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# HERV-K: The Biologically Most Active Human Endogenous Retrovirus Family

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**Summary:** The human genome contains a wide variety of endogenous retrovirus-like sequences. The human endogenous retrovirus type K (HERV-K) family comprises 30–50 members per haploid genome in humans and is highly conserved in Old World monkeys and apes. Some proviruses are displaying open reading frames (ORF) with coding capacity for viral particles. HERV-K sequences most likely code for the previously described human teratocarcinoma-derived virus (HTDV) and correlated expression of HERV-K Gag has been demonstrated by immunoelectron microscopy studies. Protease, but not yet reverse transcriptase (RT), enzymatic activity was demonstrated for recombinant HERV-K proteins. However, an ultrasensitive RT assay revealed specific polymerase activity associated with the HTDV particles. HERV-K transcription is specifically regulated by viral long terminal repeats and RNA is expressed at low steady-state levels in a variety of human tissues and tumours. In teratocarcinoma cell lines, HERV-K is highly expressed in a complex pattern showing full-length as well as subgenomic envelope (*env*) and two alternatively spliced small transcripts. The doubly spliced 1.8-kb mRNA codes for cORF protein which resembles Rev of HIV-1 and is located in the nucleolus. In addition, the cORF sequence acts as a leader and is essential for effective expression of glycosylated HERV-K Env protein. Although HERV-K sequences code for all necessary retroviral proteins, infectious particles could not yet be demonstrated. The putative implication of HERV sequences in pathophysiological processes, for example, testicular malignancies, remains to be elucidated. **Key Words:** Human endogenous retrovirus—Human teratocarcinoma-derived virus (HTDV)/Human endogenous retrovirus K (HERV-K)—Retroelement—Reverse transcriptase—Retroviral proteins—Immune response.

## THE DISCOVERY AND NOMENCLATURE OF HUMAN ENDOGENOUS RETROVIRUS (HERV) FAMILIES

HERV sequences form a substantial part of the human chromosomes in all somatic and germline cells. Numerous endogenous retroviral elements belonging to a series of families in the human genome have been detected by different experimental approaches over the past two decades (reviewed in Refs. 1 and 2). In contrast to exogenous retrovirus strains, no definite taxonomy for HERV elements has been proposed, due mainly to the lack of obvious structure–function relationships and the limited sequence information for most of the HERV elements. A current classification is based on homol-

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ogies to animal retroviruses historically originating in the isolations and characterizations of proviral sequences employing heterologous retroviral probes (2). Thus, class I families share sequence homologies with mammalian type C retroviruses, three of which constitute the ERI superfamily and have the closest relationship to murine leukemia virus (MuLV) and baboon endogenous virus (BaEV). Class II families show homologies with mammalian type B retroviruses such as mouse mammary tumor virus (MMTV) and type D retroviruses such as squirrel monkey retrovirus (SRV). Two members, HERV-K10 (3) and HERV-K(C4) (4), have been fully characterized, demonstrating limited homologies in the *gag, polymerase* (*pol*), and *env* genes. Recently, polymerase chain reaction (PCR)-based methods revealed the existence of six human MMTV-like (HML) families (5), one of which (HML-2) is identical to HERV-K. Due to the small size of PCR products in the *pol* gene, the extension of homology is unknown.

An interfering tentative nomenclature is based on the tRNA specificity of the primer binding site, adding the appropriate amino acid suffix to the acronym HERV (1). The limitations of this system are marked by the fact that distantly related families share similar PBS or cloned sequences are missing this segment. All known class II elements exhibit a lysine tRNA PBS, reflecting their relatedness to B- and D-type viruses. This definition combined with the discovery of a viral phenotype encoded by a HERV sequence has given rise to the designation HTDV/HERV-K for a functional member(s) of this family.

## THE HTDV/HERV-K FAMILY CODES FOR RETROVIRAL PARTICLES

Retrovirus-like particles in vivo were first discovered during the course of electron microscopic surveys of human placentas at the basal membrane of syncytiotrophoblasts (reviewed in Ref. 2). These particles share morphological features with retrovirus-like particles observed in testicular tumor cell lines derived from embryonic carcinomas or teratocarcinomas (6,7), in particular, the lack of an electron-lucent space between the viral core and the envelope and, obviously, frequent arrest in the budding stages (8,9) (see Fig. 1A). These particles have been designated human teratocarcinoma-derived virus (HTDV) particles (7). Mature forms with collapsed cores have been rarely detected, probably explaining the results of cocultivation experiments where HTDV could not be transmitted to other cells (9).

Recently, we have adapted a PCR-based assay to identify which of the numerous HERV families encodes the HTDV particles (10). The RU5-PCR technique was designed to amplify specifically retroviral mRNAs using primers homologous to PBS sequences. It could be shown that besides the HERV-H family, HERV-K sequences are highly expressed in teratocarcinoma cells (10), of which only HERV-K members have the capacity to code for HTDV (11,12).

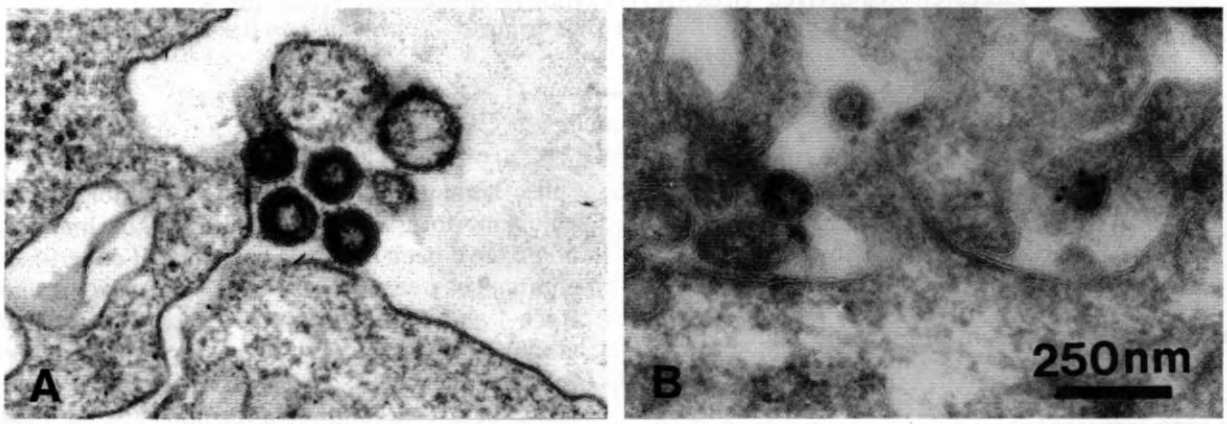


**FIG. 1.** HERV-K/HTDV in teratocarcinoma cells. **A:** HTDV particles produced by a teratocarcinoma-cell line, ultrathin section of resin-embedded cells. **B:** Ultrathin frozen section immunogold-labeled with rabbit anti-HERV-K Gag antiserum.

## CHROMOSOMAL DISTRIBUTION AND EVOLUTIONARY CONSERVATION OF HTDV/HERV-K

A series of HERV-K proviral sequences was isolated using both MMTV *pol* (13,14) and Syrian hamster intracisternal A-particle (IAP) *pol* probes (15). The fully sequenced clone HERV-K10 contains an open reading frame (ORF) of sufficient size to encode a full-length *pol* protein but is defective in the *gag* and *env* genes (3). The HERV-K family comprises 30–50 proviral sequences (15) and about 10,000 solitary LTRs in the human genome (13,16). The existence of HERV-K proviruses is restricted to the lineage of Old World monkeys and hominoids (15,17,18), with extensive conservations of *pol* and *env* gene sequences between monkeys, apes, and humans (17). The genome of gorilla harbors a proviral copy with 99% homology to HERV-K10 (19). The chromosomal distribution of HTDV/HERV-K sequences seems to be nonrandom (20,21). On chromosome Xq28, adjacent to the G6PD locus, a defective provirus devoid of *gag* was identified (22), with duplication of *pol* and long terminal repeat (LTR) sequences (23). No restriction fragment length polymorphisms (RFLP) of HERV-K loci could be detected by screening a series of human cell lines and laboratory workers from different ethnic groups (18,19).

## ORGANIZATION OF HTDV/HERV-K PROVIRUSES AND TRANSCRIPTS

The genomic organization and sequence of HTDV/HERV-K are most closely related to MMTV (24), Jaagsiekte sheep retrovirus (JSRV) (25), and SRV family members (26). Three ORFs for *gag, protease* (*pro*), and *pol* exist, implicating two frameshift events for translation of the entire precursor protein. The *pol* and *env* ORFs show a partial overlap. We have isolated cDNA and genomic clones comprising a segment between *pol* and *env* that is 292 nucleotides (nt) long, yielding ORFs for all viral genes (27,28). HTDV/HERV-K proviruses devoid of this sequence have been designated type 1 genomes (11,27). Only in type 2 genomes containing this segment can a putative envelope signal peptide be postulated (27). Type 1 genomes display fused *pol* and *env* genes and express exclusively unspliced mRNA (27). HTDV/HERV-K type 2 full-length transcripts are spliced to subgenomic *env* and two smaller mRNAs (11,27). A 1.8-kb doubly spliced transcript encompasses most of the 292-bp segment and encodes a reading frame designated cORF (central ORF) (11,27). In normal tissues such as placenta and peripheral blood lymphocytes, low steady-state levels of full-length mRNA can be detected (19). Sensitive reverse transcriptase (RT)-PCR-based assays have also revealed spliced HTDV/HERV-K transcripts in a variety of normal and tumor tissues (29). In addition, HTDV/HERV-K and related sequences seem to be expressed at different levels in several individuals studied (30). These observations suggest that type I and type II proviruses are differentially expressed.

## EXPRESSION OF HTDV/HERV-K PROTEINS

HTDV/HERV-K proviruses harbor long ORFs for viral proteins. In contrast, all other HERV elements isolated so far proved to be highly defective. However, it is unclear whether single HTDV/HERV-K genomes with ORFs for all proteins exist. To facilitate protein expression studies, *gag, pro, pol, env,* and *cORF* genes have been expressed in prokaryotic and eukaryotic systems and corresponding antisera have been raised in animals (11,27,28,31–33).

The HTDV/HERV-K Gag precursors of 76 kDa are cleaved into major core, matrix, and nucleocapsid components (12,31,33). The precursor protein is myristylated in teratocarcinoma cells (34), a prerequisite for transport to the plasma membrane and for particle production (35). Accumulation of Gag in viral particles has been revealed by immunofluorescence studies and has been further visualized by electron microscopic analyses using immunogold-labeled antibodies reacting with HTDV particles (11,12) (see Fig. 1B). In testicular tumors, Gag has been detected in the cytoplasm by immunoperoxidase staining (33).

The existence of a functional HTDV/HERV-K Protease is indicated by the presence of processed Gag proteins in teratocarcinoma cell lines. Direct evidence for an active enzyme has been provided by expression of different recombinant *protease* clones. The enzyme is expressed as a 120-kDa Gag–Protease precursor in prokaryotic expression systems and in teratocarcinoma cells and is cleaved autocatalytically to a functional 17-kDa subunit (34,36). The Polymerase ORF encodes a 160-kDa protein that is synthesized as a >200-kDa precursor by two translational frameshift events (34). Weak RT activity has been detected in HTDV particle

preparations from the supernatant of teratocarcinoma cells but was masked by the presence of a putative cellular RT inhibitor (9,37). A recently introduced ultrasensitive PCR-based RT assay (38) has been further developed to distinguish uniequivocally DNA-dependent from RNA-dependent DNA polymerases (39). Using this assay, it can be shown that HTDV particles isolated from a teratocarcinoma cell line but not control cell line supernatants contain RT-like activities (Fig. 2). HTDV/HERV-K *pol* sequences prokaryotically expressed demonstrated no specific RT activities (19), suggesting that endogenous RT-like enzymes probably require more natural conditions as they exist in the viral core. However, an indirect linkage between HERV-K *pol* sequences and HTDV particle-associated RT activities was revealed by immune affinity purification of HERV-K Polymerase from human teratocarcinoma cell membranes (Fig. 3). Immunoreactive proteins of 60–70 kDa were isolated (data not shown), which demonstrated $Mg^{2+}$-dependent RT activity in the RT-PCR assay (Fig. 3). The preference for $Mg^{2+}$ as the divalent cation for optimal RT activity is a characteristic feature of class II retroviruses (e.g., MMTV, SRV, and lentiviruses), as opposed to class I retroviruses (e.g., MuLV), which require $Mn^{2+}$ (40). Hence, it can be concluded that HTDV/HERV-K *pol* encodes a functional enzyme with weak activity.

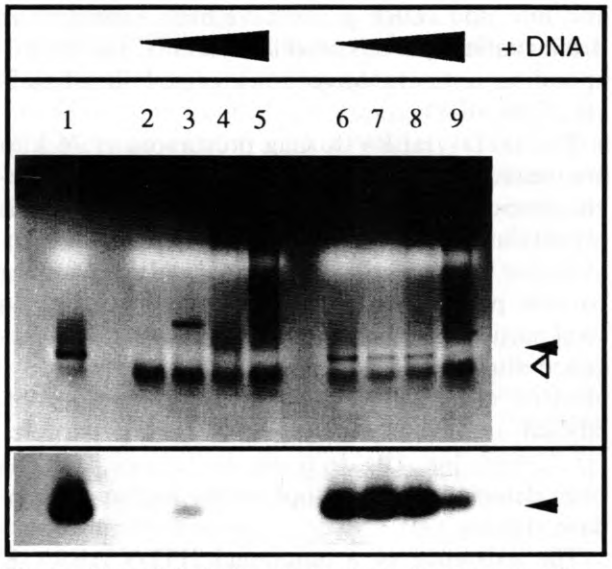


**FIG. 2.** RT activity in human teratocarcinoma versus fibroblast cell line supernatants. A specific ultrasensitive RT-PCR assay using activated DNA as competitor was employed (39). **Top:** Agarose gel electrophoresis of PCR products; arrowhead, PCR products; open triangle, free oligonucleotides. **Bottom:** Autoradiograph of corresponding Southern blot using a specific radiolabeled internal oligonucleotide probe. Lane 1, MuLV RT [0.1 mU (Life Technologies)], no DNA added; lanes 2–5, human fibroblast-cell line (MRC5; non-particle producer line) supernatant sample, with no DNA or 0.2, 1, or 5 μg DNA added, respectively; lanes 6–9, human teratocarcinoma cell line (GH; HTDV particle-producer line) supernatant sample, with no DNA or 0.2, 1, or 5 μg DNA added, respectively. Retroviral particles were collected from tissue culture supernatants by ultracentrifugation and aliquots subjected to RT-PCR assays.

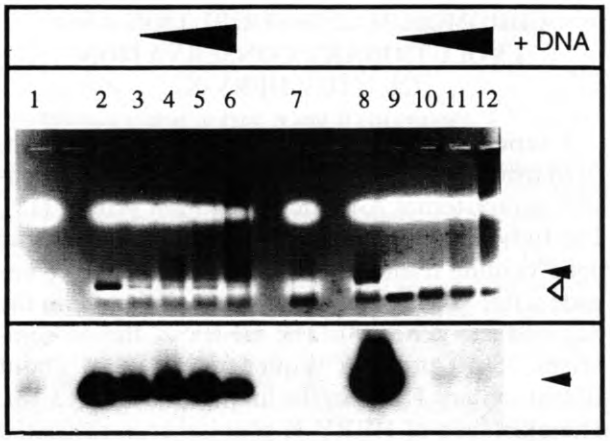


**FIG. 3.** RT activity of immune affinity-purified HERV-K Polymerase (Pol) from human teratocarcinoma cell membranes. A specific ultrasensitive RT-PCR assay using activated DNA as competitor (39) in the presence of $Mg^{2+}$ or $Mn^{2+}$ as cations was employed. **Top:** Agarose gel electrophoresis of PCR products; arrowhead, PCR products; open triangle, free oligonucleotides. **Bottom:** Autoradiograph of corresponding Southern blot. Lanes 1 and 7, no enzyme in RT reaction (negative control); lanes 2 and 8, MuL V RT (0.1 mU), no DNA added; lanes 3–6, activity of purified HERV-K Pol immunoreactive protein in the presence of $MgCl_2$, no DNA; or 0.2, 1, or 5 μg added DNA, respectively; lanes 9–12, activity of purified HERV-K Pol immunoreactive protein in the presence of $MnCl_2$, no DNA, or 0.2, 1, or 5 μg added DNA, respectively. Pol protein was purified from teratocarcinoma cell (GH) membranes by passage over an anti-HERV-K Pol immune affinity Sepharose column. The column was prepared using polyclonal goat antiserum against procaryotically expressed HERV-K Pol (19) and subsequent saturation of unspecific binding sites with MRC5 fibroblast cell membrane proteins. Aliquots of eluted protein were subjected to RT-PCR assays.

A dominant splicing event of HTDV/HERV-K full-length mRNA leads to quantitative removal of Envelope encoding sequences yielding an excess of doubly spliced 1.8-kb cORF mRNA in teratocarcinoma cells (27). This splicing pattern was mimicked in eukaryotic expression systems where recombinant cytomegalovirus (CMV) promoter-controlled HTDV/HERV-K *env* constructs produced predominantly cORF (41). Mutation of the authentic splice sites resulted in the usage of cryptic, alternative sites. Expression of HTDV/HERV-K Env protein in COS cells was achieved by insertion of CMV intron A sequences upstream of the *env* gene (41).

In contrast, high-level expression of Env was obtained using a recombinant baculovirus-based system (28). The full-length 80- to 90-kDa Env protein including the cORF leader sequence (see below) was glycosylated in insect cells. The precursor protein was not cleaved to outer surface unit (SU) and transmembrane (TM) glycoproteins, although the consensus SU/TM cleavage site is present. The protein neither appeared on the surface of infected insect cells nor was secreted into the medium (28). In teratocarcinoma cells, Env proteins are synthesized at barely detectable levels (19). Insufficient production of Env could be another reason for the lack of HTDV/HERV-K infectivity.

The doubly spliced cORF mRNA comprising two exons encodes a 12-kDa protein that is the dominant HTDV/HERV-K gene product in teratocarcinoma cells. The first exon corresponds largely to the Env signal peptide and the second exon resides in a different reading frame of the 3′ portion of the *env* gene (27). A close relationship of cORF to the ungulate lentivirus Rev proteins (42) is suggested by its structural features and accumulation in the nucleolus (27). It remains to be shown whether cORF protein can exert Rev-like functions, as the spacing of leucine residues is slightly different (27).

## ANTIBODIES AGAINST HTDV/HERV-K IN HUMANS

The humoral immune response against expressed HTDV/HERV-K proteins has been investigated by epitope mapping of Gag and Env proteins (29). Employing a synthetic peptide-based solid-phase enzyme-linked immunosorbent assay (ELISA), immunoreactive epitopes were identified at the amino and carboxy termini of Gag and in the TM region of Env, corresponding to the immunodominant domain of HIV. Antibodies were detected at very low frequencies in normal blood donors, in accordance with the finding that HTDV/HERV-K mRNA is expressed at low levels in normal tissue samples (see above). A screening survey of groups of patient sera revealed antibodies in leukemias, after pregnancies, and particularly, in patients suffering from testicular tumors (29,33,43). Although the antibody titers are elevated compared to those of normal blood donors, they hardly ever reach the titers observed after retroviral infections such as human immunodeficiency virus (HIV). Further analyses are needed to address the question whether this effect is due to unspecific reactivities or whether the antibody response is elicited against viral proteins which are expressed after induction of neonatal tolerance. In the latter case, the HERV protein-producing cell types need to be identified.

## REGULATION OF HTDV/HERV-K EXPRESSION

The possibility that expression of different proviruses is mediated by selective LTR regulation has been approached by appropriate reporter gene assays. A series of HERV-K LTRs was tested in cell lines of different origins showing a prominent upregulation of such viral promoters in embryonic tissues (44). Specific consensus binding sites for constitutive or inducible transcription factors exist in those LTRs that have the potential to trigger stimulated expression. For instance, the glucocorticoid-responsive element possibly mediates steroid hormone-induced transcription in the mammary carcinoma cell line T47D (45). It remains to be elucidated whether some proviruses are specifically transcribed in adult tissues and what cellular factors control this activation.

## PUTATIVE BIOLOGICAL SIGNIFICANCE OF HERV ELEMENTS

At present, the biological function(s) of human endogenous retroviral sequences is (are) not obvious. However, speculations may be drawn from comparison with animal retrovirus and other model systems. For instance, expressed HERV may protect their hosts against infections with a closely related exogenous retrovirus, e.g., by receptor interference (reviewed in Ref. 46) and superantigen-mediated depletion of susceptible host cells (47,48). In this respect, one has to assume that exogenous viruses related to class I and class II HERVs have been eliminated in human predecessor species or that such counterparts have not yet been detected. Another intriguing possibility concerns the impact of retrotransposition, as HERVs together with retroposons and retrotransposons serve as the main sources of RT activity. In the germline, such events will either be deleterious or remain fixed in the population if they are harmless or beneficial to the host. For example, *de novo* insertions of human LINE elements have caused a series of pathophysiological effects (49–51). As opposed to this loss of function, gain of function for instance has been described for the HERV-E family in association with altered tissue specificity of amylase gene promoters (52). Fur-

thermore, for some members of the enormous pool of solitary LTR elements, implications in specific transcriptional initiation or termination processes of adjacent cellular genes were unraveled (reviewed in Ref. 2). In the case of two HTDV/HERV-K LTRs located in the human leukocyte antigen (HLA) DQB1 region (53), it remains to be shown whether their association with susceptibility to insulin-dependent diabetes mellitus (IDDM) is directly correlated with the development of the disease (54). A putative normal cellular function of a HERV protein is represented by the single-copy HERV-R element that is selectively expressed during differentiation of placental syncytiotrophoblasts (55). Mass production of a nonglycosylated and unprocessed ENV protein in this tissue has been reported (56). As retroviral ENV proteins possess fusogenic and suppressive domains, there could be a functional correlation with some biological features of placental tissue, which exhibits considerable fusogenic and immunosuppressive properties.

In this review, the present knowledge of human endogenous retroviruses, with an emphasis on HTDV/HERV-K, has been briefly summarized. This family of elements represents a minor part in the whole spectrum of retroelements in the human genome that exhibit a broad variety of structural and functional features. Further investigations will show whether HERV families play an important role in normal and pathophysiological processes in human biology.

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