), and 17 µl AD. The resulting mixture was then amplified by incubating 10 mins at 95°C, followed by 40 cycles at 94°C for 20 sec and 64°C for 1 min, in a SD 7700 (Perkin Elmer). The calibration curve was generated by plotting the RT activity of a serial dilution of Moloney Murine Leukemia Virus (M-MLV) RT (Superscript II Gibco # 18064 - 014). Alternatively, detection of reverse transcriptase was performed with a commercially available reverse transcription assay (Boehringer Mannheim). In this procedure, dioxigenin (DIG) and biotin-labelled nucleotides synthesised by the reverse transcriptase are incorporated into the DNA molecule. Biotion-labelled DNA is then captured to the streptavidin coated surface of the microtiter plates. In a subsequent step, an antibody to DIG conjugated to peroxidase is bound to the dioxigenin-labelled DNA. In the final step, the peroxidase substrate ABTS and a substrate enhancer were added. The absorbance of the samples was determined using a microtiter plate reader. The calibration curve was generated by plotting a serial dilution of an HIV-1 derived RT with defined activity.

Supernatants were found to contain a fluctuating yet continously present RT activity, corresponding to the activity of up to 10000 µunits of M-MLV RT (Superscript II, Gibco) per ml supernatant. On the other hand, supernatants from cultured melanocytes did not contain detectable RT activity (detection limit 10^{-1} µunits). The fact that supernatants derived from melanoma cells but not from melanocytes contain pelletable RT activity shows that melanoma cells contain proviral sequences with sufficient genetic information to form particles containing a functional RT. Since no RT activity was detected in the supernatants of melanocytes, production of particles containing RT appears to be activated during transformation of melanocytes to malignant cells.

Example 2

Human melanoma cell derived particles package sequences with high homology to HERV-K

Retroviral pol genes are generally the most conserved sequences among retroviruses. In an attempt to analyse the particle preparations on a molecular level, a sequence was amplified by using degenerate primers corresponding to conserved sequences of the pol gene. Particle preparations of the melanoma cell line 518A2 and SK-Mel28 were used for RT-PCR analysis. Particles were treated with DNAse, to remove contaminating traces of genomic

DNA.

Oligonucleotide primers 3´ABDPOLS 5´dCATTCCTTGTGGTAAAACTTTCCA[T/C]TG 3´ and 5´ABDPOLAS 5´dCCCCTTGGAATACTCCTGTTTT[T/C]TG 3´ (Codon Genetic Systems, Austria), derived from conserved regions within the retroviral reverse transcriptase (RT), were adopted from Medstrand and Blomberg. In order to release viral RNA, the particle suspension was transferred to a 0.5 ml polypropylene microcentrifuge tube (Sörensen Inc., USA). Then 10 µl of sterile water containing 0.1% Triton X-100 and 10 U RNase inhibitor (Gibco) was added and incubated at 65°C for 10 min. The reverse transcription was performed in the same tubes. A mixture containing 50 mM Tris-HCl buffer pH 8.3, 50 mM KCl, 7.5 mM $MgCl_2$, 5 mM DTT, 1 mM dNTPs, 20 pmol primer 3´ABDPOLA, 10 U RNase inhibitor and 100 U of Superscript II RT (Gibco) in a final volume of 20 µl was added while the tubes were kept on ice. The reaction was performed at 42°C for 45 min.

For PCR, the reaction mixture contained 10 mM Tris-HCl buffer pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.005% (w/v) gelatin, 0.2 mM dNTPs, 25 pmol of 3´ABDPOLS and 5´ABDPOLAS, 10µl of the reverse transcription reaction and 1.25 U Taq polymerase (Boehringer Mannheim) in a final volume of 50 µl. The reaction mixes were overlaid with mineral oil and subjected to 3 thermal cycles of denaturation at 92°C for 45 s, primer annealing at 45°C for 45 s and elongation at 72°C for 60 s, followed by 45 cycles at 92°C for 45 s, 55°C for 45 s and 72°C for 60 s. 10 µl of each amplification product were analysed by electrophoresis in a 1.5 % agarose gel and visualised by ethidium bromide staining. An aliquot of the amplification product was cloned and sequenced.

RT-PCR of these preparations revealed the anticipated amplification products of 298 nucleotides. Since no amplification products were obtained when RT was omitted, possible DNA contaminations can be excluded (Fig.1a). (A) RT-PCR. Particle preparations of the melanoma cell line Mel-Juso were treated with DNAse and used for analysis. PCR (lane 1) or RT-PCR (lane 2) with degenerate primers corresponding to sequences that are conserved in retroviral polymerase (pol) genes was performed; M, molecular weight marker (Boehringer Mannheim VIII). No amplification products were obtained in the control reactions without RT, demonstrating the absence of contamination with DNA (lane 1). In contrast, RT-PCR revealed the anticipated amplification products of 298 nucleotides (lane 2). (B) Immunoblotting. Particle preparations from SK-Mel28 supernatants were separated by SDS-PAGE, blotted and HERV-K-specific proteins were detected with antiserum recognising env (lane 1), gag (lane 3) and the corresponding pre-immunesera, pre-env (lane 2) and pre-gag (lane 4). Molecular weight is indicated to the left. In addition, RT-PCR of the GAPDH gene was negative, reflecting the lack of contamination with cellular mRNA (data not shown). The amplification product was cloned into an expression vector and sequenced. Sequence analysis revealed that the amplified fragment shows high homology to the corresponding pol region of endogenous retrovirus HERV-K. Translation of the obtained nucleotide sequence revealed the amino acid sequence IKKKSGKWRMLTDLRAINSVIQPMGALQPGLPSPAIIPKNWPLVVIDLKDSFFTIPLADQDCEWFAFIIPAVNNLQPAKHF. This sequence is highly homologous with the corresponding sequences of the HERV-K HML2 and HML3 families. Within these groups the highest identity (90%) was observed for clone NMWV5 (GenBank accession AF0115998). Homology to sequences of type A, B and C retroviruses was less than 70%. The sequence analysis excludes contamination with any known non-human retrovirus and suggests an endogenous origin of the packaged RNA.

Example 3

Human melanoma derived particles contain mature gag and env proteins

Particles derived from SK-Mel28 melanoma cells were purified on iodixanol-cushions and analysed for the presence of HERV-K-specific env and gag proteins in Western blots.

Iodixanol-cushion purified particles from SK-Mel28 supernatants were pelleted, resuspended in PBS and analysed. The amount of soluble proteins was quantified by means of a modified Bradford analysis (Bio-rad, Richmond CA). 5 µg of total protein was applied per lane and separated by SDS-PAGE (10%). Proteins were transferred to PVDF membranes (Millipore, Bedford, MA) by Western blotting, and generated blots were incubated with the gag- and env-specific antisera. The membranes were washed twice with blocking solution and HERV-K-specific proteins were detected with an alkaline-phosphatase-conjugated second-step antibody.

The envelope (env) gene of retroviruses displays an ORF for the surface protein (SU) and a membrane spanning protein (TM). The env precursor is usually cleaved into the SU and TM subunits before translocation to the cell surface and incorporation into virus particles. To determine whether the env protein is present on the particles, immunoblotting with an antiserum recognising the TM domain was performed. As shown in Fig. 1b two bands are visible in the Western blot. The upper band corresponds to the precursor migrating at approximately 80 to 90 kDa. In addition, a lower band migrating at approximately 37 kDa is visible, suggesting cleavage of the precursor into subunits.

Immunoblotting with an HERV-K anti-gag antiserum revealed a double band at approximately 76 kDa, corresponding to gag precursors, as well as processed intermediate gag proteins at approximately 61 kDa, 30 kDa and one band migrating with the front (less than 19 kDa). The 30 kDa protein corresponds to the putative major core protein of HERV-K. The presence of processed gag proteins in the Western blots indicates a functional protease.

Example 4

Human melanoma cells produce retrovirus-like particles

To confirm the presence of particles electron microscopy (EM) was performed.

15µl aliquots of iodixanol-density gradient purified particle suspensions from SK-Mel28 supernatants were applied on Formvar coated grids and left there for 15min. Excess suspension was removed from the edges of the grids by filter paper. The grid with the remaining sample was air dried for 1h. The sample was either directly exposed to 1% uranyl acetate for negative staining or, for immunoelectron microscopy, fixed in paraformaldehyde-lysine-periodate for 15min, rinsed in destilled water, quenched in PBS/1% BSA and incubated in anti-env antiserum diluted 1:25 in PBS/1%BSA for 1h at RT. After washing, the grid was exposed to 5 nM colloidal gold conjugated rabbit anti-goat antibody (British Biocell, Cardiff, UK) diluted 1:50 in PBS/1%BSA for 1h. After fixation in 2.5% glutaraldehyde, washing and negative staining with 1% uranyl acetate, the grid was left to air dry. All grids were examined with a JEOL 1010 electron microscope.

For electron microscopy, immunoblotting and infection studies, cell-free supernatants were purified on iodixanol density gradients or cushions. Iodixanol is an iodinated, nonionic density gradient medium (Nycomed Pharma, Oslo, Norway). It has a low viscosity and provides iso-osmotic conditions up to densities of 1.32g/ml. The supernatants were overlaid on a cushion of 5 ml 50% iodixanol. The tubes were centrifuged in an SW28 rotor at 45000xg for 2h at 4°C and the supernatant was removed from the tubes by suction, leaving a volume of 4ml of the medium in proximity of the cushion. This fraction was harvested, pelleted in a SW41 rotor at 150000xg for 90 min at 4°C, resuspended in PBS and analysed. Alternatively, for further purification the cushion-derived fraction was loaded on iodixanol density gradients, and the fraction corresponding to a density of approximately 1.16 g/ml was harvested. The harvested fractions were diluted in PBS and pelleted in a SW41 rotor at 150000xg for 90 min at 4°C and resuspended in PBS.

Pellets were partially purified on iodixanol-cushions (Fig. 2, upper part). Alternatively, these fractions were further purified on iodixanol density-gradients (Fig. 2, lower part). These preparations were analysed by immunelectron microscopy using an env-specific antiserum and a colloidal gold conjugated reporter antibody. EM analysis of these preparations revealed the presence of retrovirus-like particles characterised by membrane bound spherical structures with diameters ranging between 80 and 120nm. The presence of immunogold on the particles indicates that the env protein is present in the envelope.

Example 5

The pol gene is expressed in tumor cells derived from melanomas

The integration sites of human endogenous retroviral elements have been found to be distributed over the whole human genome. For example, the HERV-K family was reported to be present in approximately 30 copies per human haploid genome. Based on the observation that melanoma cells (but not melanocytes) produce retrovirus-like particles, it can be hypothesised that the formation of virus-like particles might be due to the activation of retroviral genes that are usually repressed. Therefore the cloned pol sequence derived from the particle preparations was used as a probe and looked for expression of this sequence in the cytoplasm of melanoma cells by in situ hybridisation.

Touch preparations of a nevus, primary melanoma, and melanoma metastasis were made by dipping freshly excised tissue on coated slides (DAKO, Biotek Solutions). Slides were fixed in 4% paraformaldehyde for 20 mins, washed in PBS, dehydrated through graded alcohols to absolute ethanol, and were air dried.
The hybridisation mixtures consisted of Hybrisol VI (Oncor, Gaithersburg) and DIG-labelled cDNA probes, giving a final concentration of 2ng/µl. Slides were covered with glass coverslips and sealed with Gelbond (ICN). Probe and cellular material were denatured by heating to 80°C for 5 mins. Hybridisation was carried out at 37°C overnight in a humid chamber. After removal of the coverslips the slides were washed at 46°C three times with 50% formamide/2xSSC, once with 2xSSC for 10 mins, followed by a single wash step in 2xSSC containing 0,1% NP40 for 10 mins. Signal detection was performed after a blocking step in 1% blocking reagent (Boehringer Mannheim) of 30 mins at 37°C by incubation with anti-DIG antibody, conjugated to rhodamine at a dilution of 1:10 in 1% blocking reagent for 30 mins at 37°C in a humidified box. After extensive washings in PBS, slides were counterstained with 10µg/ml DAPI for 20 mins and visualised with a Zeiss fluorescence microscope using a triple bandpass filter and software from PSI.

To determine the specificity of the pol sequence for melanoma, touch preparations of a nodular melanoma were analysed, a lymph node metastasis, and a cutaneous metastasis that had been surgically removed from melanoma patients. As shown in figure 3 (Touch preparations from primary melanoma (a), lymph node metastasis (b), cutaneous metastasis (c), nevus (d), and a tumor free sentinel lymphnode (e) surgically removed from patients were analysed by in situ hybridisation with probes specific for nucleotide sequences of the pol gene (POL), melanoma inhibiting activity gene (MIA), and the influenza virus nucleoprotein (FLU). Nucleotide sequences of the pol and the melanoma inhibiting activity genes are found in primary melanoma and lymphnode and cutaneous metastases but not in the nevus and tumor free lymphnode), high copy numbers of the pol sequence were found in tumor cells of all melanoma preparations tested. In comparison, cells derived from the lymph node and the benign nevus of healthy individuals were negative. A probe specific for the nucleoprotein gene of influenza virus was always negative. As a positive control, a probe recognising the melanoma-inhibiting activity gene was used. The rate of tumor cells expressing the pol gene was in the range of 60-90%, a percentage similar to the one obtained with the melanoma-inhibiting activity specific probe. The observation that the sequences are not found in all tumor cells might be due to the sensitivity of the assay and variations in the expression levels.

Example 6

Retroviral gag, cORF, and env proteins are expressed in melanomas

To determine whether HERV-specific proteins were detectable in melanoma cells, immunofluorescence analysis with antisera recognising the gag, cORF and env proteins of HERV-K was performed.

Cells grown on chamber slides, as well as touch preparations from a nevus and primary melanoma on coated slides (DAKO) were fixed in 4% paraformaldehyde for 20 mins, washed in PBS and dehydrated through graded alcohols to absolute ethanol and air dried. In addition, five micrometer sections prepared from routinely processed paraffin wax blocks of a nevus and melanoma were placed on coated slides (DAKO). The tissues were dewaxed in xylene, rehydrated by sequential immersion in graded ethanols and PBS and subsequently permeabilised by microwave treatment.

Immunofluorescence staining was performed by incubating chamber slides, touch preparations, and paraffin sections with HERV-K-specific antibodies at a dilution of 1:100 for 1 h at 37°C in a humidified box, followed by washing three times with PBS and subsequent incubation with Alexafluor-488-conjugated antibodies at a dilution of 1:200 for 1 h at 37°C. Counterstaining was performed by mounting in Vectashield containing DAPI (Vector). Preparations were analysed by using a Zeiss fluorescence microscope with appropriate filters.

Figure 4a shows that the melanoma cell lines Mel-Juso and SK-Mel28 express the gag, cORF and env proteins. The percentage of gag-expressing cells was in the range of 1-10%, cORF was found to be present in about 20%, while expression of the env protein was detected in about 10% of the analysed cells. Expression of gag and env was found in the cytoplasm, while cORF was mainly found in the nucleus. In contrast, cultured human melanocytes (Nhem) and Vero cells did not react with any HERV-K-specific antisera. None of the corresponding preimmune-sera was reactive with any of the cells tested.

Immunofluorescence analysis was performed with touch preparation of a primary melanoma (first column); touch preparation of a nevus (second column); paraffin preparation primary melanoma (third column); paraffin preparation nevus (fourth column). Retroviral proteins are present in the melanoma preparations but absent from nevus tissue.

It was then analysed whether these proteins are also expressed in primary melanoma. Fig. 4b shows that gag, cORF, and env were detected in touch preparations and paraffin sections of a primary melanoma but not in the corresponding preparations of a nevus.

Example 7

Melanoma cell derived particles are infectious

Supernatants of approximately 10^8 melanoma cells were loaded on

30% sucrose cushions and centrifuged at 28K. The resulting pellets were washed and resuspended in 500µl PBS. For infection studies human 293 cells were grown to approximately 30% confluency and exposed overnight to the particle preparations in the presence of 8µg/ml polybrene. As controls, cells were treated with particle preparations that were heat inactivated 30 min at 60°C or mock-treated. 293 cells were exposed to particle preparations from the melanoma cell line SKMel28.

The human epithelial cell line 293 is a well established target for retrovirus infection. Immunofluorescence with HERV-K specific gag antibodies was performed three weeks post infection (p.i.). Control cells were mock-treated. 293 cells were treated with the melanoma-derived viral particles, and demonstrate that upon treatment they express the HERV-K specific gag-protein (Fig. 5a). Moreover, expression of the retroviral proteins correlated with the level of pelletable RT-activity in the supernatants (Fig. 5b), suggesting that the 293 cells were infected with the melanoma-derived particles. However, due to the high copy numbers of various HERV sequences present in the human genome it was not possible to demonstrate that expression of proteins and production of RT-containing particles was derived from de novo integrated retroviral DNA into the hosts genome. Therefore this question was addressed in bovine MDBK cells.

Example 8

Infection of MDBK cells

It was tested whether the melanoma-derived particles are infectious. Due to the high copy numbers of various HERV sequences present in the human genome it is difficult to demonstrate in human cells whether virus particles produced are derived from endogenous or exogenous virus. This question was therefore addressed in bovine MDBK cells which are free of viruses that are homologous to HERV-K. MDBK cells were exposed to melanoma derived particle preparations and passaged at a ratio of 1:10. Supernatants of these cells were analysed by RT-PCR with pol-specific HERV primers as described below:

Cell free supernatants of approximately 10^8 518A2 melanoma cells were loaded on 20% sucrose cushions and pelleted at 28K in a Beckmann SW28 rotor. The resulting pellets were washed and resuspended in 500µl PBS. For infection studies bovine MDBK cells were grown to approximately 30% confluency and exposed overnight to 100µl aliquots of the particle preparations in the presence of 0.8% polybrene. As controls, cells were treated with particle preparations that were heat inactivated. 24 hour post infection (p.i.) the inoculum was removed, the cells were washed twice with PBS and incubated with normal media. Starting at 7 days p.i. the cells were continuously passaged at a ratio of 1:10 and the supernatants were analysed for the presence of viral particles by detecting particle-associated RNA. In addition 1.5 ml of the supernatants from infected MDBK cells were put onto new (uninfected) MDBK cells, in the presence of polybrene. 24 hours later the cells were washed twice with PBS and incubated for 7 days, and passaged at a ratio of 1:10. The release of particles from the cells was determined by detecting particle-associated RNA in the supernatants by RT-PCR as described below:

For detecting particles released by the cells, supernatants from approximately 10^7 518A2 or infected MDBK cells were filtered, pelleted 2 hours at 28K and resuspended in 500µl Trizol. To this suspension 100µl chloroform were added. After vortexing and centrifugation the upper phase was precipitated with isopropanol. The precipitate was pelleted by centrifugation and washed with 70% ethanol. The pellet was resuspended, treated with DNAse in the presence of 25mM $MgCl_2$ for 45 min at 37°C, and the DNAse was inactivated for 10min at 65°C. After precipitation with 96% ethanol, the pellet was washed with 70% ethanol, resuspended in reverse transcription (RT) reaction buffer and RT was performed with the random primer p(dN)6.1/10 of the reverse transcription reaction was used as template for PCR with the oligonucleotide primers 5´108 propol 3925 5´ CCACTGTAGAGCCTCCTAAACCC 3´ (SEQ ID No 6) and 3´108 pol 4315 5´ GCTGGTATAGTAAAGGCAAATTTTTC 3´ (SEQ ID No 7) (Codon Genetic Systems, Austria) which correspond to conserved regions within the pol gene.

10 µl of each amplification product were analysed by electrophoresis in a 1.5 % agarose gel and visualised by ethidium bromide

staining. An aliquot of the amplification product was cloned and sequenced. Expression of HERV-specific RNA expressed in MDBK cells was detected by RT-PCR of total genomic RNA derived from approximately 10^7 cells in the same manner.

Figure 6 shows the result after the first passage. Lanes 1-6: After infection cells were passaged 1:10 and supernatants were analysed by RT-PCR with pol-specific primers (+). Absence of contaminating DNA was analysed by omitting the RT step (-); Lane 1, no template control; lane 2, heat inactivated particles; lane 3, particle preparation diluted 1: 100, lane 4, 1: 10 dilution, lane 5, undiluted particle preparations; lane 6, 518A2 supernatant; M, molecular weight marker; Analysis of genomic DNA with pol-specific primers by PCR. Lane 1, no template control; lane 2, no template control; lane 3, MDBK DNA; lane 4, 518A2 DNA, lane 5, no enzyme control. Infection with undiluted particle preparations revealed a pol-specific amplification product (lane 5, +), while infection with particle preparations that were diluted 1:10 (lane 4+), diluted 1:100 (lane 3+)or were heat inactivated (lane 2+) did not. None of the reactions revealed a signal when the RT step was omitted, excluding that the signals were derived from genomic DNAs contaminations (lanes 2-6, -). The presence of particle associated viral sequences in the supernatant of the melanoma-derived particle exposed MDBK cells indicates that melanoma-derived particles are capable to infect bovine cells.

Sequence analysis of the amplification product of lane 5 revealed the nucleotide sequence
5´CCACTGTAGAGCCTCCTAAACCCATACCATTAACTTGGAAAACAGAAAAACCGGTGTGGGTAA
ATCAGTGGCCGCTACCAAAACAAAAACTGGAGGCTTTACATTTATTAGCAAATGAACAGTTAGAA
AAGGGTCATATTGAGCCTTCGTTCTCACCTTGGAATTCTCCTGTGTTTGTAATTCAGAAGAAATC
AGGCAAATGGCATATGTTAACTGACTTAAAGGCCGTAAACGCCGTAATTCAACCCAT 3´
(SEQ ID No.8). This sequence is highly homologues (98%) to the sequence from HERV-K 108.

Example 9

Supernatants from infected MDBK cells contain infectious particles

Cell free supernatants from cells exposed to undiluted melanoma particle preparations were put onto new (uninfected) MDBK cells which were subsequently passaged 3 times at a ratio of 1:10

Fig. 7 shows the transfer of supernatants from melanoma-particle exposed MDBK cells to uninfected MDBK cells. 24 hours later cells were washed twice with PBS, incubated for one week, and subsequently passaged at a ratio of 1:10. Fig.7a shows pelleted supernatants from these cells which were analysed for release of particle-associated RNA by RT-PCR with pol-specific primers (lanes 1, 3, 5, 7). As negative controls supernatants from MDBK cells exposed to heat inactivated particles were transferred to MDBK cells which were treated and analysed in the same manner (lanes 2, 4, 6, 8). Lanes 1 and 2: 5 days after tranfer ; lanes 3 and 4: passage 1; lanes 5 and 6: passage 2; lanes 7 and 8: passage 3; lane M: molecular weight marker (Boehringer Mannheim VIII); lane 9: negative control: pelleted supernatant from uninfected MDBK cells; lane 10: positive control: pelleted supernatants from 518A2 cells; Fig. 7b shows the expression of HERV-specific RNAs which was analysed by RT-PCR from whole genomic RNA of MDBK cells from passage 1 with pol-specific primers. Lane 1: MDBK cells treated with supernatants from infected MDBK cells; lane 2: MDBK cells treated with supernatants from MDBK cells exposed to heat inactivated melanoma-derived particle preparations; lane M, molecular weight marker (Boehringer Mannheim VIII); lane 3, negative control: PCR of genomic DNA derived from uninfected MDBK cells; lane 4, positive control: PCR of genomic DNA derived from 518A2 cells; lane 5: non-template control. Lanes 1, 3, 5, and 7 correspond to passages 0, 1, 2, and 3 respectively. Lanes 2, 4, 6, and 8 are the negative controls and correspond to passages 0, 1, 2, and 3 of cells treated with supernatants from cells exposed to heat inactivated particle preparations.

Shown is the presence of particle associated RNA as indicated by the presence of a pol-specific sequences. Pol-specific signals were detectable after passages 0, 1, and 3, suggesting the presence of viral particles (lanes 1, 3, and 7). Analysis of passage number 2 did not reveal a pol-signal which might be due to fluctuating production levels of viral particles (lane 5). Transfer of supernatants from MDBK cells that were treated with heat inactivated particle preparations did not result in the release of particles (lanes 2, 4, 6, and 8). PCR alone did not reveal pol-specific signals, indicating that the signals obtained by RT-PCR are derived from RNA. The result that transfer of the supernatant from infected MDBK cells to uninfected MDBK cells resulted in the release of particle associated viral RNA, indicates that infection with melanoma derived particles of MDBK cells is productive.

These data show that retroviral sequences and infectious particles are expressed in melanoma cells.

Partial sequence analysis, immunoblotting and immuno-electron microscopy studies show the particles belong to the HERV-K family. In contrast to other known HERV-K like viruses, which lack infectivity, the env precursor appears to be cleaved in the melanoma derived particles, resulting in approximately equimolar amounts of the precursor and the putative transmembrane domain. Cell-free particle preparations from the melanoma cells were able to infect both human and bovine cells, as indicated by the appearance of the viral proteins gag, cORF, and env, and pelletable RT-activity in the supernatants of infected cells. Moreover, the fact that human pol and gag sequences in the genome of the infected bovine MDBK cells were detected, indicates that the melanoma-associated viruses are capable of de novo integration of their genome. This observation also suggests the presence of a functional integrase in the viral particles.

Fig. 8a shows the pCR4-Topo vector into which the MERV gag, pro, pol and env sequences isolated from the above described infected bovine cells were inserted. The primers used in the above examples are shown in the boxes of each sequence of vectors shown in fig. 8b. These vectors were deposited on 26 Spetember 2001 at the DSMZ under the names MERV-env, MERV-gag, MERV-prt and MERV-pol according to the Budapest Treaty.

Claims:

1. Polynucleotide molecule being a fragment of a nucleotide sequence of an infectious human endogenous retrovirus, which polynucleotide molecule comprises a sequence selected from the group consisting of
(a) a sequence with at least 98%, preferably 99%, still preferred 99.5%, identity to a sequence according to SEQ ID No 1,
(b) a sequence which hybridises under stringent conditions with a nucleotide sequence according to said SEQ ID No 1,
(c) a sequence which differs from said sequences (a) or (b) due to degeneration of the genetic code and
(d) a sequence comprising sequences inserted into vectors pCR4-Topo and deposited as "MERV-env", "MERV-gag", "MERV-prt" and "MERV-pol" at the DSMZ on 26 september 2001 or fragments thereof.

2. The polynucleotide molecule according to claim 1, characterised in that it comprises a sequence according to SEQ ID No 1.

3. The polynucleotide molecule, characterised in that it comprises a specific fragment of said polynucleotide molecule according to claim 1 or 2 with a length of at least 15 bp, preferably at least 20 bp, still preferred at least 30 bp.

4. A polynucleotide molecule encoding for an env protein of an infectious human endogenous retrovirus, said polynucleotide molecule comprising a sequence selected from the group consisting of
(a) a sequence with at least 98%, preferably 99%, still preferred 99.5%, identity to a sequence according to SEQ ID No 2,
(b) a sequence which hybridises under stringent conditions with a nucleotide sequence according to said SEQ ID No 2 and
(c) a sequence which differs from said sequence (a) or (b) due to degeneration of the genetic code.

5. A polynucleotide molecule encoding for a pol protein of an infectious human endogenous retrovirus, said polynucleotide molecule comprising a sequence selected from the group consisting of
(a) a sequence with at least 99%, preferably 99.5%, still preferred 99.8%, identity to a sequence according to SEQ ID No 3,

(b) a sequence which hybridises under stringent conditions with a nucleotide sequence according to said SEQ ID No 3 and
(c) a sequence which differs from said sequence (a) or (b) due to degeneration of the genetic code.

6. A polynucleotide molecule encoding for a gag protein of an infectious human endogenous retrovirus, said polynucleotide molecule comprising a sequence selected from the group consisting of
(a) a sequence with at least 98%, preferably 99%, still preferred 99.5%, identity to a sequence according to SEQ ID No 4,
(b) a sequence which hybridises under stringent conditions with a nucleotide sequence according to said SEQ ID No 4 and
(c) a sequence which differs from said sequence (a) or (b) due to degeneration of the genetic code.

7. A polynucleotide molecule encoding for a pro protein of an infectious human endogenous retrovirus, said polynucleotide molecule comprising a sequence selected from the group consisting of
(a) a sequence with at least 98%, preferably 99%, still preferred 99.5%, identity to a sequence according to SEQ ID No 5,
(b) a sequence which hybridises under stringent conditions with a nucleotide sequence according to said SEQ ID No 5 and
(c) a sequence which differs from said sequence (a) or (b) due to degeneration of the genetic code.

8. A polynucleotide molecule, characterised in that it comprises a sequence which is complementary to said sequence as defined in any one of claims 1 to 7.

9. The polynucleotide molecule according to any one of claims 1 to 8, characterised in that it comprises a detectable label.

10. A polynucleotide molecule coding for a ribozyme, characterised in that it comprises two sequence sections, each of which has a length of at least 10 to 15 base pairs and which are complementary to specific sequence sections of said polynucleotide molecule according to any one of claims 1 to 7 so that said ribozyme complexes and cuts the mRNA transcribed by a natural infectious endogenous retrovirus DNA.

11. A primer pair, characterised in that the primers specifically hybridise to a polynucleotide molecule according to any one of claims 1 to 8 for an amplification reaction of a fragment of said polynucleotide molecule.

12. A biologically functional vector comprising a polynucleotide molecule according to any one of claims 1 to 10.

13. A biologically functional vector comprising a polynucleotide molecule according to any one of claims 1 to 8 in inverse orientation with respect to the promotor.

14. A recombinant host cell stably transfected with said biologically functional vector according to claim 12 or 13.

15. The host cell according to claim 14, characterised in that it is a mammalian cell.

16. A method for producing an infectious human endogenous retrovirus or at least one fragment thereof comprising the steps of
(a) transfecting a host cell with a vector according to claim 12,
(b) selecting and cultivating cells which are stably transfected with said vector and
(c) isolating and purifying said retrovirus or said fragment.

17. An infectious human endogenous retrovirus comprising as RNA a polynucleotide molecule according to anyone of claims 1 to 8.

18. An infectious human endogenous retrovirus being encoded by a polynucleotide molecule according to claim 1 or 2.

19. A preparation comprising an infectious human endogenous retrovirus according to any one of claims 16 to 18.

20. An env protein encoded by a polynucleotide molecule according to claim 4.

21. A pol protein encoded by a polynucleotide molecule according to claim 5.

22. A gag protein encoded by a polynucleotide molecule according to claim 6.

23. A peptide characterised in that it is a specific fragment of said retrovirus according to any one of claims 16 to 18.

24. An antibody, characterised in that it is directed against an antigen derived from said retrovirus according to any one of claims 16 to 18.

25. An antibody fragment, characterised in that it is directed against an antigen derived from said retrovirus according to any one of claims 16 to 18.

26. A solid support, characterised in that it has attached to its surface said antibody according to claim 24 or said antibody fragment according to claim 25.

27. A method for diagnosing cancerous cells comprising the steps of
(a) providing a sample of said cells to be tested or supernatant thereof,
(b) analysing whether or not said infectious endogenous retrovirus according to claim 16 or 17 or fragment thereof is present in said sample whereby
(c) the presence of said retrovirus or fragment thereof in said sample diagnoses cancerous cells.

28. The method according to claim 27, characterised in that said analysing step comprises an antigen-antibody reaction using an antibody according to claim 24 or an antibody fragment according to claim 25 in order to detect proteins or protein fragments of said retrovirus.

29. The method according to claim 27, characterised in that said analysing step comprises detecting the polynucleotide sequence of said retrovirus and a fragment of the polynucleotide sequence specific for said retrovirus, respectively, whereby a hybridisation reaction or amplification reaction is carried out using a polynucleotide according to any one of claims 1 to 8.

30. The method according to claim 29, characterised in that said polynucleotide sequence is transformed into cDNA in a reverse transcription step prior to said hybridisation step and amplification reaction, respectively.

31. The method according to claim 27, characterised in that said analysing step comprises
(a) providing cells devoid of said endogenous retrovirus,
(b) adding said supernatant of said sample to said cells,
(c) cultivating said cells and
(d) detecting a polynucleotide molecule according to any one of claims 1 to 7 in the DNA of said cells.

32. The method according to claim 31, characterised in that said cells are non-human cells.

33. The method according to any one of claims 27 to 32, characterised in that said sample is a skin tissue sample from a patient.

34. The method according to claim 32, characterised in that said sample comprises melanocytes.

35. A method for diagnosing cancer in a patient comprising the steps of
(a) providing a sample of said patient to be tested,
(b) analysing whether or not at least one antibody according to claim 24 or antibody fragment according to claim 25 is present in said sample whereby
(c) the presence of said antibody or fragment thereof in said sample diagnoses cancer.

36. The method according to claim 35, characterised in that said sample is a blood sample of said patient.

37. The method according to claim 35, characterised in that said analysing step comprises an antigen-antibody reaction using an antigen derived from said retrovirus according to any one of claims 16 to 18.

38. A kit for carrying out a method according to any one of claims 27 to 34 comprising
(a) a first reagent comprising a polynucleotide molecule according to any one of claims 1 to 8, an antibody according to claim 24 or an antibody fragment according to claim 25 or a solid support according to claim 26 and
(b) as a positive control a second reagent comprising a polynucleotide comprising a sequence which is complementary to the sequence of said polynucleotide in said first reagent or a retrovirus according to any one of claims 16 to 18, an env protein according to claim 20, a pol protein according to claim 21, a gag protein according to claim 22 or a peptide according to claim 23.

39. A kit for carrying out a method according to claim 37 comprising
(a) a first reagent comprising an antigen derived from said retrovirus according to any one of claims 16 to 18 and
(b) as a positive control a second reagent comprising an antibody according to claim 24 or an antibody fragment according to claim 25 specific for said antigen in said first reagent.

40. The kit according to claim 39, characterised in that said antigen is labelled with a detectable marker.

41. The kit according to claim 39 or 40, characterised in that said antigen is immobilised to a solid support.

42. A PNA molecule, characterised in that it comprises a base sequence complementary to the sequence of said polynucleotide molecule according to any one of claims 1 to 7.

43. A pharmaceutical composition comprising a polynucleotide according to any one of claims 1 to 9, a vector according to claim 11 or 12, an antibody according to claim 24, an antibody fragment according to claim 25 and a PNA molecule according to claim 42, respectively, and a pharmaceutically acceptable carrier.

44. The pharmaceutical composition according to claim 43, char-

acterised in that it further comprises at least one antiviral substance.

45. A sun cream, characterised in that it comprises a polynucleotide according to any one of claims 1 to 9, a vector according to claim 11 or 12, an antibody according to claim 24, an antibody fragment according to claim 25 and a PNA molecule according to claim 42, respectively and at least one UV-protection substance.

46. The sun cream according to claim 45, characterised in that it further comprises at least one antiviral substance.

47. A vaccine, characterised in that it comprises as an antigen an attenuated retrovirus according to claim 17 olr 18, an env protein according to claim 20, a pol protein according to claim 21, a gag protein according to claim 22 or a peptide according to claim 23, respectively, and optionally an adjuvant.

FIG. 1

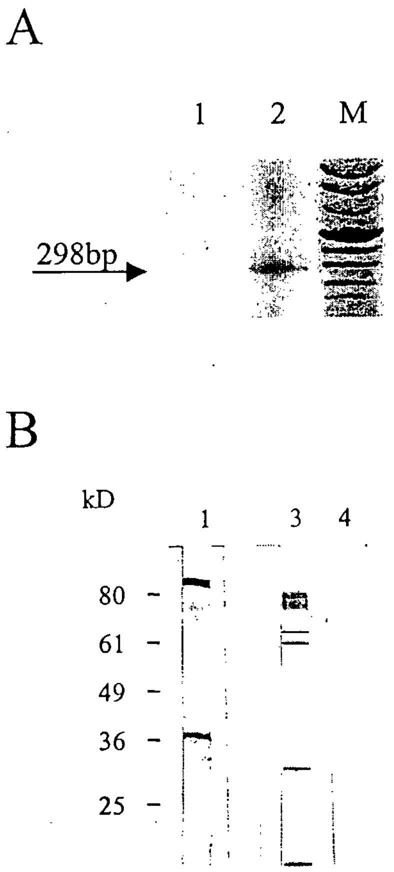


FIG. 2

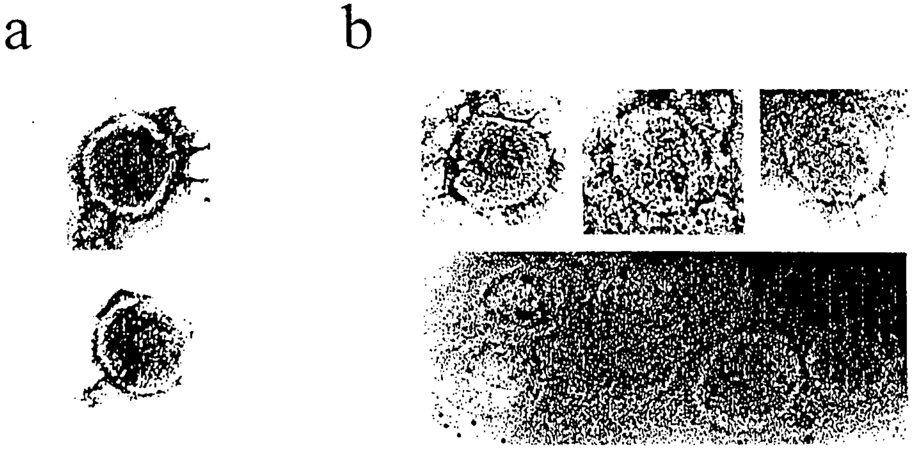
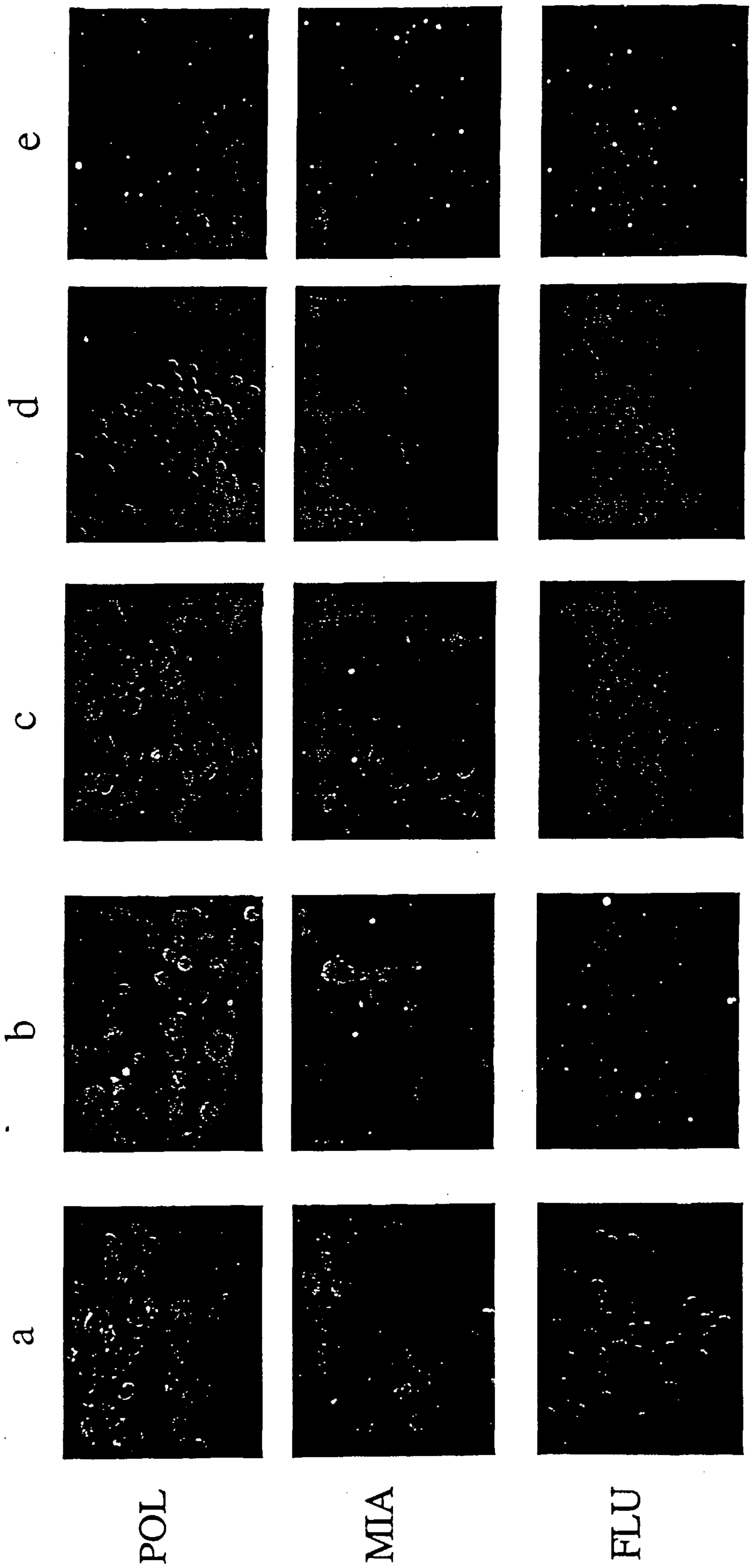


FIG. 3



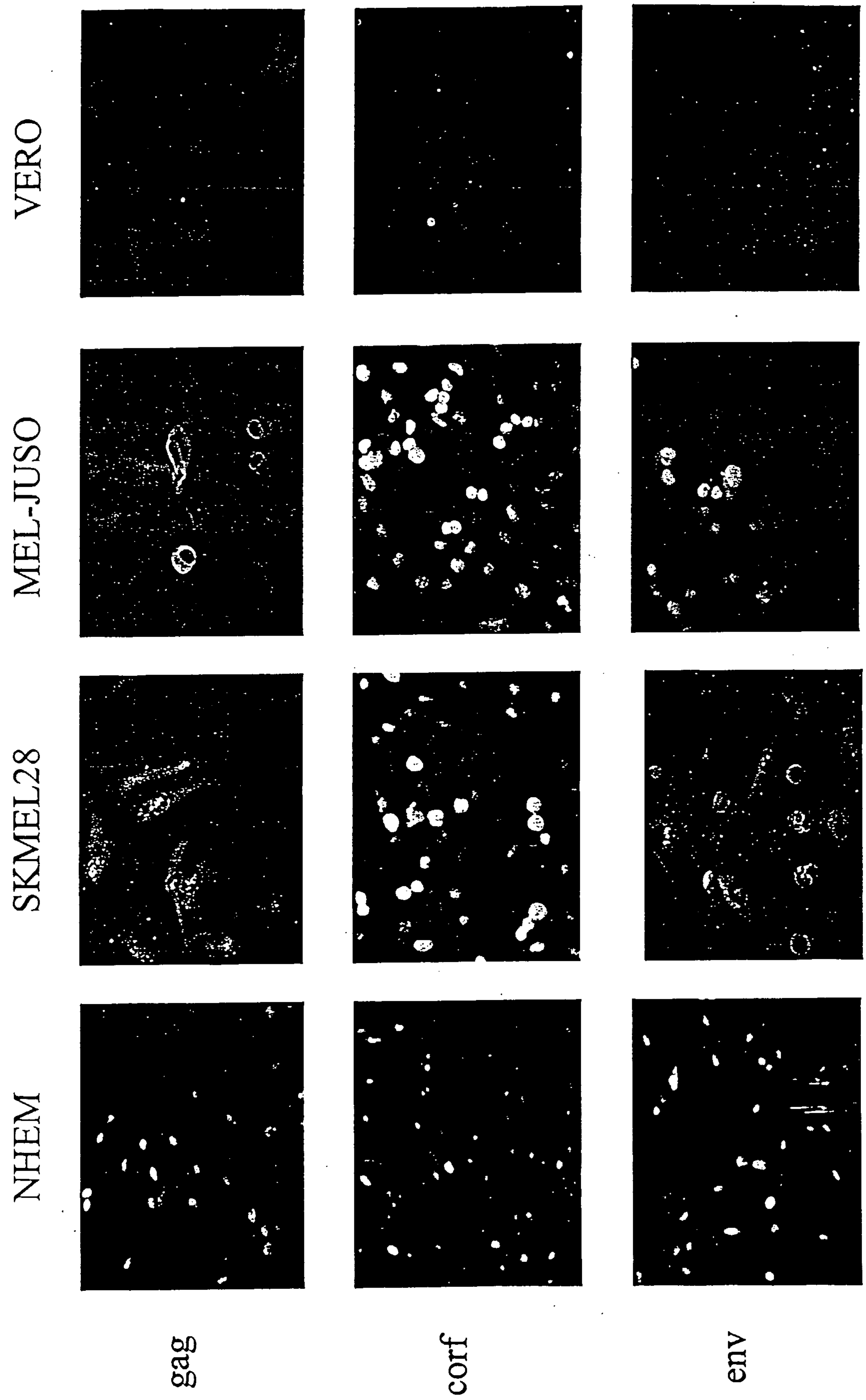


FIG. 4A

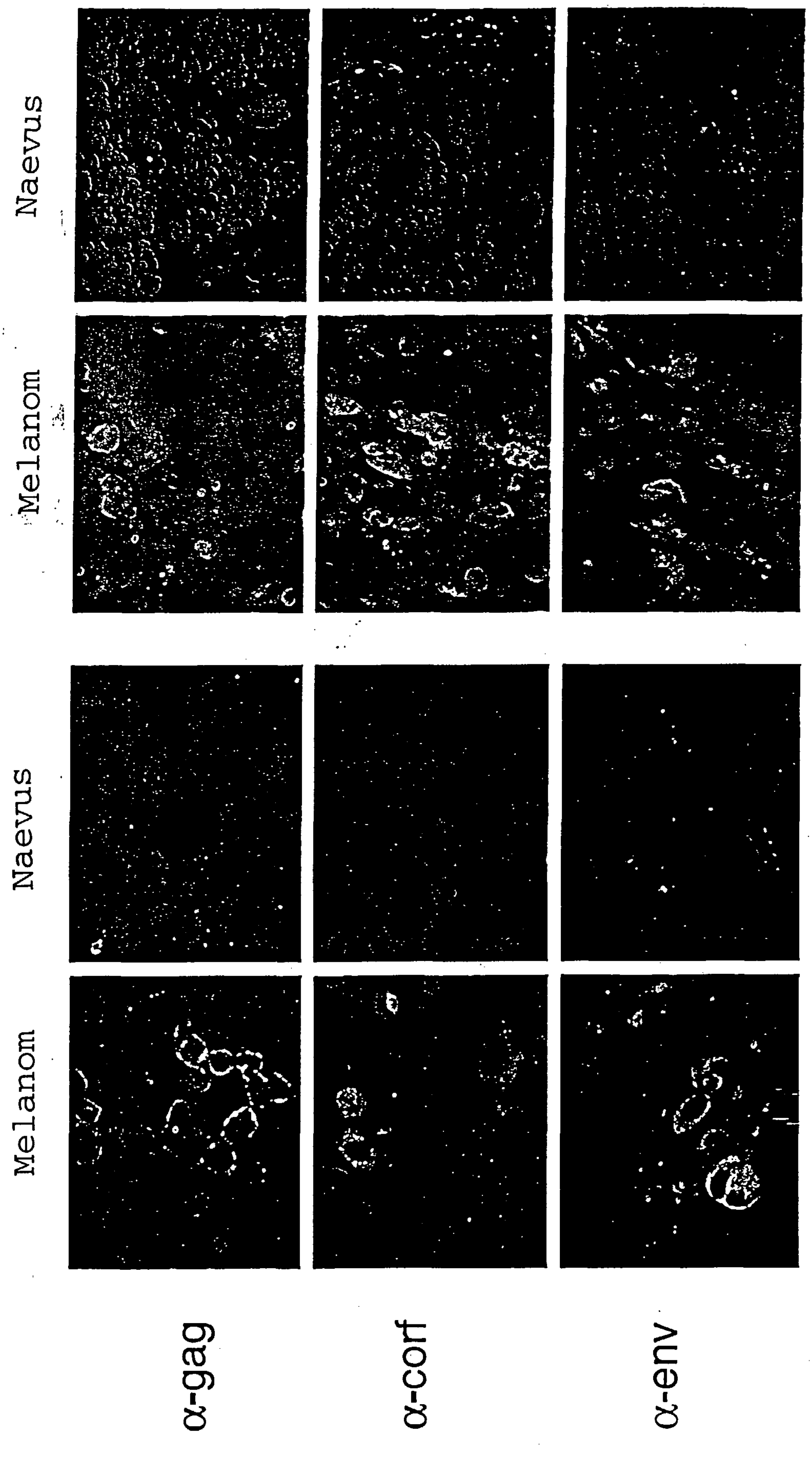
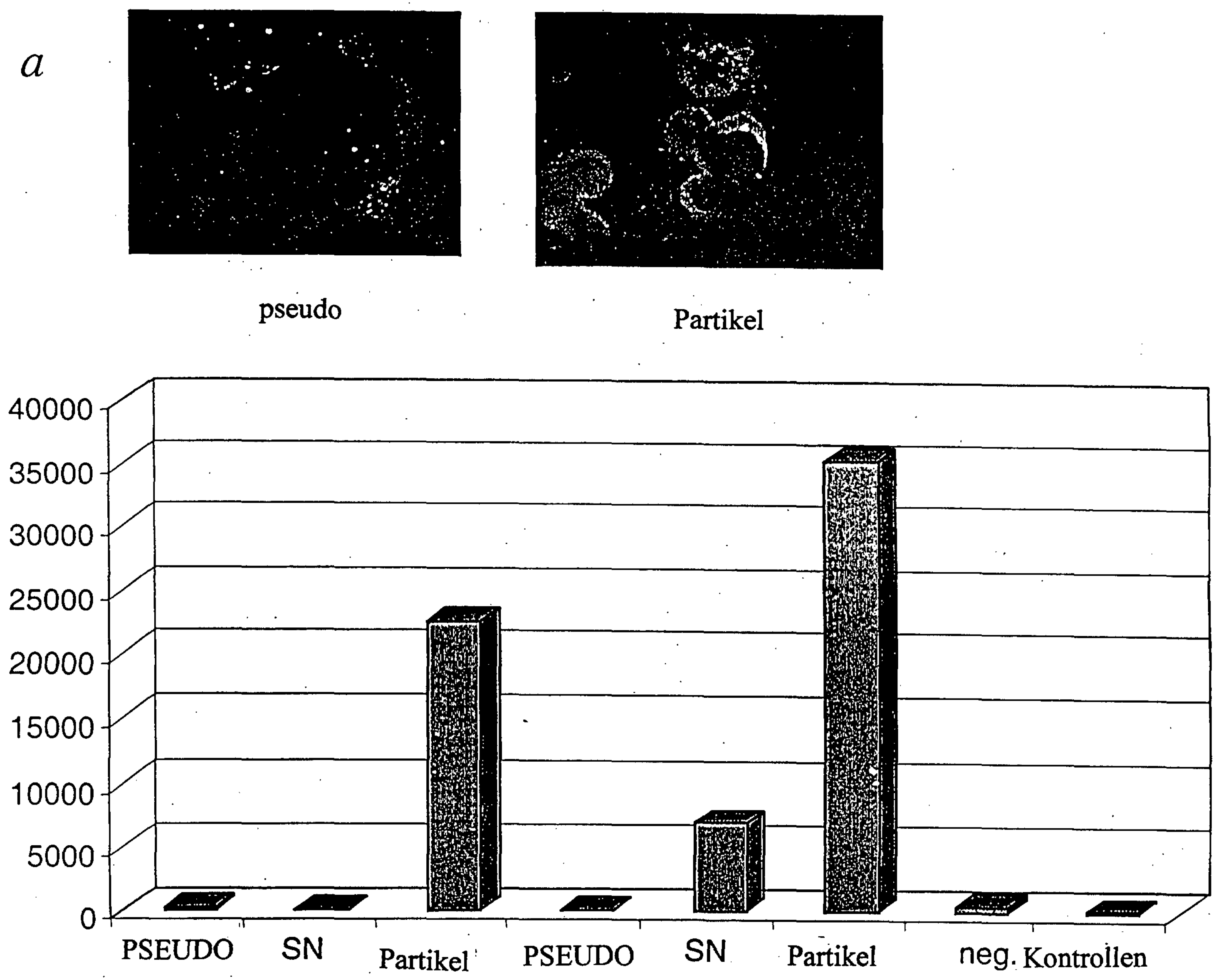


FIG. 4B

FIG. 5


a
pseudo
Partikel
b
RT-Aktivität
40000
35000
30000
25000
20000
15000
10000
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0
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SN
Partikel
PSEUDO
SN
Partikel
neg. Kontrollen

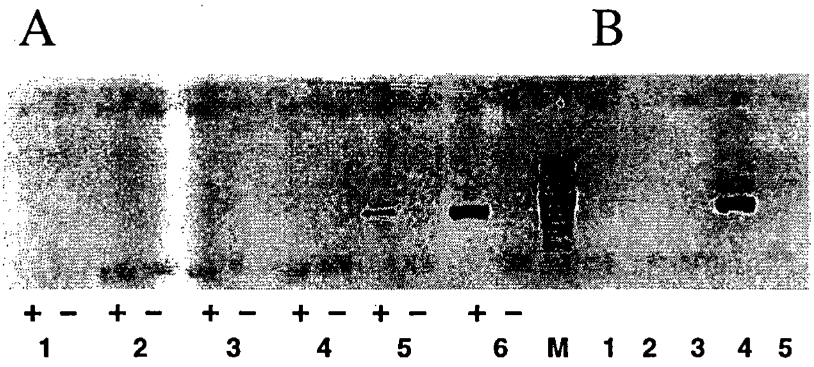


FIG. 6

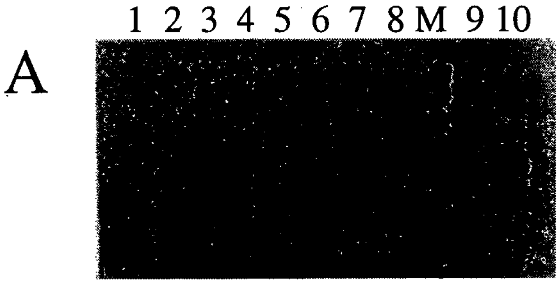


FIG. 7A

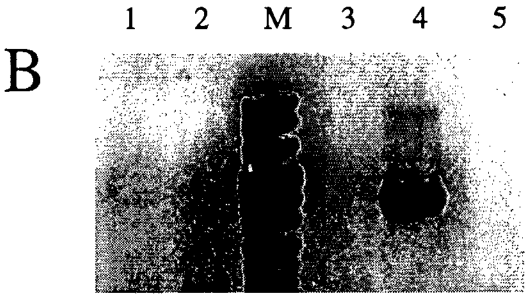
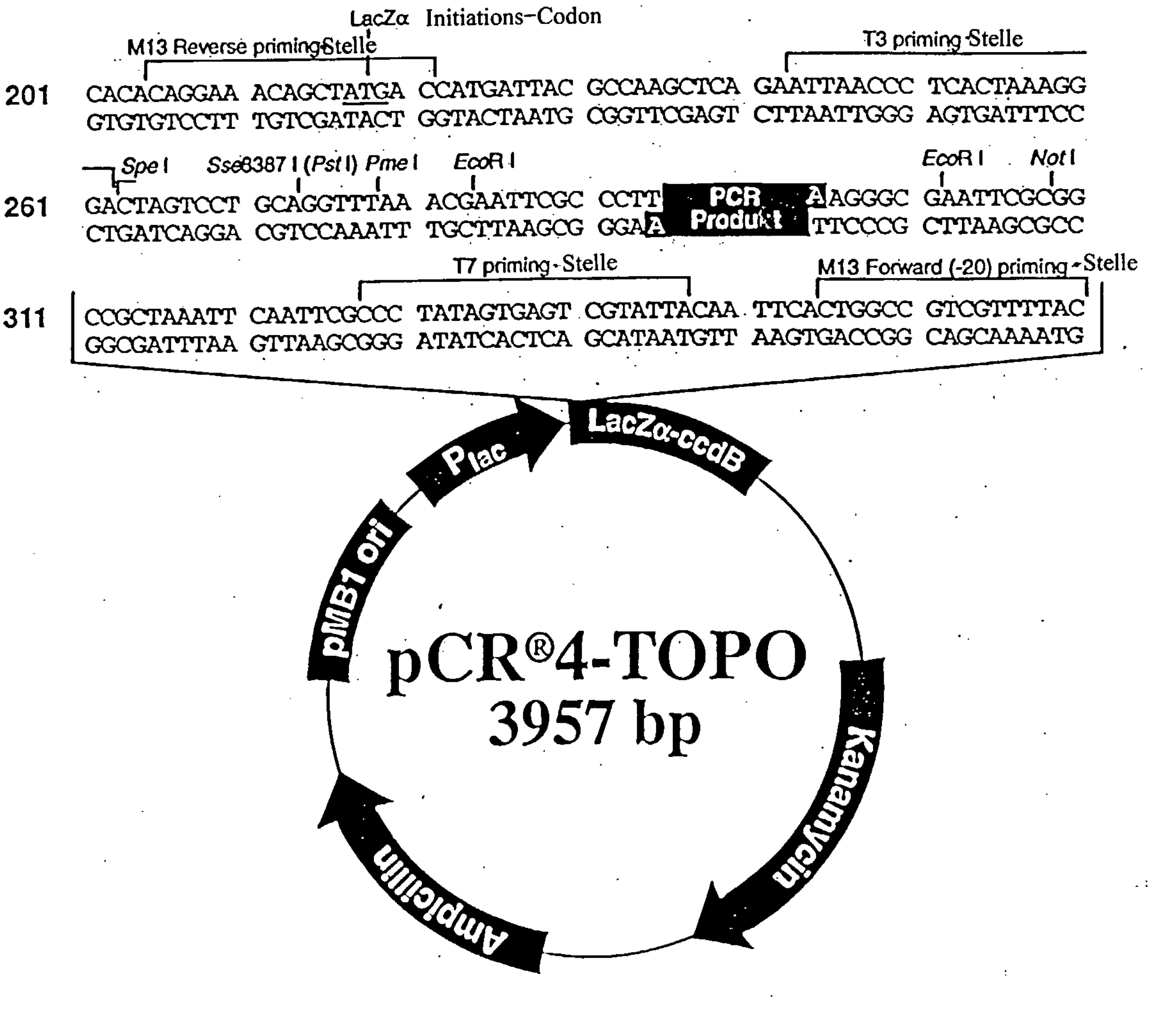


FIG. 7B

FIG. 8A


LacZα Initiations-Codon
M13 Reverse priming-Stelle
T3 priming-Stelle
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Ampicillin

FIG. 8B

MERV gag

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Vektor pCR4-TOPO	Primer	gag-Sequenz

...AAGCCAGTTACCACAATACAACAATTGTCCCCCACCA	CAAGCGGCAGTGCAGCAGTAG	AAGGGCGAATCGCGGCCGCT...
gag-Sequenz	Primer	Vektor pCR4-TOPO

MERV prot

...TTAAACGAATTCGCCCTT	CAAGCGGCAGTGCAGCAGTAG	ATTTATGTACTATACAAGCAGTCTCTCTGCTTCCAGGGG...
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prot-Sequenz	Primer	Vektor pCR4-TOPO

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Vektor pCR4-TOPO	Primer	pol-Sequenz

...CTTCCTGTTTGGATACCCACTAGACATTTGAAGTT	CTACAATGAACCCATCAGAGAGC	AAGGGCGAATCGCGGCCGCT...
pol-Sequenz	Primer	Vektor pCR4-TOPO

MERV env

...TTAAACGAATTCGCCCTT	CTACATGAACCCATCAGAGATGCAAAGAAAAGC	ACCTCCGCGGAGACGGAGNACACCGCA...
Vektor pCR4-TOPO	Primer	env-Sequenz

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env-Sequenz	Primer	Vektor pCR4-TOPO

SEQUENCE LISTING

<110> Wolff Prof., Klaus
Muster Prof., Thomas

<120> Infectious HERV

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ccacagggca gggcgccgta ccctcagccg cccactagga gacttaatcc tacggcacca 780
cctagtagac agggtagtga actacatgaa attattgata aatcaagaaa ggaaggagat 840
actgaggcat ggcaattccc agtaatgtta gaaccgatgc cacctggaga aggagcccaa 900
gagggagagc ctcccacagt tgaggccaga tacaagtctt tttcaataaa aatgctaaaa 960
gatatgaaag aaggagtaaa acagtatgga cccaactccc cttatatgag gacattatta 1020
gattccattg ctcatggaca tagactcatt ccttatgatt gggagattct ggcaaaatcg 1080
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caggtccaaa gaaatagggc tgccaatcct ccagttaaca tagatgcaga tcaactatta 1200
ggaataggtc aaaattggag tactattagt caacaagcat taatgcaaaa tgaggccatt 1260
gagcaagtta gagctatctg ccttagagcc tgggaaaaaa tccaagaccc aggaagtacc 1320
tgcccctcat ttaatacagt aagacaaagt tcaaaagagc cctatcctga ttttgtggca 1380
aggctccaag atgttgctca aaagtcaatt gccgatgaaa aagcccgtaa ggtcatagtg 1440
gagttgatgg catatgaaaa cgccaatcct gagtgtcaat cagccattaa gccattaaaa 1500
ggaaaggttc ctgcaggatc agatgtaatc tcagaatatg taaaagcctg tgatggaatc 1560
ggaggagcta tgcataaagc tatgcttatg gctcaagcaa taacaggagt tgttttagga 1620
ggacaagtta gaacatttgg aggaaaatgt tacaattgtg gtcaaatcgg tcacttaaaa 1680
aagaattgcc cagtcttaaa caaacagaat ataactattc aagcaactac aacaggtaga 1740
gagccacctg acttatgtcc aagatgtaaa aaaggaaaac attgggctag tcaatgtcgt 1800
tctaaatttg ataaaaatgg gcaaccattg tcgggaaacg agcaaagggg ccagcctcag 1860
gccccacaac aaactggggc attcccaatt cagccatttg ttcctcaggg ttttcaggga 1920
caacaacccc cactgtccca agtgtttcag ggaataagcc agttaccaca atacaacaat 1980
tgtcccccac cacaagcggc agtgcagcag tag 2013

<210> 5
<211> 1224
<212> DNA
<213> Human endogenous retrovirus

<400> 5
caagcggcag tgcagcagta gatttatgta ctatacaagc agtctctctg cttccagggg 60
agcccccaca aaaaatcccc acaggggtat atggcccact gcctgagagg actgtaggac 120
taatcttggg aagatcaagt ctaaatctaa aaggagttca aattcatact ggtgtggttg 180
attcagacta taaaggcgaa attcagttgg ttattagctc ttcaattcct tggagtgcca 240
gtccaggaga caggattgct gaattattac tcctgccata tattaagggt ggaaatagtg 300
aaataaaaag aacaggaggg tttggaagca ctgatccgac aggaaaggct gcatattggg 360

caagtcaggt ctcagagaac agacctgtgt gtaaggccat tattcaagga aaacagtttg 420
aagggttggt agacactgga gcagatgtct ctatcattgc tttaaatcgg tggccaaaaa 480
attggcctaa ccaaaaggct gttacaggac ttgtcggcat aggcacagcc tcagaagtgt 540
atcaaagtac ggagatttta cattgcttag ggccagataa tcaagaaagt actgttcagc 600
caatgattac ttcaattcct cttaatctgt ggggtcgaga tttattacaa caatggggtg 660
cggaaatcac catgcccgct ccatcatata gccccacgag tcaaaaaatc atgaccaaga 720
tgggatatat accaggaaag ggactaggga aaaatgaaga tggcattaaa attccagttg 780
aggctaaaat aaatcaagaa agagaaggaa tagggtatcc tttttagggg cggccactgt 840
agagcctcct aaacccatac cattaacttg gaaaacagaa aaaccggtgt gggtaaatca 900
gtggccgcta ccaaaacaaa aactggaggc tttacattta ttagcgaatg aacagttaga 960
aaagggtcat attgagcctt cattctcacc ttggaattct cctgtgtttg taattcagaa 1020
gaaatcaggc aaatggcgta tgttaactga cttaagggcc gtaaacgccg taattcaacc 1080
catggggcct ctccaacctg ggttgccctc tctggccatg atcccaaaag actggccttt 1140
aattataatt gatctaaagg attgcttttt taccatccct ctggcggagc aggattgcga 1200
aaaatttgcc tttactatac cagc 1224

<210> 6
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:primer

<400> 6
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<210> 7
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:primer

<400> 7
gctggtatag taaaggcaaa tttttc 26

<210> 8
<211> 250
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:oligonucleotide

<400> 8
ccactgtaga gcctcctaaa cccataccat taacttggaa aacagaaaaa ccggtgtggg 60

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taaatcagtg gccgctacca aaacaaaaac tggaggcttt acatttatta gcaaatgaac 120
agttagaaaa gggtcatatt gagccttcgt tctcaccttg gaattctcct gtgtttgtaa 180
ttcagaagaa atcaggcaaa tggcatatgt taactgactt aaaggccgta aacgccgtaa 240
ttcaacccat                                                        250
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FIG. 2

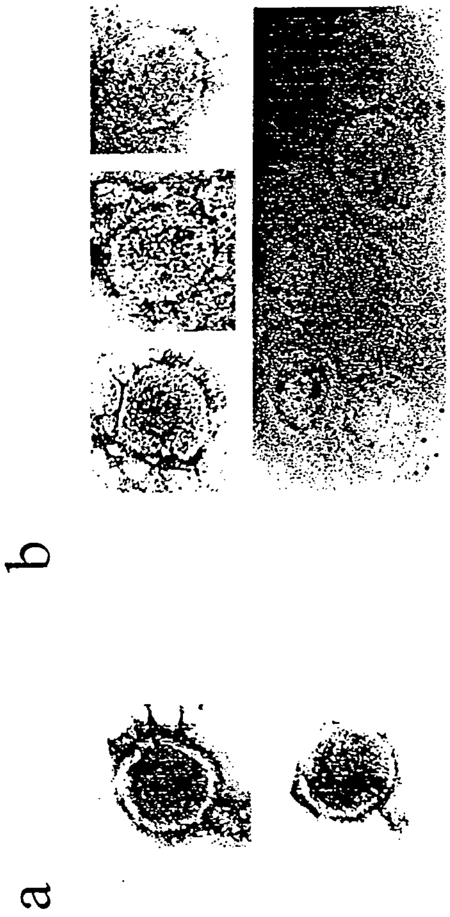


FIG. 5

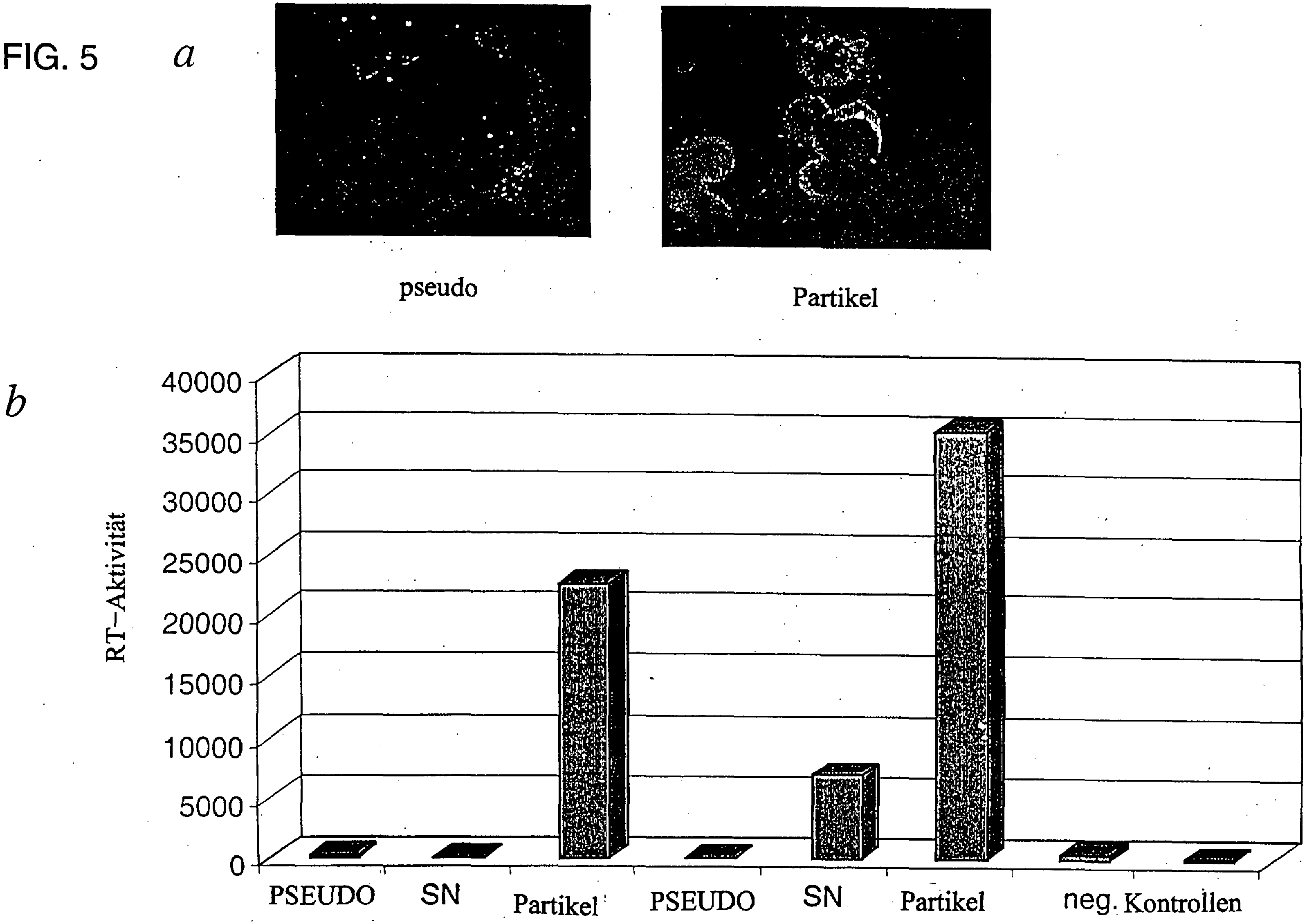
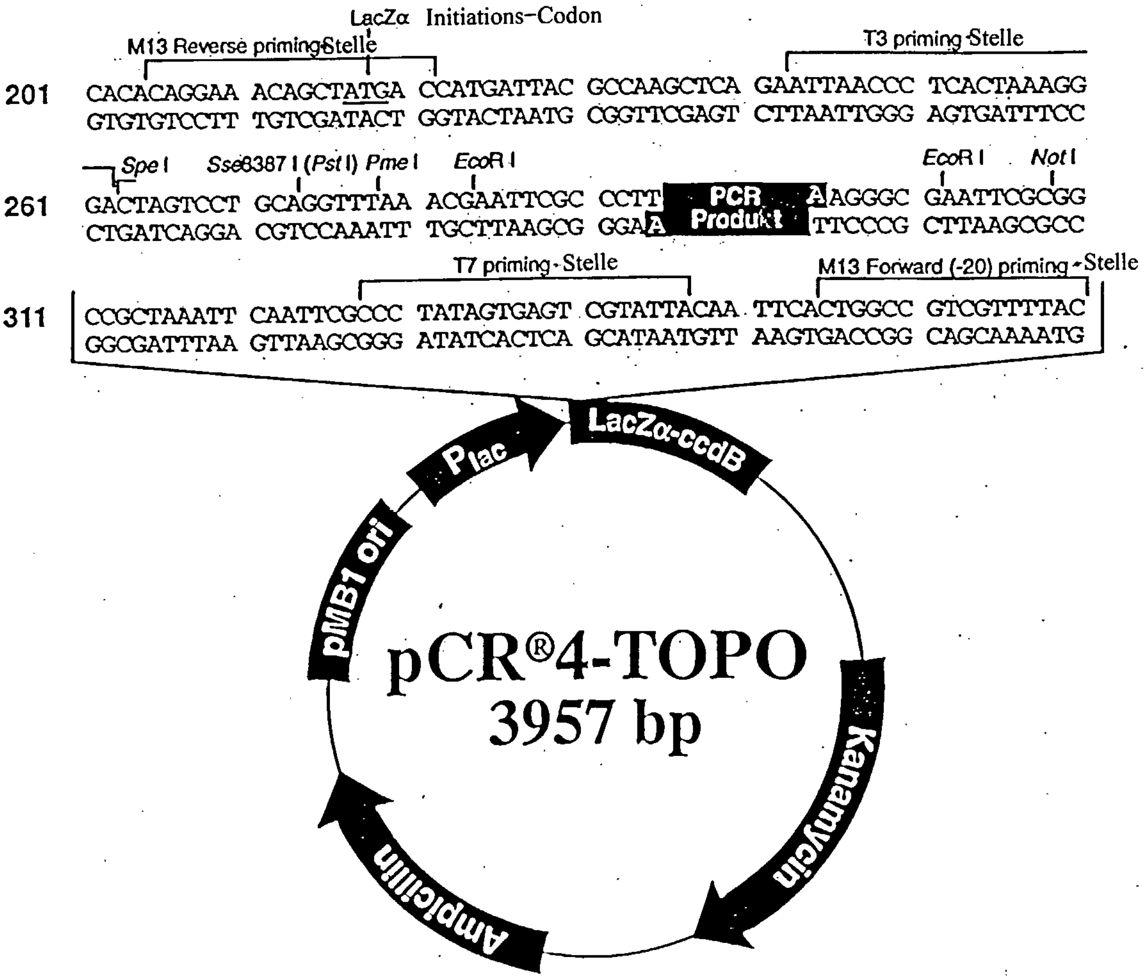

a
pseudo
Partikel
b
RT–Aktivität
40000
35000
30000
25000
20000
15000
10000
5000
0
PSEUDO
SN
Partikel
PSEUDO
SN
Partikel
neg. Kontrollen

FIG. 8A


LacZα Initiations-Codon
M13 Reverse priming-Stelle
T3 priming-Stelle
201
CACACAGGAA ACAGCTATGA CCATGATTAC GCCAAGCTCA GAATTAACCC TCACTAAAGG
GTGTGTCCTT TGTCGATACT GGTACTAATG CGGTTCGAGT CTTAATTGGG AGTGATTTCC
Spe I
Sse8387 I (Pst I)
Pme I
EcoR I
EcoR I
Not I
261
GACTAGTCCT GCAGGTTTAA ACGAATTCGC CCTT
PCR Produkt
A
AGGGC GAATTCGCGG
CTGATCAGGA CGTCCAAATT TGCTTAAGCG GGA
A
TTCCCG CTTAAGCGCC
T7 priming-Stelle
M13 Forward (-20) priming-Stelle
311
CCGCTAAATT CAATTCGCCC TATAGTGAGT CGTATTACAA TTCACTGGCC GTCGTTTTAC
GGCGATTTAA GTTAAGCGGG ATATCACTCA GCATAATGTT AAGTGACCGG CAGCAAAATG
Plac
LacZα-ccdB
pMB1 ori
pCR®4-TOPO
3957 bp
Kanamycin
Ampicillin

INTERNATIONAL SEARCH REPORT

Interr pplication No
PCT/EP 02/10899

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/11 C12N15/48 C12N15/63 C12Q1/68 C12N5/10
C12N7/00 C07K14/15 C07K2/00 C07K16/10 C07K17/00
G01N33/53 G01N33/574 A61K39/21 A61K48/00 A61K9/06

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

BIOSIS, MEDLINE, EMBASE, EPO-Internal, WPI Data, PAJ, SEQUENCE SEARCH, EMBL

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE EMBL 'Online! 1 September 1999 (1999-09-01) BARBULESCU M., TURNER G., SEAMAN M.I., DEINARD A.S., KIDD K.K., LENZ J.: "Homo sapiens endogenous retrovirus HERV-K108, complete sequence." retrieved from EBI Database accession no. AF164614 XP002233324 cited in the application abstract & BARBULESCU M ET AL: "MANY HUMAN ENDOGENOUS RETROVIRUS K (HERV-K) PROVIRUSES ARE UNIQUE TO HUMANS." CURRENT BIOLOGY, vol. 9, no. 4, 1999, pages 861-868, XP000953273 the whole document --- -/--	1-22, 24-26,42

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search 3 March 2003	Date of mailing of the international search report 17/03/2003
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer Brenz Verca, S

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	MUSTER T ET AL: "Detection of viral sequences and virus-like particles in human melanoma." JOURNAL OF INVESTIGATIVE DERMATOLOGY, vol. 114, no. 4, April 2000 (2000-04), page 860 XP009006717 61st Annual Meeting of the Society for Investigative Dermatology.;Chicago, Illinois, USA; May 10-14, 2000 ISSN: 0022-202X the whole document ---	1-47
A	KOMURIAN-PRADEL F ET AL: "RAPID COMMUNICATION. MOLECULAR CLONING AND CHARACTERIZATION OF MSRV-RELATED SEQUENCES ASSOCIATED WITH RETROVIRUS-LIKE PARTICLES" VIROLOGY, ACADEMIC PRESS,ORLANDO, US, vol. 260, no. 1, 1999, pages 1-9, XP000891352 ISSN: 0042-6822 the whole document ---	1-22
A	REUS KATRIN ET AL: "Genomic organization of the human endogenous retrovirus HERV-K(HML-2.HOM) (ERVK6) on chromosome 7." GENOMICS, vol. 72, no. 3, 15 March 2001 (2001-03-15), pages 314-320, XP002233321 ISSN: 0888-7543 table 1 ---	
A	LOEWER ROSWITHA ET AL: "The viruses in all of us: Characteristics and biological significance of human endogenous retrovirus sequences." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES, vol. 93, no. 11, 1996, pages 5177-5184, XP002233322 1996 ISSN: 0027-8424 cited in the application the whole document ---	
P,A	WO 01 70941 A (DISSING SORENSEN PERNILLE ;MS RES AS (DK); ANDERSSON POUL (DK); CH) 27 September 2001 (2001-09-27) examples 1,2,4-6 ---	

-/--

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
P,A	TURNER GEOFFREY ET AL: "Insertional polymorphisms of full-length endogenous retroviruses in humans." CURRENT BIOLOGY, vol. 11, no. 19, 2 October 2001 (2001-10-02), pages 1531-1535, XP002233323 ISSN: 0960-9822 page 1533, left-hand column, paragraph 2; figure 2 -----	

Int[illegible]al application No.
PCT/EP 02/10899

INTERNATIONAL SEARCH REPORT

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 27-37 are directed to a diagnostic method practised on the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. ☒ Claims Nos.: 1 23 47
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 1 23 47

With regard to claim 1, the initial phase of the search revealed a very large number of documents relevant to the issue of novelty. Indeed, since the origin of a polynucleotide is not a technical feature and is therefore irrelevant, the only technical feature defining the polynucleotide of said claim 1 is the sequence of SEQ ID No:1.
So many sequences hybridizing under stringent condition to SEQ ID No:1 and thus falling into the scope of the claim were retrieved that it is impossible to define a complete state of the art in this sense.
The EMBL database entry AF164614 cited in the search report is thus only a selected representative of a large state of the art.
Furthermore, since the sequences of part (d) of claim 1 are defined by an arbitrary denomination given by the Applicant and not by a deposit number, no search could be carried out for said claim part.

Claim 23 relates to a peptide defined by no technical feature but only by being a fragment of an infectious human endogenous retrovirus. As mentioned before, the origin does not represent a technical feature, thus claim 23 basically embraces all possible peptides, starting from dipeptides up to peptides of undefined length. Such a search is economically not feasible, consequently said claim was excluded from search.
For the same reason, claim 47 was also excluded from search inasfar it concerns a vaccine comprising a peptides according to claim 23.

The remaining claims were not searched inasfar they refer to the matter of claim 1 which was excluded from search for the reasons explained above.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Internati[illegible] Application No
PCT/EP 02/10899

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 0170941 A	27-09-2001	AU 5829201 A WO 0170941 A2 EP 1265989 A2	03-10-2001 27-09-2001 18-12-2002

专利名称(译)	感染性人内源性逆转录病毒的多核苷酸片段		
公开(公告)号	EP1430127A1	公开(公告)日	2004-06-23
申请号	EP2002779436	申请日	2002-09-27
[标]申请(专利权)人(译)	GREENHILLS BIOTECH RES DEV TRADE		
申请(专利权)人(译)	GREENHILLS生物技术研究开发贸易GMBH		
当前申请(专利权)人(译)	GREENHILLS生物技术研究开发贸易GMBH		
[标]发明人	GRASSAUER ANDREAS MUSTER THOMAS		
发明人	WOLFF, KLAUS,UNI. FÜR DERMATOLOGIE AKH WIEN PEHAMBERGER, H.,UNI. FÜR DERMATOLOGIE AKH WIEN GRASSAUER, ANDREAS MUSTER, THOMAS		
IPC分类号	A61K38/00 A61K39/00 C07K14/15 C12N7/00 C12N15/48 G01N33/569 G01N33/574 C12N15/11 C12N15/63 C12Q1/68 C12N5/10 C07K2/00 C07K16/10 C07K17/00 G01N33/53 A61K39/21 A61K48/00 A61K9/06		
CPC分类号	A61K38/00 A61K39/00 A61K2039/5254 A61P31/14 C07K14/005 C12N7/00 C12N2740/10021 C12N2740/10022 G01N33/56983 G01N33/5743 G01N2333/15		
优先权	2001001539 2001-09-27 AT		
外部链接	Espacenet		

摘要(译)

描述了多核苷酸分子，其是感染性人内源性逆转录病毒的多核苷酸序列的片段，所述多核苷酸分子包含选自具有至少98%，优选99%，仍优选99.5%的序列的序列的序列。根据SEQ ID No 1的序列，ba序列，其在严格条件下与根据所述SEQ ID No 1的核苷酸序列杂交，ca序列与所述序列a或b不同，因为遗传密码的变性和包含序列的da序列插入载体pCR4-Topo并于2001年9月26日在DSMZ或其片段上保藏为“MERV-env”，“MERV-gag”，“MERV-prt”和“MERV-pol”。