Originally published as:

Kurth, R., Bannert, N.
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(2010) International Journal of Cancer, 126 (2), pp. 306-314.

DOI: 10.1002/ijc.24902

Beneficial and detrimental effects of human endogenous Retroviruses

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Abstract

In this mini review, we aim to evaluate the structure and function of Human Endogenous Retroviruses (HERVs) with respect to the benefit they may have for humans or the damage they may cause. Emphasis is laid on their putative roles, if any, in pregnancy, in gene regulation and in cancer. As a basis for this discussion it will first be necessary to briefly describe the structure and function of retroelements, including HERVs, before addressing their positive or negative effects at the cellular and organismal level. Finally, we will give an outlook in which we will attempt to define priorities for future research.

The recent sequencing of the entire human genome revealed that almost half consists of transposable elements (TEs), namely DNA transposons (2.8%; 0.3 x 10^6 copies) and the more abundant retroelements (42.2%; 2.7 x 10^6 copies).1,2,3 DNA transposons amplify without RNA intermediates, whereas retroelements (as the name implies) require a reverse transcriptase to retrotranscribe RNA into DNA copies that will subsequently integrate into chromosomal DNA. TEs have often been regarded as ''selfish DNA'' or ''junk DNA,'' but it remains unclear whether they are really all ''junk,'' because upon becoming part of our genome, like all genes they are subject to natural selection and can be co-opted for the benefit of the host. Indeed, it may well turn out that TEs, in addition to other already measurable positive effects (some of which are described below), may play a major role in shaping our genome by increasing its plasticity and in the evolution of mammalian gene regulation networks.4–7

HERVs belong to the retroelements, which can be subdivided into those with regulatory long terminal repeats (LTRs, 8.3% of our DNA; 0.3 x 10^6 copies) and retroelements without LTRs (33.9%; 2.4 x 10^6 copies) (Fig. 1). Among the non- LTR members, short and long interspersed elements (SINEs and LINEs, respectively) are present in very high copy numbers. SINEs cannot code for proteins whereas LINEs encode a reverse transcriptase (RT) that can be utilized by both SINEs and LINEs for retrotranspositions or for the formation of pseudogenes. It is unknown whether the LINE RT can also be used by additional retroelements like HERVs for retrotransposition. The LTR containing retroelements can be grouped into 6 superfamilies.8 Class I–III HERVs possess limited nucleotide sequence homologies to C-, B- or spumaretroviruses, respectively. The other superfamilies MER4, MST and MLT represent ancient retrotransposons not known to be still functional in humans today.

Basic Genomic Organization and Replication of HERVs

To discuss the functions of HERVs that make up 8% of the human genome, one needs to look at their genetic organization. 1,9 All exogenous and preserved endogenous retrovirus strains have the basic genetic order 50-gag-pro-pol-env-30. Gag codes for matrix and capsid proteins, pro for protease, pol for reverse transcriptase, RNase H and integrase and env for envelope, as illustrated in Figure 2 for the youngest and most active human endogenous retrovirus family HERV-K. In particular, exogenous viruses, notably HIV, possess additional non-structural accessory genes that facilitate their replication or impair host defenses (reviewed in Ref. 10). These accessory genes are rare in endogenous virus strains, with the exception of HERV-K (see below).

Retroviruses are characterized by the outstanding and unique features of their replication that have consequences for their hosts. Virus particles contain 2 copies of RNA of ~8 to 10 kb in length. Reverse

transcription from fragile single-strand viral particle RNA into relatively stable doublestrand DNA reverts the usual flow of genetic information and leads to constitutive integration of the DNA provirus into host chromosomes. Most retroviruses prefer active genes or transcription start sites for integration.11,12 Proviruses in gene-rich regions of the chromosomes tend to become inactivated or excised, resulting over time in a disproportionate
accumulation of proviral sequences in gene-sparse regions, consistent with an integration effect on gene expression that typically reduces fitness. There is no other virus family that constitutively requires chromosomal integration for their replication. Only retroviruses exist in exogenous plus endogenous form, the latter in all cells of (usually) all members of a given host species as a result of germ-line infection of ancestors millions of years ago.4,13,14

Mechanisms of Retroviral Malignant Cell Transformation

Although most retroviruses tend to integrate in gene-rich regions, insertional mutagenesis has nevertheless been more often demonstrated for LINEs than for HERVs in humans and other animals (see below). Newly integrated proviral DNA possesses identical long terminal repeats at the 30- and 50-ends with primer binding sites and promoter and enhancer domains.

Downstream promotion or expression enhancement of cellular genes can be a consequence of proviral chromosomal integration.15–17 Furthermore, retroviruses, like other viruses such as herpesviruses, are capable of incorporating cellular genes, albeit at the expense of their structural genes. When retroviruses transduce cellular proto-oncogenes during reverse transcription due to template-switching of the RT
they become dependent on co-infecting wild-type viruses to provide structural proteins. Historically, much of our knowledge of oncology has been gained by the investigation of acutely transforming retroviruses carrying oncogenes (reviewed in Ref. 18–20).

A few replication-competent retroviruses encode their own oncogenic protein, notably Rous sarcoma viruses of chickens with their src-oncogene, the tax gene of the exogenous human T-cell lymphoma virus (HTLV) or the env gene of the Jagsiekte sheep retrovirus (reviewed in Ref. 21). Oncogenic retroviral proteins stimulate cell proliferation, often by influencing signaling pathways or levels of cytokine production that leads to growth stimulation and/or immune suppression. When searching for novel human retroviruses or investigating the role of HERVs in human cancer one has to take into account the different strategies developed by retroviruses to induce cellular proliferation and cancer and be aware that novel strains may use strategies as yet unknown.22

Germ-Line Infections of Humans

The discovery of replication-competent exogenous retroviruses that can be oncogenic in natural hosts such as mice, sheep or cats (Fig. 3) and at the same time exist in endogenous form stimulated the search for corresponding viruses in humans, i.e., the HERVs. In the pre-PCR era, several approaches were used for their discovery.

Low stringency hybridization of human genomic libraries using probes derived from conserved pol-regions of animal retroviruses led, for example, to the detection of retroviral sequences now known to belong to the youngest HERV-K family.23 In another experimental approach, highly conserved animal retrovirus proteins such as Gag were used in immunological
assays to screen for cross-reacting antibodies in healthy human blood donors and in patients.24,25 The detection of such antibodies in patients suffering from germ cell tumors led to attempts to grow these tumor cells in vitro and this resulted in the discovery of ‘‘Human Teratocarcinoma-Derived Retroviruses’’ (HTDV) in the late 1970s,26 which subsequently were shown to belong to the HERV-K family.27

Members of the HERV-K family of human ERVs possess a number of distinguishing features that make them attractive for intensive research. Ono et al. sequenced the first HERVK10 genome23,28 and found it to be surprisingly well conserved. The presence of only a few nonsense mutations suggested either recent infection or an ongoing process of purifying selection. HERV-K first integrated into the germ-line of Old World primate ancestors some 35 million years ago. Since then, and in contrast to other HERV members, repeated proliferation bursts have resulted in more than 60

proviral copies and over 2,500 solitary LTRs.4,29 Re-infection of the germ-line (rather than retrotransposition) by replication- competent HERV-K was predominantly responsible for this amplification. Indeed, since the divergence of the human and chimpanzee lineages some 6 million years ago, the insertional rate of new integrations has remained constant, amounting by now to over 70 human-specific viral insertions of which 15–20 are highly preserved full-length proviruses. 30,31 A replication-competent infectious virus clone has not yet been rescued from the human genome but single cycle infectivity has recently been demonstrated by consensus sequence clones of human-specific HERV-K(HML-2), the youngest known active group of HERV-K elements.32,33

About 10% of the human-specific HML-2 loci are polymorphic, i.e., are present only in a minority of human alleles as provirus, single LTRs or as result of an other recombination event.34 These most recently integrated elements have not (yet) penetrated the entire human population. Their timepoint of germ-line infection can be estimated by the degree of homology between their LTR sequences at the 50-
and 30-ends as these LTRs are identical at the time of integration and about 300,000 years are needed for each mismatch. In particular, HERV-K113, which is located on chromosome 19 in about 30% of African and 10% of Caucasian populations, appears to be one of the youngest additions to the HERV family with age estimates for the initial germ-line infection of human ancestors ranging from 200,000 years35 to 2 million years (own observations) ago. HERV-K, including HERV-K113 on chromosome 19, is the only human ERV with proviral elements retaining open reading frames for all viral genes.36

A comparison of amino acid changes between various human-specific HERV-K(HML-2) loci has enabled us to define the original sequence at the time of initial integration. A correspondingly modified and cloned ancestral HERVK113 sequence then allowed a functional analysis of viral
RNAs and proteins.32 Although viral RNAs, proteins and viral particles (Fig. 4A) could be detected in transfected cells, productive replication has not yet been observed—due to viral processing defects or inhibition by host cellular factors.

Over the last 10 years, a variety of laboratories have added evidence that the various loci of HERV-K elements can code for all structural, regulatory and enzymatic viral proteins (reviewed in Ref. 4, 22, 37) and it was stated that ‘‘HERV-K 113 is an excellent candidate for an endogenous retrovirus that is capable of reinfecting humans today’’ (from Ref. 36).
An infectious HERV-K in turn would be the most likely candidate for a newly detected oncogenic retrovirus.22,38

Beneficial Functions of HERVs

To explain their evolutionary conservation, it is necessary to consider the potential beneficial functions that HERVs provide for their hosts. In particular, we need to explain why animal and human ERVs have, over millions of years, retained open reading frames and all necessary functional domains in some of their proviral chromosomal loci. What functions do the corresponding viral RNAs and proteins have?

A more broadened view of transposable elements (TEs), including HERVs, demonstrates that it is the autonomous LINE 1 family that accounts for a large part of the human genome (500,000 copies) and that by self-mobilization and trans-mobilization of nonautonomous Alu (1.1 million copies) TEs and processed pseudogenes (11,000 copies) drive the shaping of the human genome by providing reverse transcriptase and promoter function. The vast majority of retrotransposition events are either neutral or deleterious to the genome and the latter will be eliminated by negative selection. 39,40 In addition, L1-L1 recombination contributes to sequence deletions, a process that generates genomic variation. 39 The complexity and redundancy of human gene regulation networks and the long evolutionary time-scale required to recognize marginally improved phenotypes makes it difficult to decipher the possible benefit afforded by such a generation of variation by deletion.

Fortunately, there are recognizable beneficial innovations resulting from the action of TEs, including HERVs.41–43 For example, at least 50% of the human-specific (i.e., young on an evolutionary time-scale) HERV-K(HML-2) LTRs act in vivo as active promoters for host non-repetitive DNA transcription, 44 demonstrably contributing to the expression of nearby genes (reviewed in Ref. 8, 16). Retrotransposon-mediated sequence transduction and gene duplication lead to the creation of novel genes41,45 and fosters the diversity of multigene families such as MHC- or T-cell receptor

genes.46,47 TEcoded RT was also shown to repair chromosomal breaks.48 It has also been suggested that the telomerase required to replicate
and stabilize the ends of chromosomes is derived from TE RT.49 HERV LTRs often contain binding sites for the p53 regulator.7 In fact, HERV LTRs account for over 30% of all p53 binding sites genome-wide and may have been co-opted as regulatory sequences to expand the p53 transcriptional network.7 Thus, these HERV LTRs may contribute to the anti-oncogenic function of the stress-responsive p53 pleiotropic regulator.

Furthermore, HERV-W envelope glycoproteins can confer cellular resistance to superinfection by exogenous retroviruses as shown in vitro.50 Interference inhibition of infection by exogenous, potentially oncogenic or immunosuppressive retrovirus strains is a general feature in animal retrovirology.

A clinically impressive example of cancer immunotherapy has very recently been described.51 HERV-E, which is apparently activated in a proportion of renal cancer cells, was shown to provide target antigens recognizable by cytotoxic T-cells from donors after allogeneic hematopoetic stem cell transplantation. This therapeutic approach led to complete (10%) or partial (30%) tumor regression in patients and underlines that the natural host is apparently not immunologically tolerant to HERV.

Of even greater significance is the fact that the placentas of primates, including humans,52 produce retrovirus particles. 53–55 Originally observed by electron microscopy and later supported by the demonstration that the trophoblast specific human growth factor pleiotrophin (PTN) is under the control of a HERV LTR,56 these results prompted studies of HERV expression in placentas. In syncytiotrophoblasts, but not in cytotrophoblasts, high levels of HERV-W and HERV-FRD envelope proteins are demonstrable, named syncytin- 1 and syncytin-2, respectively,57–59 and additional HERV env proteins were subsequently detected in syncytiotrophoblasts. 58,60 Retrovirus envelope proteins are anchored in the lipid bilayer membrane of both viral particles and cell surface membranes and initiate the fusion of viral and cellular membranes during the infection process. In cases where the cell surface viral envelope protein can interact with its receptor on adjacent cells, they have also been shown to cause fusion of infected and uninfected cells. Thus, the cell–cell fusogenic activity mediated by HERV Env proteins probably contributes to the physiological placenta morphogenesis by mediating fusion of cytotrophoblasts to
syncytiotrophoblasts.61

Placentas are, from the viewpoint of the mother, allogeneic organs and the reasons for maternal tolerance are still only poorly understood. The fetal multinucleated villous syncytiotrophoblast layer acts as the fetal-maternal interface and is responsible, among other functions, for trophic and hormonal exchange. At this boundary, immunological tolerance has to be effective to prevent allogeneic rejection of the fetus. It is therefore not at all surprising that the already known immunosuppressive property of retroviral Env proteins could also be demonstrated for syncytin-2 and other HERV Env proteins, although not for syncytin-1.61 HERVs may therefore be instrumental in safe-guarding placenta morphogenesis, physiology and fetal–maternal tolerance. Downregulation and abnormal intracellular localization of placental syncytin expression may contribute to the etiology of pre-eclampsia.62 Similar fusogenic and immunosuppressive endogenous retrovirus proteins were recently detected in all rodents tested63 as well as in sheep,64 which suggests positive selection over millions of years.

Detrimental Effects of HERVs

HERVs have not, of course, evolved primarily for the benefit of the host and are therefore likely to also have detrimental effects (Table 1). These can best be understood by first considering the disease-inducing properties of exogenous human retroviruses.

It is sufficient to summarize that there are 4 well-characterized exogenous retroviruses, namely human T-lymphotropic virus Type 1 (HTLV-1) and HTLV-2 and the notorious human immunodeficiency viruses HIV-1 and HIV-2. The only unequivocal human tumor retrovirus is HTLV-1, causing monoclonal adult T-cell leukemia in a minority (1– 2%) of the infected people, often after latency periods of up to 50 years. It is the Tax protein of HTLV-1 that promotes cellular proliferation by activating a variety of cellular genes. HTLV-1 may also occasionally cause neurological diseases.65

HTLV-2 was isolated from a patient with hairy cell leukemia but probably does not possess significant, if any, oncogenic potential. HTLV-2 may also cause neurological myelopathy or paraparesis.66

HIV-1 and HIV-2 are obviously the cause of AIDS, the greatest medical catastrophe of modern times. It is amazing that these 4 virus strains were all isolated within a very brief time period between 1980 (HTLV-1) and 1986 (HIV-2), during an intense period of scientific activity (described, for example, by Anders Vahlne67). It is perhaps surprising that opportunistic tumors in AIDS are not directly caused by HIV-1 or HIV-2, given that billions of virus particles are produced by billions of HIV-infected cells in millions of HIVpositive people every day. There should be ample opportunity for insertional mutagenesis of tumor suppressor genes or downstream promotion or incorporation of cellular protooncogenes. For reasons not readily understood, these events appear to be thankfully very rare in HIV infection and may in fact be due to the very short life of most productively infected cells.68 Opportunistic tumors defining AIDS are instead the consequence of immune dysfunction, a fact that underlines the significance of an effective immune system in tumor surveillance.

A recently discovered presumably exogenous retrovirus may now have to be added to this list: XMRV (Fig. 4B), a virus related to a xenotropic murine leukemia virus (over 95% sequence homology), has been isolated from stromal cells surrounding human prostate cancer tissues and from the hematopoetic cells of these patients.69,70 Positive specimens were, for the most part, obtained from patients with a polymorphism in the RNaseL gene that results in an impaired innate immune system because of a reduced interferonresponse to infections.70 PCR amplification of viral sequences from different patients revealed >98% nucleotide sequence homology and the sequence can easily be distinguished from murine retrovirus genomes because of a characteristic deletion upstream of the gag-gene and unique point mutations. XMRV integration sites have been mapped from tumor tissues69 and after in vitro infection of the human prostate cancer cell line DU14571 and have been shown to locate preferably in GC-islands, DNase hypersensitive sites and gene-dense regions close to transcription start sites. However, a common integration site near cellular proto-oncogenes or tumor suppressor genes was not found. High-titer XMRV production by a prostate carcinoma cell line has also been very recently reported.72

These results still need to be confirmed by other laboratories and an etiological role in prostate cancer has yet to be demonstrated. As XMRV is detected in only about 1% of prostatic stromal tissue and in hematopoetic but not carcinoma
cells,70,71 an indirect paracrine mechanism for viral oncogenicity has been suggested.73 A preference for XMRV integration in the regulatory region of transcriptionally active genes could alter gene expression, resulting in a stromal microenvironment favorable for tumor initiation and/or progression and escape from immune surveillance.73,74

We have recently screened more than 600 clinical tumor samples, including prostate cancer biopsies, for XMRV infection, without a single positive result. About 13% of the patients were homozygous and 35% were heterozygous for the functionally impaired RNaseL allel R462Q previously described to be present in the vast majority of XMRV-positive prostate cancer patients. The reason for this discrepancy is not yet clear. It is possible that geography might play a role, as we studied patients from Germany whereas the Silverman laboratory69 had a cohort of patients from the United States. As the mode of transmission of XMRV is entirely unclear and its prevalence is unknown, additional studies of prostate cancer patients from other areas, including the US, are urgently needed. In addition, the mechanism of tumorigenesis needs to be clarified, as does the origin of XMRV, which may be an example of zoonotic transmission from mice to humans. Indeed, it is possible that XMRV is associated with other human diseases, even with other human tumors.

Human Endogenous Retroviruses and Cancer

HERVs have been associated for decades with 2 types of chronic human diseases: autoimmunity and cancer. The scientific literature has been littered with premature claims associating known or novel retroviruses or their footprints (viral DNA- or RNA-sequences, reverse transcriptase activity, proteins, etc.) with human disease. Little has withstood subsequent scientific scrutiny and in the times of advanced PCRand genome-wide chip technologies we will focus on those situations in which the involvement of HERVs in tumor development can be discussed seriously.

The possible role of HERVs in autoimmune disease would also be beyond the scope of this mini review and the topic has anyway been addressed in a detailed, comprehensive and elegant review.19 The take-home message of the authors is that ‘‘the notion that the cause of any of these complex diseases is due to a single infectious etiological agent is most likely naive’’ (from Ref. 22).

As mentioned earlier, oncogenic animal retroviruses can transform normal cells by 3 main mechanisms: (i) capture of cellular oncogenes resulting in acutely transforming, replication incompetent virus strains requiring co-infection with wild-type helper viruses for replication, (ii) insertional mutagenesis destroying tumor suppressor genes, or, in a related mechanism (iii) downstream promotion of adjacent growthpromoting cellular genes. Only animal endogenous retroviruses that concomitantly exist also in an exogenous, horizontally transmissible form can use all 3 mechanisms. Notable examples include mouse leukemia and mouse mammary tumor viruses (MLV, MMTV), feline leukemia viruses (FeLV), porcine endogenous retroviruses (PERV) and koala retroviruses (KoRV).75,76 The exogenous strains only induce tumors rarely in outbred animal populations and usually replicate without causing severe symptoms. Unambiguous evidence for a causal oncogenic role of endogenous virus strains has only
been found in somewhat artificial models, namely in mice bred for rapid tumor development caused, for example, by MLV or MMTV or the mouse melanoma-associated MelARV virus strain, a recombinant between different endogenous MLVs.

In this regard, it is interesting that very high titers of pelletable HERV-K RNA and reverse transcriptase activity can be found in plasma of patients with certain lymphomas and breast cancers77 and these titers drop dramatically after cancer treatment. A causal relationship between HERV-K expression and tumor development has not (yet) been established and these observation urgently need confirmation by other laboratories.

As mentioned earlier, there is no evidence that an infectious, horizontally transmissible HERV actually exists, although this cannot be categorically excluded as such a strain would probably not be very prevalent in the human population because of strong negative selection. Cellular proteins such as the APOBEC (apolipoprotein B mRNA-editing enzyme catalytic polypeptide-like) cytidine deaminases enzyme family and the tripartite motif (TRIM) 5-alpha protein can inhibit retrovirus replication in human cells, including
HERVs33,78 and attempts to isolate replication-competent HERVs by co-cultivating putatively infected cells with other human indicator cells may therefore be doomed to failure.

As replication-competent HERVs have not yet been unambiguously detected, HERV sequences are assumed to be able to play a causative role in cancer development after retrotransposition to novel chromosomal loci a subsequent enhancement of growth-promoting cellular genes or inhibition of tumor suppressor genes. Furthermore, recombination between the thousands of HERV loci may lead to gain-offunction sequences. Among all known HERV families, HERV-K(HML-2) is the youngest and probably still most
active provirus. As mentioned before, HERV-K 113 on chromosome 19 has all reading frames open and possesses all the regulatory domains required for an infectious retrovirus. Furthermore, the HERV-K(HML-2) family possesses 2 accessory viral proteins not found in the other HERVs, namely Rec and Np9 (reviewed in Ref. 38). Rec, initially named cORF,79 is a protein functionally related to HTLV Rex and HIV Rev, responsible for the nuclear export of unspliced or singlespliced viral mRNA into the cytoplasm. Rat-1 cells transfected with rec grew into tumors in nude mice,80 whereas these cells transfected with gag or env remained non-tumorigenic. In a related approach, Galli et al.81 could demonstrate that rec expressed facultatively in transgenic mice may directly contribute to germ cell tumor formation as such animals experienced dysfunctions in germ cell development and developed carcinoma in situ in a manner reminiscent of precursor lesions of human germ cell tumors. It was subsequently shown, again by the laboratory of Mueller- Lantzsch80,82 that both Rec and Np9 could bind to the promyelocytic leukemia zinc finger protein (PLZF) which is known to be both a tumor suppressor and a transcriptional suppressor of the c-myc proto-oncogene. Interestingly, PLZF may also be positively involved in regulating spermatogonial stem cell homeostasis in mice.83

Co-expression of both Rec and Np9 proteins abrogated transcriptional repression of the c-myc gene by PLZF resulting in increased synthesis of c-Myc protein and of proteins regulated downstream of c-Myc such as p53. Likewise, cells stably transfected with PLZF and rec exhibited increased cell proliferation and reduced apoptosis.

Np9-, but not rec- or gag-transcripts were found exclusively in about 50% of a variety of cell lines derived from germ cell tumors, breast cancer and leukemias, but not in normal cells or cell lines.84 This exclusive restriction to tumor cell lines is both astounding and unusual and definitely calls for an intensified study of additional tumor lines and biopsies as well as a modern transcriptome analysis.37 Taken together, Rec and Np9 may even act in concert as oncoproteins in GCT via inhibition of PLZF and possibly through interference of Np9 with the Numb/Notch pathway essential for proliferative Ras signaling.85 Inhibition of rec- and np9-RNAs by siRNA may revert the transformed phenotype of CGT cell lines back to normal.

Only HERV-K has been shown to be able to produce retrovirus particles.9,38 On the basis of the ratio of nonsynonomous to synonomous nucleotide changes (>1) that implies purifying selection during replication and because of the allelic polymorphism (refer previous section) it has been suggested that HERV-K can still replicate via re-infection.30,31 However, a comparison of HERV-K113 and HERV-K115 frequencies in breast cancer and seminoma patients and in unmatched controls showed no differences in their sites of chromosomal integration as would be expected if new horizontal infections were occurring. A search for functional HERVs should therefore concentrate on those tumors in which virus proteins and particle production has been observed, e.g., ingerm cell tumors and in melanomas.

HERV-K-like particles in human melanomas were first described some time ago86 and, more recently, HERVK(HML-2) mRNA and proteins were detected in primary melanomas, in their metastases and in melanoma cell lines.87 Indeed, antibodies and cytotoxic T-lymphocytes specific for HERV-K antigens could be detected in melanoma patients.88–90 Attempts to isolate infectious HERV-K particles from melanoma cells were not successful but this should now be attempted again by focusing on the HERV-K 113 locus in chromosome 19 and the other most recently acquired elements.

HERV may also indirectly facilitate tumor development by the immunosuppressive function of its Env proteins. As much as this immunosuppression may be advantageous in syncytiotrophoblasts at the fetal–maternal interface as mentioned earlier, it may be detrimental to the immune surveillance of tumors. This has recently been shown in elegant experiments using chemically induced allogeneic mouse tumors that were rejected after injection into immunocompetent mice. These tumors transfected with HERV-K env or with env from Moloney MLV or Mason-Pfizer Monkey Virus (MPMV) suppressed tumor rejection and grew in the recipient mice.91,92 The immunosuppressive domain is localized in the transmembrane (TM) portion of retroviral Envs93 and the immunosuppressive effects of endogenous retroviral envelope proteins have also been demonstrated in mouse melanoma and neuroblastoma models (reviewed in Ref. 38).

Conclusions and Outlook

We have come a long way since Barbara McClintock's epochal discovery of transposable elements in maize in the 1950s.94 Considering the rapidly increasing knowledge of transposable elements in the creation, modulation, regulation and inactivation of eukaryotic genes, we should stop describing them as ''junk'' DNA. Transposable elements are not necessarily useless. They can shape the structure, function and networking of the human genome with their promoters, enhancers, polyadenylation signals and polymerases.1,5,7,54

ERVs are also TE but do not transpose as easily or as often as LINE or SINE elements. ERVs in animal models are involved in tumorigenesis at various levels ranging from infectious, oncogenic ERVs to retrotransposition of ERV sequences leading to insertional mutagenesis and downstream promotion of genes that themselves promote cell growth. ERV Env proteins are fusogenic and immunosuppressive, facilitating tumor escape from immunological surveillance.

In the absence of an infectious HERV, future experimental studies must focus on the indirect mechanisms of malignant cell transformation. Because of the high background of thousands of mutated HERV sequences, retrotransposition events will be very difficult to detect directly, except when they lead to an unusual cellular phenotype by inhibiting or activating cellular gene networks in a measurable way. HERVs may act as (initial) co-factors in the complex multi-step development of tumors and further analyses should include, for example, inhibition of HERV-K RNA in normal and malignant cell lines by siRNA and—in a more general approach—genomewide expression screening using proteome and transcriptome technologies.

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Figures and Tables

Figure 1. Classification of the major retroelements, their share of the human genome (in percent) and their copy number estimates.

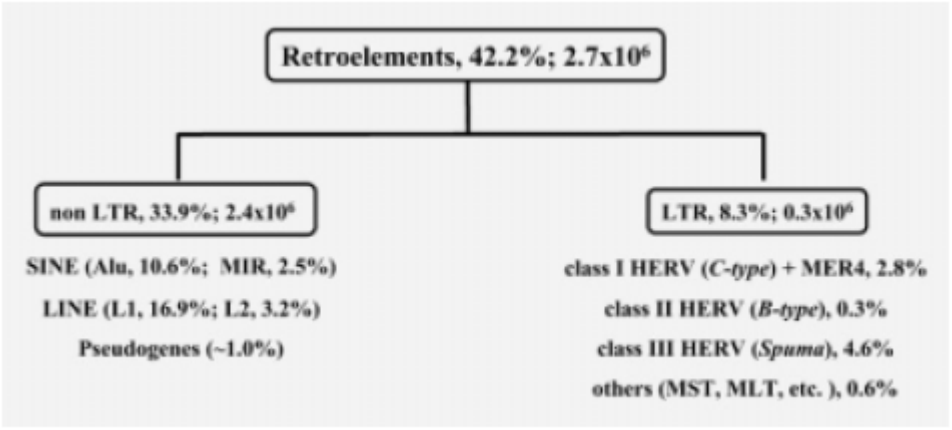

Figure 2. Genomic structure of the HERV-K provirus. The accessory protein Rec is functionally related to the HIV protein Rev and the HTLV protein Rex. It shuttles RNA transcripts out of the nucleus. Refer text for details.

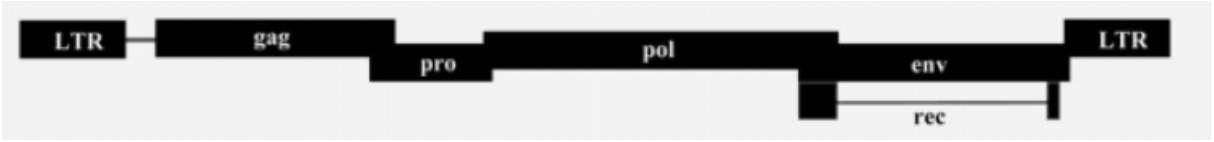

Figure 3. Retrovirus strains existing in both endogenous and exogenous replicating forms. With the exception of PERV, an association with malignancies has been reported.

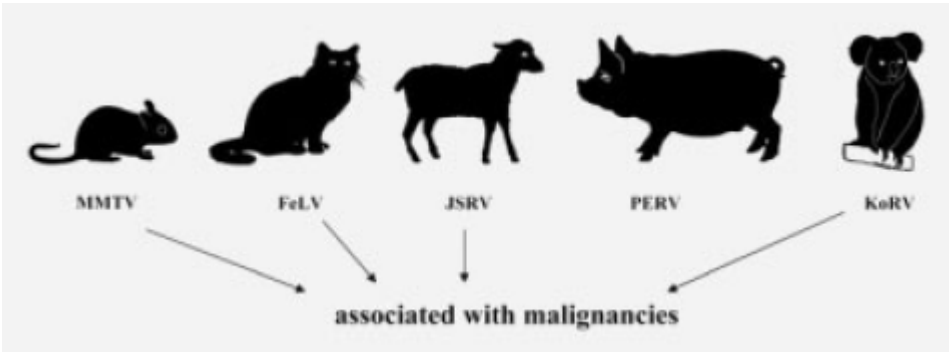

Figure 4. Thin section electron microscopy of retroviral particles. (a) Reconstituted ancestral HERV-K113 particles. Mature virions with condensed cores are shown in the left hand panel, whereas an immature virion and a budding particle are shown in the right hand panel. (b) Image of a mature XMRV particle. (Bar: 100 nm).

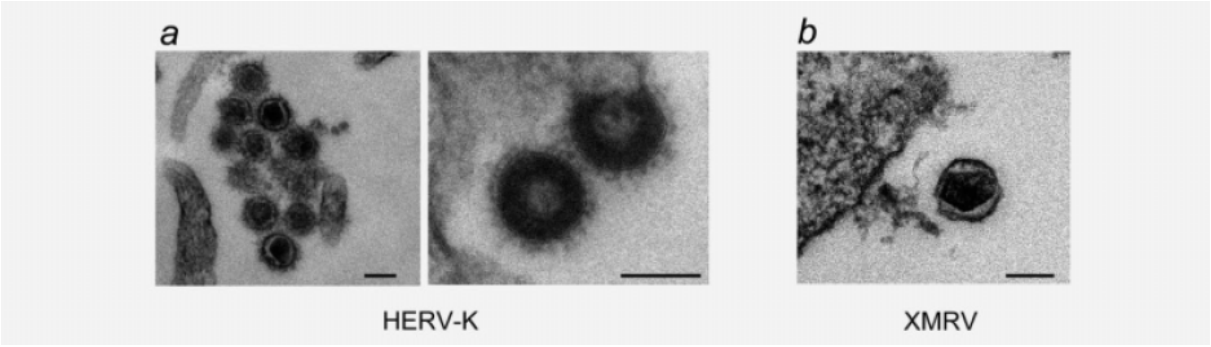

Table 1. Representative HERV families and their detrimental or beneficial effect

HERV family	Copy number[1]	Suspected effect	Refs.[2]
Gammaretroviral **(Class I)**			
HERV-E	250 (1000)	Opitz syndrome	17
HERV-W	40 (1100)	Placenta formation	60
HERV-FRD	50 (2000)	Placenta formation	57
HERV-H	1000 (1000)	Gene expression	42
Betaretroviral **(Class II)**			
HERV-K(HML-2)	60 (2500)	Carcinogenesis	81
Spumaviral **(Class III)**			
HERV-L	580 (6000)	Fv-1 restriction in mice	43

1Copy number of solo LTRs is given in parentheses. 2For additional details and examples see Ref. 1.