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Endogenous retroviruses and cancer

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Abstract. The genomes of vertebrates contain sequences that are similar to present-day exogenous retroviruses. Such sequences, called endogenous retroviruses (ERVs), have resulted from ancestral germ line infections by exogenous retroviruses which have thereafter been transmitted in a Mendelian fashion. By analogy to exogenous tumorigenic retroviruses, ERVs have been implicated in the pathogenesis of cancer. Cumulative evidence from animal models indicates that ERVs may participate in the process of malignant transformation or promote tumor growth, e.g. through insertional mutagenesis or via counteracting tumor immunosurveillance. Here, we review the role of ERVs in tumorigenesis with focus on human ERVs (HERVs) in human cancer. Although available data suggest a potential role of HERVs in human cancers, in particular germ cell tumors, the contributions of HERVs to human tumorigenesis warrant further elucidation. (Part of a Multi-author Review)

Keywords. Human endogenous retrovirus, cancer, tumorigenesis, germ cell tumor, melanoma.

Introduction

The development of cancer is a complex multistep process. Accumulation of mutations in oncogenes and tumor suppressor genes, resulting in malignant transformation of cells, as well as escape of malignant cells from immunological control mechanisms that normally protect from deregulated growth contribute to tumorigenesis [1, 2]. Inherited and environmental factors have been involved in the etiology of cancer, the relative importance of each depending on the type of malignancy. Evidence for a tumorigenic capacity presently exists for three broad categories of environmental agents: radiation, chemical carcinogens, and viruses. Among the tumorigenic viruses, the retrovirus family has been of exceptional importance for the shaping of the prevailing concepts of tumorigenesis. Much of what is known today about normal and aberrant cellular growth and its control has in fact originated from studies of regulatory genes first identified in tumorigenic animal retroviruses [3].

The hallmark of the retroviral replication cycle is reverse transcription of retroviral genomic RNA into a double-stranded DNA copy which is stably integrated into chromosomal DNA of the host cell to form a provirus. Notably, the genomes of all vertebrate species analyzed so far contain proviral sequences closely related to those of exogenous retroviruses. Such sequences, termed endogenous retroviruses (ERV), have arisen from infections of germ-line cells by exogenous retroviruses during the evolutionary past, followed by fixation of some of those retroviruses in the host's genome and subsequent vertical transmission from parent to offspring in a Mendelian fashion [4–6]. By analogy to exogenous retroviruses, ERVs have been implicated in the pathogenesis of diseases, in particular cancer and autoimmunity [7–9]. ERVs are of interest as potential disease-inducing agents since, on one hand, they are endogenous inherited factors, whereas on the other hand they may have retained potentially pathologic

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properties of their exogenous infectious retroviral ancestors. Here, we review the subject of ERVs and cancer, with a special emphasis on the role of human endogenous retroviruses (HERVs) in human cancers.

Background: exogenous retroviruses and cancer

Historically, the initial detection of ERVs in chicken in the late 1960 s [4] and the discovery of ERV elements in humans in the early 1980 s [10, 11] originated from precedent extensive studies on exogenous retroviruses causing cancer in animals. Indeed, retroviruses were originally identified as the causative agents of transmissable tumors of chicken [12, 13] and mice [14], which led to their designation as 'RNA tumor viruses' [15]. Oncogenic animal retroviruses induce cancer by two main mechanisms: oncogene capture and insertional mutagenesis [16]. Oncogene capture applies to acute transforming retroviruses that upon infection rapidly cause tumors in their host. These retroviruses carry a viral oncogene (v-*onc*) whose expression results in aberrant growth of infected cells. The v-*onc* gene often disrupts the viral genome, necessitating co-infection with an undisrupted (wild-type) helper virus for transmission. The finding that v-*onc* genes are derived from cellular genes which became inserted into the retroviral genome by recombination during reverse transcription (hence the term oncogene capture) has had a profound impact on the general understanding of cancer [3]. Indeed, the cellular homologues of v-*onc* genes, termed c-*onc* or proto-oncogenes, frequently play pivotal roles in the control of cellular growth and differentiation. It is these proto-oncogenes that in many types of cancer are also common targets for chemical carcinogens, radiation, and genetic alterations.

Insertional mutagenesis is a pathogenic mechanism of nonacute retroviruses which do not carry a v-*onc* and induce tumors considerably slower than acutely transforming viruses. Integration of proviral genomes at a site close to cellular proto-oncogenes can activate their expression via regulatory sequences within the viral long terminal repeat (LTR), while integration in tumor-suppressor genes may disrupt and thereby inactivate them. Viruses that cause tumors by such insertional mutagenesis are called *cis*-activating viruses and are replication-competent.

The finding of oncogenic retroviruses in higher mammals stimulated intensive searches for exogenous retroviruses causing tumors in humans in the 1960 s and 1970 s. While initial high hopes were not fulfilled, the first infectious human retrovirus, human T cell leukemia virus 1 (HTLV-1), was described in 1980 [17] and shown to be the etiologic agent of adult T cell lymphoma/leukemia (ATL) and also of a neurological disease called HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) [18, 19]. Induction of ATL by HTLV-1 does not seem to result from oncogene capture or insertional mutagenesis. Rather, the nonstructural regulatory protein Tax acts as an oncoprotein and is currently regarded the primary HTLV-1-encoded factor responsible for malignant transformation [20]. Tax is able to transform and immortalize rodent fibroblasts and human T lymphocytes and has been shown to transactivate cytokines and cytokine receptors through interaction with several different cellular pathways (e.g. NF-κB, CREB). Tax may also stimulate cell growth by binding to cyclin-dependent kinase holoenzymes or inactivation of tumor suppressors (e.g. p53, DLG) [21]. Also, transgenic expression of Tax in developing thymocytes of mice results in T cell malignancies with features characteristic of ATL [22].

Infection with human immunodeficiency virus type 1 (HIV-1) is associated with an increased incidence of 'opportunistic tumors', thought mainly to result from deficient immunological control of neoplastic cells. Beside this indirect mechanism, HIV-1 has been suggested to promote oncogene activation in non-Hodgkin lymphomas by insertional mutagenesis [23]. However, such mechanism probably contributes to malignant transformation in only a small subset of HIV-associated non-Hodgkin lymphomas [24]. A number of 'novel' retroviruses that have previously been associated with human cancers presently remain under scrutiny (for review, see [25]). Nevertheless, as yet unidentified retroviruses of potential relevance for human tumors may still await their discovery. A xenotropic MLV-related retrovirus (XMRV) was recently identified in prostate cancers from patients with a genetic susceptibility to prostate cancer related to mutations that impair RNase L, an enzyme involved in the innate antiviral immune response [26]. Cloning of XMRV integration sites from prostate cancer tissue has provided strong evidence for *bona fide* infections of human cells by XMRV [27]. It remains unclear though whether XMRV plays any causal role in human prostate cancer.

With respect to potential tumorigenic properties of HERVs, it is noteworthy that, to date, HTLV-1 represents the only firmly established example of an exogenous retrovirus directly causing human cancer. Moreover, ATL is a rare outcome of HTLV-1 infections, observed in only ~1–3% of HTLV-1 seropositive individuals and usually after a long period of latency, indicating that HTLV-1 may be a necessary but not sufficient cause for this disease and that co-factors or host genetic factors may also be required [3]. It may be speculated that the relative paucity of

exogenous tumorigenic retroviruses in humans and the overall low frequency of ATL in HTLV-1 infected individuals could at least in part be due to a number of restricting factors, such as the APOBEC (apolipoprotein B mRNA-editing enzyme catalytic polypetide-like) cytidine deaminases enzyme family and the tripartite motif (TRIM)5α protein, which both can actively inhibit retrovirus replication in human cells (for review, see [28]). Of note, these factors also target ERVs, as demonstrated by the inhibition of murine retroelements by APOBEC3G [29] and the inhibition of a HERV-K(HML-2) consensus element by APOBEC3F [30]. Such defense mechanisms may thus likely contribute to the control of potential pathogenic effects of ERVs in humans.

Endogenous retroviruses and cancer: lessons from animal models

Despite millions of years of residence in their host genomes, many ERVs still show clear similarities, e.g. in terms of their internal genome organization (*gag, pro, pol,* and *env*) and the presence of flanking control elements (LTRs) with exogenous infectious retroviruses. It is therefore not surprising that they have been suspected to be involved in human cancer and other human diseases [7–9]. However, unambiguous evidence for a causal role of ERVs in tumorigenesis has so far only been obtained from a few inbred murine systems [4]. Two classical examples are leukemias induced by endogenous murine leukemia virus (MLV) in AKR mice, and mammary tumors caused by endogenous mouse mammary tumor virus (MMTV) in GR mice. Notably, in both cases there exist well-studied exogenous variants of the respective viruses that likewise induce tumors. Leukemogenesis in AKR is a multistep process depending on the generation of leukemogenic recombinant viruses (known as mink cell focus forming [MCF] viruses) from at least three endogenous MLV proviruses. *De novo* integration of MCF recombinant viruses may subsequently activate proto-oncogenes, eventually resulting in leukemia [4, 31]. Likewise, expression of endogenous MMTV-related proviruses in lactating mammary glands may lead to the release of infectious virus, followed by reinfection, novel proviral insertions, and activation of cellular proto-oncogenes [4].

Two more recent studies using either a murine melanoma or a murine neuroblastoma model have provided additional evidence for mobility and amplification of ERVs in murine tumor cells [32, 33]. Murine B16 melanomas from C57BL/6 mice spontaneously produce an ERV, termed melanoma-associated retrovirus (MelARV). Analysis of the provirus content and insertion sites of MelARV in B16 melanoma cells, and a subline of these cells with enhanced metastatic activity (B16F10), indicated that this element can give rise to novel proviral insertions. Importantly, MelARV insertions in B16F10 cells targeted and altered the transcriptional profiles of genes potentially involved in metastatic spread [33]. Likewise, a cell line (Neuro-2a) derived from a spontaneous A/J mouse neuroblastoma produced an infectious ERV, dubbed Neuro-2a-associated retrovirus (NeRV). Southern blot analyses showed that NeRV was strongly amplified in Neuro-2a cells, in accord with retrotransposition or reinfection of NeRV in Neuro-2a cells [32]. Interestingly, both MelARV and NeRV were most likely generated by recombination events between N-tropic endogenous MLV proviruses and parts of *gag* sequences from B-tropic endogenous MLV. Since A/J and C57BL/6 mice are only permissive for retroviruses with B-tropic, but not with N-tropic Gag proteins, these recombination events likely conferred the ability to replicate in A/J and C57BL/6 mice cells [32, 33]. Evidence suggesting that MelARV and NeRV may also promote tumor growth at the level of host immunosuppression will be discussed below.

Overall, a common pathogenetic characteristic of the discussed animal models is the presence of replication-competent ERVs which are able to form novel proviral insertions that induce tumors by insertional mutagenesis, or that promote tumor growth by host immunosuppression. In the case of MMTV in GR mice, such a replication-competent ERV is apparently inherited, whereas in the other instances replication-competency of ERVs relies on recombination events between different endogenous proviral loci.

HERVs and cancer: evolutionary perspective

The association of ERVs with some forms of cancer in animals was without doubt one of the driving forces for identifying ERVs in humans [6]. Still, current knowledge on the role of HERVs in human cancers remains rather limited and, as of yet, conclusive evidence for a causative function of HERVs in human cancer – and other human diseases – could not be produced [5, 6, 25]. To estimate the pathogenic potential of HERVs in humans it appears helpful to look at them from an evolutionary perspective.

In a broad sense, HERVs can be considered to be part human gene and part virus [7]. As far as HERVs are genes, they are subject to the same evolutionary forces as any other gene in the human genome. Accordingly, any overtly pathogenic HERVs would undergo negative selection, prohibiting fixation of such HERVs in

the population. HERV elements in the human genome can thus be viewed as the domesticated and tamed remnants of once active elements and would be expected to be neutral at least [34]. However, if a pathogenic effect of a HERV element would only become relevant after the reproductive phase, preservation of such an element might be more likely. Similarly, if HERVs would provide some evolutionary advantages at the population level, occasional collateral pathogenic effects of HERVs might be outbalanced by those advantages [7]. Several beneficial effects of HERVs have been proposed. First, HERVs may contribute to modelling and plasticity of the genome. For instance, HERVs constitute sequences that are substrates for genomic rearrangements. Furthermore, regulatory elements located in the HERV LTRs can provide tissue-specific enhancers, alternative promotors, or alternative polyadenylation signals for nearby genes [5]. It has been found that more than one-third of the binding sites in the human genome for the transcription factor and tumor suppressor p53 are accounted for by HERV LTR regions. These HERV LTR p53 sites are likely part of the p53 transcriptional program and regulate p53 target genes [35]. Second, the presence of ERVs may protect from infections by related exogenous retroviruses, for instance by receptor interference, a mechanism well documented in animal models [28, 36]. Third, functional properties of ERVs – such as the fusogenic activity of retroviral Env proteins – may have been diverted by the host organism to its benefit. Indeed, the *env* sequence of a HERV-W provirus on chromosome 7q21.2 appears to have turned into a *bona fide* gene whose protein product, termed Syncytin-1, is highly expressed in the placenta, where it may participate in the fusion of the cytotrophoblast into the syncytiotrophoblast [37, 38]. Another retroviral Env protein, dubbed Syncytin-2 and belonging to the HERV-FRD family, may similarly take part in placenta formation and has furthermore been hypothesized to be involved in fetomaternal tolerance by means of a immunosupressive domain located in its transmembrane (TM) subunit [39, 40].

Interestingly, expression of Syncytin-1 has also been detected in a proportion of human breast cancers as well as in endometrial carcinomas. Cell culture experiments suggest that this expression may result in breast cancer-endothelial fusions or endometrial carcinoma cell-cell fusions [41, 42]. Such cell fusions may modify the biological behavior of tumor cells and promote tumor growth [43]. The fusogenic properties of Syncytin-1, which serve a physiological goal when active in the placenta, may therefore foster a pathological process in the case of a possibly dysregulated expression of Syncytin-1 in tumor cells. Similarly, an aberrant expression of Syncytin-1 in glial cells may contribute to the pathogenesis of multiple sclerosis, a chronic inflammatory demyelinating CNS disease, by induction of proinflammatory cytokines and redox reactant-mediated oligodendrocyte damage [44]. These ambivalent features of Syncytin-1 appear to be in accord with the idea that occasional unfavorable effects associated with a dysregulated HERV expression in human diseases may be the toll for the potentially favorable effects of HERVs at the population level. In this respect, HERV genes may mimick cellular proto-oncogenes.

HERV expression in human cancers: cause or coincidence?

A large and constantly growing number of reports have described detection of RNA transcripts from various HERV families in many different types of human cancers or tumor cell lines, e.g. [45–58]. Nevertheless, even when HERV RNA expression was found to differ from non-diseased control tissue, it remains in many cases unknown whether this merely represents an epiphenomenon or indicates a genuine role in the process of tumorigenesis. Furthermore, the repetitive nature of most HERVs poses additional considerable challenges in terms of differentiating between potentially disease-relevant and irrelevant RNA transcripts. In fact, there are about 30 distinct HERV families present in the human genome, with each family containing few to several hundred similar elements [59]. The vast majority of HERVs have accumulated mutations, deletions, or truncations, and thus do not contain uninterrupted open reading frames (ORFs) for full lengths retroviral proteins. For instance, out of ~4000 endogenous retroviral *env* loci in the human genome, only 16 encompass an intact full-length ORF that may potentially encode a functional Env protein [60]. The ratio between full-length and interrupted ORFs is even lower for HERV *gag* (17 vs. 9500) and *pol* (13 vs. 20900) genes [61]. Under the premise that putative pathological functions of HERVs may rely on their capacity to encode intact full-lengths proteins, only very few elements thus appear to have the capacity to exert such functions. It should also be stressed that HERV transcripts have been found in every tissue and cell type analyzed so far [62, 63]. As outlined above, the large majority of transcribed HERV sequences are not expected to contain uninterupted ORFs. Although a role of RNA transcripts from such defective elements cannot be ruled out [64], they are often considered non-functional and their biological relevance is largely elusive. Since methods applied for the detection of HERV

Table 1. HERV-encoded proteins previously detected in human tumors and tumor cell lines.

Tumor type	HERV protein*	References
Germ cell tumor tissue	HERV-K(HML-2) Gag	[106]
Germ cell tumor cell lines (GH, Tera-1, NCCIT, 2102Ep)	HERV-K(HML-2) Gag HERV-K (HML-2) Env HERV-K(HML-2) Rec HERV-K(HML-2) Np9	[106] [120] [121, 131] [126]
Melanoma tissue, melanoma cell lines (SK-Mel-28, Mel-Juso)	HERV-K (HML-2) Env (TM) HERV-K(HML-2) Gag HERV-K(HML-2) Rec	[110] [130] [131]
Breast cancer tissue, breast cancer cell lines (MCF-7, MDA-MB-231)	HERV-W Env	[41]
Ovarian cancer tissue	HERV-K Env	[135]
Endometrial carcinoma tissue	HERV-W Env	[42]
Astrocytoma tissue	HERV-W Env	[136]
Neuroblastoma cell line (IMR32)	HERV-W Gag HERV-W Env	[137]

* HERV-encoded proteins were detected by immunohistochemistry and/or Western blot.

RNA frequently do not allow to differentiate from which loci within a given HERV family the detected RNA transcripts are derived, the biological significance of HERV RNA expression in diseased tissues often remains obscure. Future studies may thus concentrate on the identification of individual transcriptionally active proviral loci from which HERV RNA transcripts in different types of cancers originate. This strategy might downsize the complexity inherent in analyses of repetitive elements and enable to focus further characterizations, such as genetic or functional studies, to apparently relevant HERV loci. In some cases, HERV-encoded proteins have been detected by immunohistochemistry or immunoblot in different human cancers (see Table 1). While expression of HERV proteins in tumor tissue provides an argument for some functional significance, a crucial question remains whether such expression is just a chance event, perhaps resulting from deregulated gene expression in tumor cells, or directly contributes to human tumorigenesis.

HERVs and cancer: potential mechanisms

So, how might HERVs be active in tumorigenesis? Below we discuss possible mechanisms by which HERVs could contribute to the development of human cancer. An outline of potential mechanisms of HERVs in human tumorigenesis is provided in Figure 2.

Insertional mutagenesis

As detailed above, tumorigenic properties of ERVs in animal models frequently depend on retroviral movement, i.e. *de novo* formations of proviral copies in somatic cells of the host organism [4]. However, it must be emphasized that to date no replication-competent HERV has been identified in the human genome, and evidence for novel proviral HERV formations in present-day human beings is as yet lacking. The value of the findings originally obtained in animal models for human tumorigenesis has consequently been questioned [65]. While the issue of whether there are replication-competent HERV sequences in present-day human individuals remains unresolved, it continues to be of considerable interest in the context of a potential pathogenic role of HERVs in human disease.

Among all currently known HERV families, HERV-K(HML-2) (see also Fig. 1) is regarded to be the most probable candidate for replication-competent elements [6]. For that and other reasons, the HERV-K(HML-2) family has been the subject of numerous studies (for review, see [6, 66]). HERV-K(HML-2) has been dubbed the most 'active' HERV family, with new proviral integrations having occurred after the divergence of humans from chimpanzees, approximately 6 million years ago [34, 67, 68]. Some HERV-K(HML-2) insertions are only present in a proportion of human individuals, indicating that they are not yet fixed in the human population, and some of these elements appear to have invaded the human lineage quite recently in evolutionary terms (less than 200 000 years ago) [69, 70]. Remarkably, several proviral HERV-K insertions are almost or completely intact, i.e. they contain full-length intact ORFs for retroviral Gag, Pro, Pol, and Env proteins [69, 71–73]. Furthermore, HERV-K(HML-2) is currently the only HERV family that has conclusively been demonstrated to be capable of producing retrovirus-like particles [74–76]. Based on analyses of insertional polymorphism within the

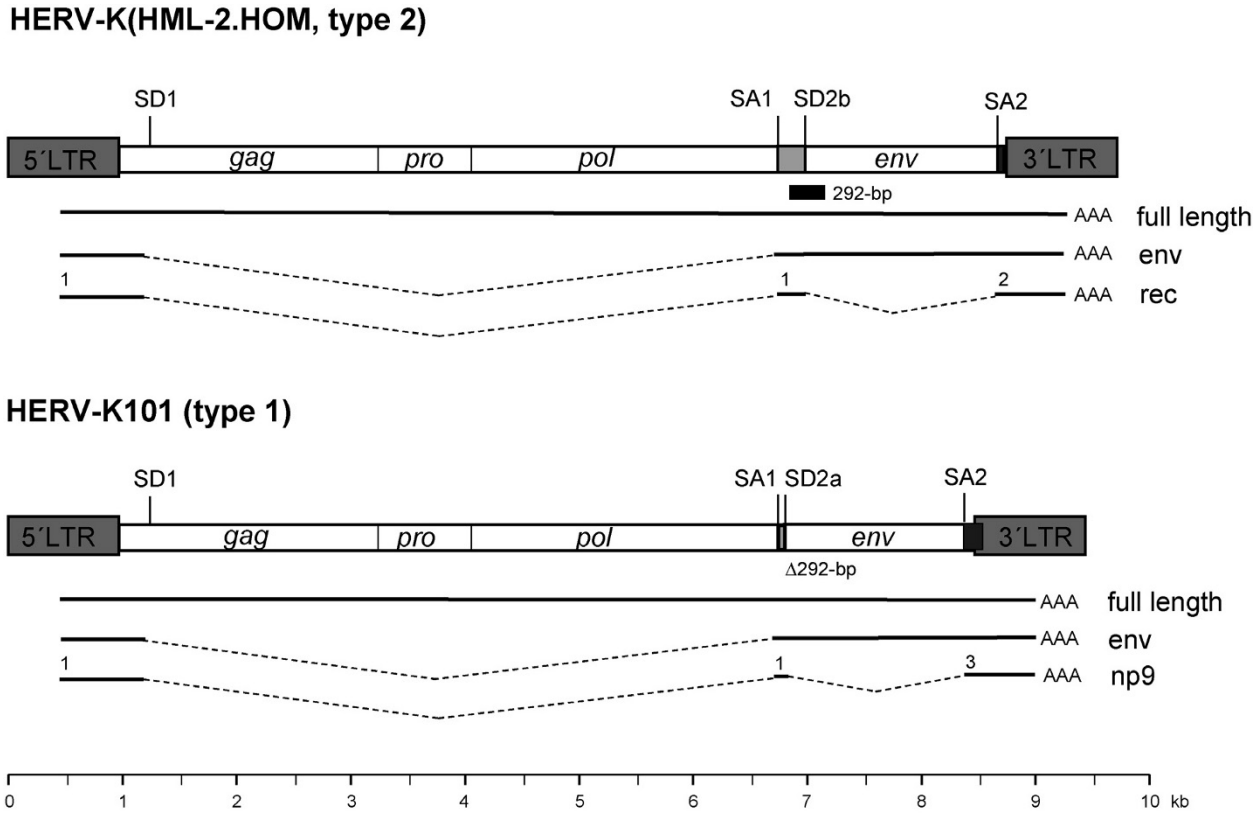

Figure 1. Schematic representation of full-length HERV-K(HML-2) type 1 and 2 proviruses and their respective mRNA transcripts. Location of 5′ and 3′ LTRs, *gag*, *pro*, *pol*, and *env* genes, and splice donor (SD) and splice acceptor (SA) sites are shown. There are two types of HERV-K (HML-2) proviruses in the human genome differing by the presence (type 2; black rectangle) or absence (type 1; Δ292-bp) of a 292-bp sequence at the *pol-env* boundary [119]. HERV-K(HML-2.HOM) is an example of a type 2 provirus, HERV-K101 an example of a type 1 provirus. The 292-bp sequence harbors a splice donor site (SD2b) involved in the generation of doubly spliced transcripts coding for the two exons (light grey and dark grey boxes) of the accessory ~15 kDa protein Rec by some type 2 proviruses [138, 139]. Rec is a functional homologue of the RNA-binding lentiviral nuclear export proteins Rev (HIV-1) and Rex (HTLV-1) [140]. In type 1 proviruses, an alternative splice donor (SD2a) located upstream of the 292-bp stretch is used to generate mRNAs coding for Np9 [54]. Np9 is an ~9 kDa, 74-amino acid protein that shares its 14 N-terminal amino acids (exon 1, light grey box) with Rec and Env, whereas the 60 C-terminal amino acids (exon 2, dark grey box) are derived from the third (non-Rec, non-Env) ORF. Numbers on top of *rec* and *np9* transcripts indicate the reading frames.

HERV-K(HML-2) family and the ratio of nonsynonymous to synonymous nucleotide changes as well as the acquisition of stop codons in HERV-K(HML-2) genes, HERV-K(HML-2) has been suggested to be still active in present-day humans and to most likely proliferate via re-infection, i.e. extracellular movement from one cell to another [34, 77]. However, while engineered HERV-K(HML-2) consensus sequences, or a chimeric construct of three recombined HERV-K(HML-2) proviruses, have indeed been shown to be infectious and to form new proviruses, a replication-competent 'natural' HERV-K(HML-2) allele could not be identified so far [30, 78]. Even the most complete HERV-K(HML-2) provirus known to date, HERV-K113 (which harbors full-length ORFs for all retroviral genes), appears to be replication-incompetent [79]. Nevertheless, absence of infectious HERVs in the published human genome sequences does not rule out that such elements exist. Indeed, Belshaw et al. proposed that there is a pool of HERV-K(HML-2) elements in the human germ line that is still active and infectious until today and that such elements may cause disease in individuals carrying them. Because these HERV-K(HML-2) elements may be deleterious to their hosts, they are unlikely to reach high allele frequencies in the human population as a whole. Only if an infectious HERV-K(HML-2) element acquires inactivating mutations it may, in its neutral state, reach high allele frequencies and eventually become fixed [34].

In conclusion, while indirect evidence seems suggestive of the existence of functional HERV-K(HML-2) alleles in humans [30, 34, 77, 80], direct experimental prove in support of that hypothesis is lacking. A search for such functional HERV-K(HML-2) elements might possibly concentrate on tumors in which HERV-K(HML-2) proteins and particles have been detected (e.g. germ cell tumors [GCT] or melanomas, see

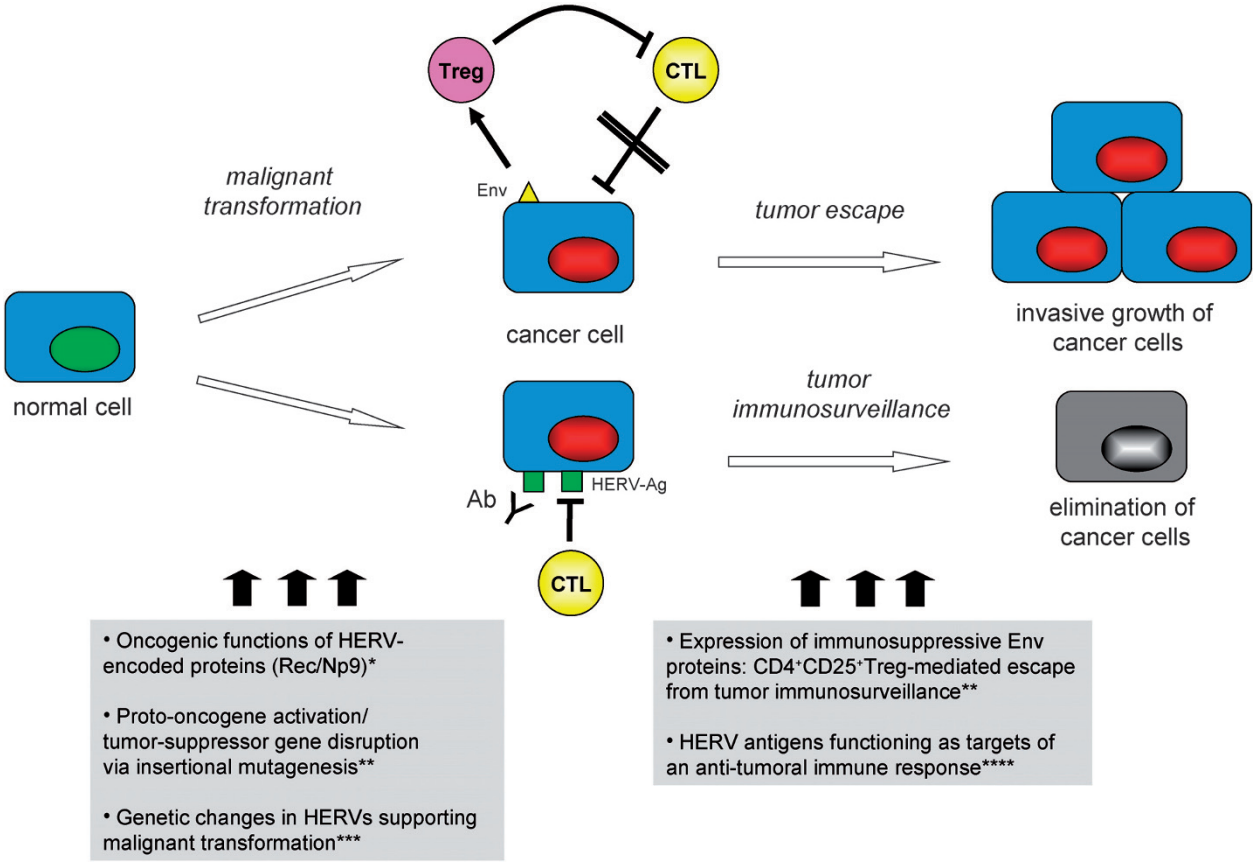

Figure 2. Possible mechanisms by which HERVs could be involved in tumorigenesis. A simplified scheme of tumorigenesis and potential fates of malignant cells is depicted to indicate steps in tumorigenesis in which HERVs may play a role. The process by which a normal cell (green nucleus) turns into a cancer cell (red nucleus) is referred to as malignant transformation. Possible mechanisms by which HERVs contribute to malignant transformation are listed. Malignant cells are normally controlled by the immune system ('tumor immunosurveillance'). Expression of HERV antigens (HERV-Ags, green squares) on tumor cells may elicit an antitumor immune response involving, among others, antibodies (Ab) and $CD8^+$ cytotoxic T cells (CTL). Ideally, antitumor immune responses result in the elimination of cancer cells. Expression of immunosuppressive endogenous retroviral envelope (Env, yellow triangle) proteins on tumor cells may lead to a $CD4^+CD25^+$ regulatory T cell (Treg)-mediated suppression of antitumor CTL, leading to escape of tumor cells from immunosurveillance ('tumor escape') and consecutive invasive cancer growth. The level of evidence of the outlined mechanisms for human cancers is indicated. * Some evidence for potential relevance in human cancer (germ cell tumors). ** Evidence in animal models, so far no evidence in humans. *** Hypothetical mechanism. **** Some evidence for anti-HERV immune responses in certain human cancers; very limited data concerning their functional relevance.

below). No doubt, demonstration of novel integrations of HERV elements, such as HERV-K(HML-2), in genomic DNA of distinct malign somatic cells along with corresponding empty pre-integration sites in genomic DNA of other somatic cells in the same human individual would provide strong evidence for a lingering mobility of some HERVs and possible ensuing pathogenic functions. Although the clonality of tumor cells may facilitate the detection of *de novo* proviral HERV integrations in the genome of tumor cells, this will certainly be no easy task, given the large number of highly similar elements within each HERV family already present in the human genome.

Oncogenic proteins encoded by HERVs

Among pathogenic mechanisms that do not necessitate HERV movement, direct oncogenic effects of HERV-encoded proteins have to be considered. Indeed, expression of the oncogenic protein Tax currently represents the most firmly established mechanism by which an exogenous retrovirus (HTLV-1) can induce cancer in humans [20, 21]. As for potentially oncogenic HERV proteins, two proteins from the HERV-K(HML-2) family, termed Rec and Np9, have been studied in more detail and will be discussed in the context of HERV-K(HML-2) in GCTs (see below).

As outlined above, recent work also suggests that retroviral Env proteins with fusogenic properties (e.g. Syncytin-1) might contribute to cell fusion events in

tumors [41, 42]. While it is conceivable that retroviral Env proteins may thus modulate the biological behaviour of cancer cells, phenotypical consequences of such cell fusions need to be further determined.

The Env protein of Jaagsiekte sheep retrovirus (JSRV), the etiologic agent of pulmonary adenocarcinoma, a contagious lung cancer of sheep, is an active oncogene whose expression is sufficient to transform cell lines *in vitro* and to induce lung cancer in sheep and mice *in vivo* [81, 82]. Intriguingly, a subtype of human pulmonary adenocarcinoma, called bronchoalveolar carcinoma (BAC), resembles lung cancers induced by JSRV in sheep. Such similarities provoked speculations that JSRV, or a related betaretrovirus, may play a role in human lung cancer [82]. While immunohistochemical studies revealed reactivity with an antiserum directed against the JSRV Gag protein in about 30 % of human BAC and lung adenocarcinoma samples [83], studies of JSRV DNA or RNA expression in human lung cancers generally do not support an association with JSRV [82]. This may indicate that the observed immunoreactivity with an antiserum against a betaretroviral Gag results from a cross-reactive human endogenous betaretrovirus, e.g. HERV-K (presumably HML-2) [84]. Of note, JSRV and HERV-K share a conserved motif in the N-terminal region of the surface (SU) domain of their Env proteins, which may suggest that these proteins use the same receptor, i.e. the putative tumor suppressor Hyal2 [7]. Since malignant transformation of human bronchial epithelial cells by JSRV Env appears to involve Hyal2 [85], it has been proposed that endogenous betaretroviral Env proteins could have oncogenic properties as well [7]. Nevertheless, these issues have not been addressed experimentally so far, and it is unknown whether endogenous betaretroviral Env proteins are expressed in human lung cancers.

Tumor immune escape mediated by immunosuppressive endogenous retroviral Env proteins

In the immunocompetent host, development of cancer is controlled by the immune system, a phenomenon known as cancer immunosurveillance. Consequently, the evasion from such immunological control mechanisms (termed tumor escape) very likely is an important step towards uncontrolled growth of transformed cells, eventually resulting in clinically detectable tumors [2].

Early studies established that the envelope transmembrane (TM) protein p15E of feline leukemia virus (FeLV) has immunosuppressive properties (for review, see [86]). Within the TM domain, a ~20-amino acid region is highly conserved among different retroviruses and a synthetic 17-amino-acid peptide (CKS-17) derived from this region has been shown to have immunosuppressive effects *in vitro* [87]. This suggests that the immunosuppressive portion of retroviral TM resides, at least partially, within this 17-amino acid sequence, also referred to as the immunosuppressive domain (ISD). ISDs are present in animal ERVs as well. They have also been identified in 10 out of 16 HERV elements containing complete *env* genes in the human genome, with only the putative 6 full-length Env proteins from the HERV-K(HML-2) family not harboring an ISD [60]. Immunosuppressive functions of animal and human endogenous retroviral Env proteins have essentially been implicated in two processes: induction of immune tolerance at the materno-fetal barrier via a physiological expression in the placenta [88] and suppression of an antitumoral immune response through aberrant expression in cancers. Somewhat reminiscent of the possibly context-dependent physiological and non-physiological consequences of cell fusions mediated by Syncytin-1, the immunosuppressive effects of ERV Env proteins, which might possibly serve an important physiological goal by induction of fetomaternal tolerance, could have deleterious consequences when active in tumors.

Indeed, in a series of pivotal studies, it has been elegantly shown that murine tumor cells that stably express transduced Env proteins of a Moloney murine leukemia virus (MoMLV), the simian retrovirus Mason-Pfizer monkey virus (MPMV), and a HERV (HERV-H), when injected into immunocompetent recipient mice, escape from rejection by the immune system, resulting in tumor growth *in vivo*. In contrast, in tumor cells transduced with irrelevant transmembrane proteins or empty vector, no enhanced tumor growth could be observed [89–91]. Using this *in vivo* tumor-rejection assay, it was also demonstrated that Syncytin-2, but not Syncytin-1, has immunosuppressive functions. Furthermore, the minimal ISD active *in vivo* could be delineated to a 20-amino acid stretch in the TM domain of MoMLV, which comprises the CKS-17 sequence. Comparison of those 20 amino acids in immunosuppressive and non-immunosuppressive Env proteins enabled identification of key amino acid residues responsible for the absence or presence of immunosuppressive activity in Syncytin-1 and Syncytin-2 [40]. The relevance of expressed ERVs in cancer cells for promotion of tumor growth via subversion of cancer immunosurveillance is also suggested by a study using B16 murine melanoma cells of C57Bl/6 origin, which spontaneously produce the endogenous melanoma-associated retrovirus (MelARV) [92]. Knocking down expression of MelARV in B16 melanoma cells by RNA interference led to rejection of these cells in immunocompetent mice,

while control cells developed into lethal tumors. This effect could be partially reverted by re-expressing the MelARV *env* gene in MelARV knockdown B16 melanoma cells, again indicating that tumor escape is mediated by Env [93]. In this model, tumor escape appears to depend on regulatory $CD4^+CD25^+$ T cells (Tregs), since adoptive transfer of Tregs from mice engrafted with B16 wild-type cells to mice engrafted with B16 MelARV knockdown cells enhanced the growth of B16 MelARV knockdown cells to levels of B16 wild-type cells [93]. Such Tregs likely contribute to tumor growth through suppression of cellular antitumor immune reponses [94]. However, it will be interesting to further clarify the molecular mechanisms involved in the interaction of retroviral Env proteins with Tregs. In addition, immunosuppressive properties of endogenous retroviral Env proteins may also inhibit humoral immune responses: mice injected with recombinant ectodomains of Syncytin-1 and Syncytin-2 revealed 10- to 30-fold higher IgG titers against the non-immunosuppressive than against the immunosuppressive variant of each ectodomain [40]. Remarkably, results strikingly similar to those from the B16 murine melanoma line could also be obtained with the Neuro-2a cell line derived from a spontaneous A/J mouse neuoblastoma which produces a functional ERV (NeRV) [32]. Knockdown of NeRV in Neuro-2a cells likewise resulted in reduced tumor growth in A/J immunocompetent mice. This effect was again mediated by the immune system, as NeRV knockdown Neuro-2a cells showed growth rates similar to Neuro-2a control cells in X-irradiated mice. Altogether, these examples from animal models provide rather compelling evidence that ERVs, even if they do not participate in the process of malignant transformation proper, may directly promote tumor progression by subverting tumor immunosurveillance via expression of immunosuppressive Env proteins. Obviously, an important question is whether such mechanisms may also be active in human cancer. Surprisingly little is known so far about the expression of endogenous retroviral Env proteins containing ISDs in human tumors (see Table 1). In contrast, a growing body of evidence indicates that Tregs can impair antitumor immunity in human cancers and thus promote tumor growth (for review, see [95]). Future studies may therefore clarify whether immunosuppressive HERV Env proteins are expressed in human cancers and whether such expression may, by analogy to the murine B16 melanoma model [93], be mechanistically linked to Treg-mediated tumor immune escape. This could open the possibility for novel therapeutic strategies directed not only against Tregs [96, 97] but also against immunosuppressive HERV Env proteins.

A role for polymorphic HERVs?

Even if tumorigenic effects of HERV proteins should eventually be corroborated in human malignancies, a conceptual problem might be posed by the fact that integration sites of HERVs are largely identical in all human individuals [65, 98]. In other words, if all humans harbor the same HERVs, why should proteins encoded by those elements contribute to tumorigenesis only in the proportion of humans that actually develop cancers? However, pitfalls of that argument are underlined by many examples of proto-oncogenes and tumor suppressor genes that are present in the human genome and yet contribute to tumorigenesis only in specific instances. In addition, it has been proposed that polymorphic HERVs (HERV insertions present in the genome of only a proportion of human individuals) may explain a more selective association of HERVs with certain diseases [98]. Insertional polymorphisms have so far essentially been described in the HERV-K(HML-2) family, with currently more than 15 polymorphic loci known [34, 69, 70, 99]. In an investigation of two polymorphic HERV-K(HML-2) loci, HERV-K113 and HERV-K115, in 102 patients with breast cancer and 102 controls, no significant difference in the frequency of HERV-K113 and HERV-K115 was observed between the two groups [100]. We have determined the frequencies of HERV-K113 and HERV-K115 in patients with seminomas ($n = 27$) and healthy male controls ($n = 80$). HERV-K113 was present in 3/27 patients and 15/80 controls ($p = 0.4$; two-tailed Fisher's exact test) and HERV-K115 was present in 2/27 patients and 6/80 controls ($p = 1$; two-tailed Fisher's exact test) [J. Klatt and M. Sauter, unpublished]. These data similarly refute an association of HERV-K113 or HERV-K115 with seminomas. Of further note, an initial report on an association of HERV-K113 with multiple sclerosis [101] could not be confirmed in a subsequent larger study [102], pointing towards the need for larger studies to clarify associations of polymorphic HERVs with disease. Given the fact that, depending on ethnicity, certain polymorphic elements may be present in up to 30% of human individuals [69], any overt pathogenic effects of these polymorphic insertions overall appear rather unlikely. A typical human cancer contains about 80 mutations leading to amino acid exchanges in protein encoding genes. Whereas only ~15 of these mutations, called 'drivers', are likely to be responsible for tumor initiation, maintenance, and progression, the remaining ~65 mutations represent harmless 'passengers' [1]. In theory, there might be mutated HERVs in tumor cells that could possibly function as drivers. However, no experimental data concerning this issue are available to date. Also, several single-nucleotide poly-

morphisms (SNPs) have been described in HERVs, and some of them are located in protein-encoding HERV genes [37, 103]. Nevertheless, whether these SNPs are of any functional significance in a pathologic context is as yet unknown.

HERV-encoded tumor antigens: potential targets of tumor immunosurveillance

HERVs may be involved in human cancer as targets of an antitumor immune response. Indeed, the de-differentiated status of tumor cells may lead to the production of endogenous retroviral proteins or epitopes that are normally not expressed in healthy human tissues. A dysregulated expression of HERVs may, for instance, be related to hypomethylation of HERV genes in tumor cells [104, 105]. Such tumor-associated aberrantly expressed HERV epitopes could elicit an antitumor immune response when exposed to the immune system. In accord with this notion, antibodies against HERV-K(HML-2) Gag and Env have been detected in ~50–80% of patients with GCTs [106–108]. Interestingly, the proportion of antibody-positive patients declined considerably (~10%) after tumor removal [107] and antibody titers also appear to decrease upon tumor remission under chemotherapy [108, 109]. Antibodies against HERV-K(HML-2) Gag and Env are also present in less than 5% of healthy individuals, but such individuals did not produce antibodies against the TM portion of HERV-K(HML-2) Env, whereas anti-TM antibodies were universally found in GCT patients [109]. This may indicate that an HERV-K(HML-2) Env protein containing an immunogenic TM domain is aberrantly expressed in GCTs. Interestingly, antibodies against the TM portion of HERV-K(HML-2) Env are also produced in a proportion of patients with melanomas [110]. Nevertheless, the functional significance of these findings, i.e. whether the production of anti-HERV-K(HML-2) antibodies may contribute to the immunological control of tumor growth, currently remains elusive.

We have searched for antibodies against Syncytin-1 in sera from patients with different types of cancer (including breast cancer) and autoimmune diseases [M. Sauter and N. Mueller-Lantzsch, in preparation] [111]. Antibodies against Syncytin-1 were detected in less than 1% of individuals studied, and no associations with specific diseases could be observed. This suggests that Syncytin-1, which can be regarded as a physiologically expressed placental self-antigen, might be immunologically tolerated, whereas other HERV proteins (e.g. HERV-K[HML-2] Env when expressed in GCTs) are not. The mechanisms underlying immunological tolerance, a potential loss-of-tolerance, and the immunogenicity of different HERV proteins are only incompletely understood and deserve further study.

The first evidence that HERV-encoded peptides expressed in human tumor cells can also be targets for antitumor cytotoxic $CD8^+$ T cell (CTL) responses came from a study that identified a HERV-K(HML-6)-encoded HLA-A2-restricted peptide (termed HERV-K-Mel) which was recognized in autologous melanoma cells by CTLs from two patients with melanomas [64]. This peptide is encoded by a very short ORF within an otherwise highly defective HML-6 provirus on chromosome 16. This is of interest as it indicates that also truncated proteins produced from short HERV ORFs may in specific circumstances exert a biologically significant function. HERV-K-MEL RNA is not expressed in normal tissues with the exception of testis and some skin samples, but was found in a majority of cutaneous and ocular melanoma samples, suggesting that it might be a possible target for immunotherapeutic approaches. Second, CTLs against HERV-K Gag-derived peptides have been detected in a proportion of patients with a history of seminoma and a minority of healthy individuals [112]. Finally, a third study has recently characterized a HLA-A11-restricted 10-mer peptide (termed CT-RCC-1) which appears to be targeted by donor lymphocytes in patients undergoing nonmyeloablative hematopoietic stem cell transplantation for metastatic renal cell carcinoma, consistent with a graft-versus-tumor reaction [113]. The CT-RCC-1 peptide is encoded by an HERV-E locus on chromosome 6q whose RNA is expressed in renal cell carcinomas, but not in normal kidney and other normal human tissues. Expansion of CTLs recognizing CT-RCC-1 in a patient with prolonged regression of the metastatic renal cell carcinoma seems compatible with a possible involvement of such CTLs in tumor regression [113].

In summary, current evidence suggests that HERV proteins may function as tumor antigens, which implies that by eliciting an antitumoral immune response HERVs could presumably participate in tumor biology also in a beneficial way. Additionally, such immune responses may have the potential to serve as tumor markers. Further studies are needed to clarify the functional consequences of HERV-directed immune responses in terms of tumor growth control and the clinical usefulness of such immune responses as tumor markers.

Specific examples of HERVs in tumorigenesis

Although HERVs have tentatively been involved in quite a number of specific tumor entities, research has

especially focussed on two types of human cancers, GCTs and melanomas, which are discussed in more detail below.

HERV-K (HML-2) and testicular germ cell tumors

Testicular GCTs represent the most common solid tumors among young men in western industrialized countries and are thought to be derived from cells in the germ-cell lineage that are blocked in maturation. GCT in adults may initiate already in primordial germ cells during fetal development. Histologically, GCTs are classified into two main subtypes: seminomas and a group collectively referred to as non-seminomas. Both types progress through a non-invasive precursor lesion, called carcinoma *in situ* [114]. Early electron microscopical studies which had revealed the presence of particles with retroviral morphology budding from human placental trophoblasts [115] prompted investigations on retrovirus production in human tumors that contain embryonal or placental tissues [116]. This resulted in the detection of retrovirus-like particles in several cell lines derived from human GCTs [117, 118]. Consequently, such particles were designated human teratocarcinoma-derived virus (HTDV) particles, but re-named HTDV/HERV-K after they were found to be encoded by HERV-K(HML-2) [74, 119] (for review see [66]). HTDV/HERV-K particles from all different GCT lines analyzed so far seem to be defective, displaying either an immature morphology and/or lacking surface spikes [75, 120]. Accordingly, attempts to prove their infectivity have failed up to now [116]. Almost 30 years after their initial discovery, the significance, if any, of these particles for the development of GCTs remains unknown. Still, an association of HERV-K(HML-2) with GCTs was further supported by the detection of HERV-K(HML-2) mRNA and proteins (see Table 1) in GCT tissue and cell lines and by a specific immune response against HERV-K(HML-2) Gag and Env proteins in GCT patients (see above) [57, 74, 106–110, 120, 121]. Subsequent investigations on the functional relevance of HERV-K(HML-2) for GCT tumorigenesis especially concentrated on two accessory HERV-K(HML-2) proteins, Rec and Np9 (Fig. 1). As of yet, HERV-K(HML-2) is the only HERV family that has been shown to code for such accessory proteins [6].

An oncogenic potential of Rec was first suggested by experiments demonstrating that HERV-K(HML-2) *rec*- (but not *gag*-, *env*-, or empty vector-) transduced Rat-1 cells grew into tumors following injection in nude mice [122]. In contrast, Rat-1 fibroblasts transduced with HERV-K(HML-2) *rec*, *gag*, *env*, or an empty vector showed similar growth chracteristics *in vitro*. The mechanisms underlying altered proliferation of *rec*-transduced cells *in vivo* but not *in vitro* are not clear, but likely rely on the interaction with host factors. Strong evidence that the expression of Rec may directly contribute to the development of GCT *in vivo* was provided by a study on transgenic mice that inducibly express Rec. Such mice show disturbed germ cell development and exhibit, by 19 months of age, changes reminiscent of carcinoma *in situ*, the predecessor lesion of GCTs in humans [121]. To begin to unravel the molecular mechanisms underlying these oncogenic effects, proteins interacting with Rec were searched for. This led to the identification of the promyelocytic leukemia zinc finger protein (PLZF) as a protein interaction partner of Rec [122] as well as of Np9 [123]. PLZF is a tumor suppressor and transcriptional repressor of the c-*myc* proto-oncogene and, intriguingly, also plays an essential role in the regulation of spermatogonial stem cell maintenance in mice [124, 125]. Co-expression of Rec and Np9 with PLZF abrogated the transcriptional repression of the c-*myc* gene promotor by PLZF and resulted in c-Myc overproduction, leading to upregulation of c-Myc regulated genes like p53, PCNA and IκBα. Accordingly, cells stably transfected with PLZF and Rec showed increased cell proliferation and a reduced apoptosis rate compared to cells stably transfected with PLZF alone (Fig. 3) [123].

In a survey of normal and malignant cells, *np9* transcripts were exclusively detected among malignant cells [54]. Regarding possible functional consequences of Np9 expression in tumors, Np9 (but not Rec) was found to bind to and functionally interfere with the ligand of Numb protein X (LNX), a RING-Type E3 ubiquitin ligase that regulates the transcription factor Notch via degradation of the Notch-antagonist Numb [126]. The Numb/Notch pathway is an essential part of proproliferative Ras signalling and has also been suggested to be involved in GCTs by causing dysfunction of the mitotic/meiotic switch and subsequent genetic instability [127].

In summary, these data are compatible with the idea that Rec and Np9 may act as oncoproteins in GCTs via inhibition of the tumor suppressor and spermatogonial stem cell regulator PLZF, and possibly also, in the case of Np9, through interference with the Numb/Notch pathway. Although the studies on Rec and Np9 provide strong hints for a contribution of HERVs in GCT development, it is unknown whether Rec and Np9 are causally involved in GCTs in humans and which factors induce or regulate the expression of these HERV-K(HML-2) accessory proteins in GCTs.

HERV-K and melanoma

Melanoma is the most malignant type of skin cancer in humans and arises from pigment-producing melano-

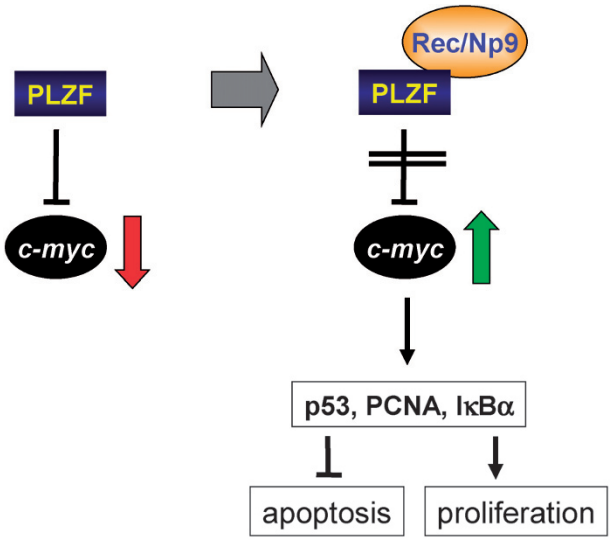

Figure 3. Oncogenic effects of Rec or Np9 may be mediated by interaction with the promyelocytic leukemia zinc finger protein (PLZF). PLZF is a stem cell regulator and tumor suppressor that represses transcription of the c-*myc* proto-oncogene (left side) [124, 125]. The HERV-K(HML-2) accessory proteins Rec and Np9 can both bind to PLZF [122, 123]. This binding abrogates the transcriptional repression of the c-*myc* gene promotor by PLZF, resulting in c-Myc overproduction, which in turn leads to upregulation of c-Myc-regulated genes, like p53, PCNA and IκBα. Phenotypically, this is associated with increased proliferation and reduced apoptosis of cells stably co-expressing Rec and PLZF compared to cells stably expressing PLZF alone [123].

cytes in the epidermis. Reports dating back to the 1970 s have described retrovirus-like particles in human melanomas [128, 129]. Several more recent studies have detected HERV-K(HML-2) mRNA and proteins in human primary melanomas, lymph node metastases from melanomas, and melanoma cell lines (Table 1), but not in melanocytes or normal lymph nodes [110, 130, 131]. Furthermore, antibodies against HERV-K(HML-2) Env protein could be detected by Western blot [110] and ELISA [132] in a proportion of melanoma patients. As discussed above, a peptide encoded by a different HERV-K subfamily, HML-6, was recognized on melanoma cells by CTL [64]. In addition, retroviral particles purified from the melanoma cell line SK-Mel28 were identified by electron microscopy and shown to contain HERV-K(HML-2) Env and partially processed Gag proteins [130]. This indicates that, similar to GCT cell lines, melanoma cell lines may produce HERV-K-encoded particles. Moreover, particles derived from the melanoma cell line 518A2 were reported to infect bovine MDBK cells, suggesting that 518A2 cells might harbor an infectious variant of HERV-K (termed melanoma ERV [MERV]) [130]. Retroviral particles from melanoma cells contain a variety of different HERV-K RNA sequences [133]; however, a distinct MERV clone capable of infecting cell cultures *in vitro* has so far not been described. Also, retroviral particles derived from SK-MEL28 melanoma cells did not infect MDBK cells [110]. The issue of an infectious HERV-K variant in human melanomas therefore appears to require further scrutiny.

While studies of murine melanomas clearly suggest a role of ERVs in this type of cancer in mice (see above) [33, 93], recent work also argues for an oncogenic function of HERV-K(HML-2) in human melanomas [134]. A-375 human melanoma cells that were stably transduced with a construct expressing an siRNA sequence targeted against HERV-K(HML-2) *gag* did not show a change of proliferation and differentiation compared to wild-type or control-transduced A-375 cells *in vitro*. However, upon injection in nude mice, tumor growth of HERV-K(HML-2) knock-down A-375 cells was reduced compared to wild-type A-375 cells or to control-transduced A-375 cells. It will be interesting to analyze in more detail the mechanisms underlying this reduced tumor growth *in vivo*.

Conclusion

In summary, cumulative evidence from animal models clearly indicates that ERVs may be involved in the process of tumorigenesis at various levels. The potential role of HERVs in human cancer, however, appears more complex. To date, evidence for a role of HERVs in human cancers (and other diseases) mediated by insertional mutagenesis is lacking. In contrast, and as exemplified by oncogenic properties of HERV-K(HML-2) accessory proteins Rec and Np9, available data are compatible with the idea that HERVs may contribute to human cancers, in particular GCTs and melanomas, by virtue of HERV-encoded oncoproteins. Much remains to be learnt, though, about the regulation and effector pathways of suspected HERV-encoded oncoproteins. Given the strong evidence for growth promotion of tumors by immmunosuppressive endogenous retroviral Env proteins in animals, it is tempting to speculate that similar mechanisms may also operate in human cancers. Future studies should clarify this issue. Whereas a causative role *sensu strictu* of HERVs in human tumors has not been demonstrated up to now, it seems plausible that certain HERVs may act as distinct co-factors in the complex multi-step processes leading to human cancers. Further unravelling of these contributions may provide important new insights not only into the biology of human cancer but also into the peculiar evolutionary interplay between HERVs and their human hosts.

1 Wood, L. D., Parsons, D. W., Jones, S., Lin, J., Sjoblom, T., Leary, R. J., Shen, D., Boca, S. M., Barber, T., Ptak, J. et al. (2007) The genomic landscapes of human breast and colorectal cancers. Science 318, 1108–1113.
2 Dunn, G. P., Old, L. J. and Schreiber, R. D. (2004) The immunobiology of cancer immunosurveillance and immunoediting. Immunity 21, 137–148.
3 Vogt, P. K. (1997) Historical introduction to the general properties of retroviruses. In: Retroviruses, pp. 1–25, Coffin, J. M., Hughes, S. H. and Varmus, H. E. (eds.), Cold Spring Harbour Laboratory Press, New York.
4 Boeke, J. D. and Stoye, J. P. (1997) Retrotransposons, endogenous retroviruses, and the evolution of retroelements. In: Retroviruses, pp. 343–435, Coffin, J. M., Hughes, S. H. and Varmus, H. E. (eds.), Cold Spring Harbour Laboratory Press, New York.
5 de Parseval, N. and Heidmann, T. (2005) Human endogenous retroviruses: from infectious elements to human genes. Cytogenet. Genome Res. 110, 318–332.
6 Bannert, N. and Kurth, R. (2004) Retroelements and the human genome: new perspectives on an old relation. Proc. Natl. Acad. Sci. USA 101 Suppl. 2, 14572–14579.
7 Blomberg, J., Ushameckis, D. and Jern, P. (2005) Evolutionary aspects of human endogenous retroviral sequences (HERVs) and disease. In: Retroviruses and Primate Genome Evolution, pp. 204–238, Sverdlov, E. D. (ed.), Eurekah.com.
8 Löwer, R. (1999) The pathogenic potential of endogenous retroviruses: facts and fantaisies. Trends Microbiol. 7, 350–356.
9 Nakagawa, K. and Harrison, L. C. (1996) The potential roles of endogenous retroviruses in autoimmunity. Immunol. Rev. 152, 193–236.
10 Martin, M. A., Bryan, T., Rasheed, S. and Khan, A. S. (1981) Identification and cloning of endogenous retroviral sequences present in human DNA. Proc. Natl. Acad. Sci. USA 78, 4892–4896.
11 Callahan, R., Drohan, W., Tronick, S. and Schlom, J. (1982) Detection and cloning of human DNA sequences related to the mouse mammary tumor virus genome. Proc. Natl. Acad. Sci. USA 79, 5503–5507.
12 Ellermann, V. and Bang, O. (1908) Experimentelle Leukämie bei Hühnern. Zentralbl. Bakteriol. Parasitenkd. Infectionskr. Hyg. Abt. Orig. 46, 595–609.
13 Rous, P. (1911) A sarcoma of the fowl transmissible by an agent separable from the tumor cells. J. Exp. Med. 13, 397–411.
14 Bittner, J. J. (1936) Some possible effects of nursing on the mammary gland tumour incidence in mice. Science 84, 162.
15 Weiss, R., Teich, N., Varmus, H. and Coffin, J. M. (1984) RNA tumor viruses. Cold Spring Harbor Laboratory Press, New York.
16 Rosenberg, N. and Jolicoeur, P. (1997) Retroviral Pathogenesis. In: Retroviruses, pp. 475–585, Coffin, J. M., Hughes, S. H. and Varmus, H. (eds.), Cold Spring Harbor Laboratory Press, New York.
17 Poiesz, B. J., Ruscetti, F. W., Gazdar, A. F., Bunn, P. A., Minna, J. D. and Gallo, R. C. (1980) Detection and isolation of type C retrovirus particles from fresh and cultured lymphocytes of a patient with cutaneous T-cell lymphoma. Proc. Natl. Acad. Sci. USA 77, 7415–7419.
18 Robert-Guroff, M., Nakao, Y., Notake, K., Ito, Y., Sliski, A. and Gallo, R. C. (1982) Natural antibodies to human retrovirus HTLV in a cluster of Japanese patients with adult T cell leukemia. Science 215, 975–978.
19 Gessain, A., Barin, F., Vernant, J. C., Gout, O., Maurs, L., Calendar, A. and de The, G. (1985) Antibodies to HTLV-1 in patients with tropical spastic paraparesis. Lancet 2, 407–410.
20 zur Hausen, H. (2006) Infections Causing Human Cancer, Wiley-VCH, Weinheim, pp. 306–337.
21 Grassmann, R., Aboud, M. and Jeang, K. T. (2005) Molecular mechanisms of cellular transformation by HTLV-1 Tax. Oncogene 24, 5976–5985.
22 Hasegawa, H., Sawa, H., Lewis, M. J., Orba, Y., Sheehy, N., Yamamoto, Y., Ichinohe, T., Tsunetsugu-Yokota, Y., Katano, H., Takahashi, H. et al. (2006) Thymus-derived leukemia-lymphoma in mice transgenic for the Tax gene of human T-lymphotropic virus type, I. Nat. Med. 12, 466–472.
23 Shiramizu, B., Herndier, B. G. and McGrath, M. S. (1994) Identification of a common clonal human immunodeficiency virus integration site in human immunodeficiency virus-associated lymphomas. Cancer Res. 54, 2069–2072.
24 Killebrew, D. and Shiramizu, B. (2004) Pathogenesis of HIV-associated non-Hodgkin lymphoma. Curr. HIV Res. 2, 215–221.
25 Voisset, C., Weiss, R. A. and Griffiths, D. J. (2008) Human RNA 'rumor' viruses: the search for novel human retroviruses in chronic disease. Microbiol. Mol. Biol. Rev. 72, 157–196.
26 Urisman, A., Molinaro, R. J., Fischer, N., Plummer, S. J., Casey, G., Klein, E. A., Malathi, K., Magi-Galluzzi, C., Tubbs, R. R., Ganem, D. et al. (2006) Identification of a novel Gammaretrovirus in prostate tumors of patients homozygous for R462Q RNASEL variant. PLoS Pathog. 2, e25.
27 Dong, B., Kim, S., Hong, S., Das Gupta, J., Malathi, K., Klein, E. A., Ganem, D., Derisi, J. L., Chow, S. A. and Silverman, R. H. (2007) An infectious retrovirus susceptible to an IFN antiviral pathway from human prostate tumors. Proc. Natl. Acad. Sci. USA 104, 1655–1660.
28 Goff, S. P. (2004) Retrovirus restriction factors. Mol. Cell 16, 849–859.
29 Esnault, C., Heidmann, O., Delebecque, F., Dewannieux, M., Ribet, D., Hance, A. J., Heidmann, T. and Schwartz, O. (2005) APOBEC3G cytidine deaminase inhibits retrotransposition of endogenous retroviruses. Nature 433, 430–433.
30 Lee, Y. N. and Bieniasz, P. D. (2007) Reconstitution of an infectious human endogenous retrovirus. PLoS Pathog. 3, e10.
31 Fan, H. (1997) Leukemogenesis by Moloney murine leukemia virus: a multistep process. Trends Microbiol. 5, 74–82.
32 Pothlichet, J., Heidmann, T. and Mangeney, M. (2006) A recombinant endogenous retrovirus amplified in a mouse neuroblastoma is involved in tumor growth in vivo. Int. J. Cancer 119, 815–822.
33 Pothlichet, J., Mangeney, M. and Heidmann, T. (2006) Mobility and integration sites of a murine C57BL/6 melanoma endogenous retrovirus involved in tumor progression in vivo. Int. J. Cancer 119, 1869–1877.
34 Belshaw, R., Dawson, A. L., Woolven-Allen, J., Redding, J., Burt, A. and Tristem, M. (2005) Genomewide screening reveals high levels of insertional polymorphism in the human endogenous retrovirus family HERV-K(HML2): implications for present-day activity. J. Virol. 79, 12507–12514.
35 Wang, T., Zeng, J., Lowe, C. B., Sellers, R. G., Salama, S. R., Yang, M., Burgess, S. M., Brachmann, R. K. and Haussler, D. (2007) Species-specific endogenous retroviruses shape the transcriptional network of the human tumor suppressor protein p53. Proc. Natl. Acad. Sci. USA 104, 18613–18618.
36 Spencer, T. E., Mura, M., Gray, C. A., Griebel, P. J. and Palmarini, M. (2003) Receptor usage and fetal expression of ovine endogenous betaretroviruses: implications for coevolution of endogenous and exogenous retroviruses. J. Virol. 77, 749–753.
37 Mallet, F., Bouton, O., Prudhomme, S., Cheynet, V., Oriol, G., Bonnaud, B., Lucotte, G., Duret, L. and Mandrand, B. (2004) The endogenous retroviral locus ERVWE1 is a bona fide gene involved in hominoid placental physiology. Proc. Natl. Acad. Sci. USA 101, 1731–1736.
38 Blond, J. L., Lavillette, D., Cheynet, V., Bouton, O., Oriol, G., Chapel-Fernandes, S., Mandrand, B., Mallet, F. and Cosset, F. L. (2000) An envelope glycoprotein of the human endogenous retrovirus HERV-W is expressed in the human placenta and fuses cells expressing the type D mammalian retrovirus receptor. J. Virol. 74, 3321–3329.

39 Blaise, S., de Parseval, N., Benit, L. and Heidmann, T. (2003) Genomewide screening for fusogenic human endogenous retrovirus envelopes identifies syncytin 2, a gene conserved on primate evolution. Proc. Natl. Acad. Sci. USA 100, 13013–13018.

40 Mangeney, M., Renard, M., Schlecht-Louf, G., Bouallaga, I., Heidmann, O., Letzelter, C., Richaud, A., Ducos, B. and Heidmann, T. (2007) Placental syncytins: Genetic disjunction between the fusogenic and immunosuppressive activity of retroviral envelope proteins. Proc. Natl. Acad. Sci. USA 104, 20534–20539.

41 Bjerregaard, B., Holck, S., Christensen, I. J. and Larsson, L. I. (2006) Syncytin is involved in breast cancer-endothelial cell fusions. Cell. Mol. Life Sci. 63, 1906–1911.

42 Strick, R., Ackermann, S., Langbein, M., Swiatek, J., Schubert, S. W., Hashemolhosseini, S., Koscheck, T., Fasching, P. A., Schild, R. L., Beckmann, M. W. et al. (2007) Proliferation and cell-cell fusion of endometrial carcinoma are induced by the human endogenous retroviral Syncytin-1 and regulated by TGF-beta. J. Mol. Med. 85, 23–38.

43 Duelli, D. and Lazebnik, Y. (2003) Cell fusion: a hidden enemy? Cancer Cell 3, 445–448.

44 Antony, J. M., van Marle, G., Opii, W., Butterfield, D. A., Mallet, F., Yong, V. W., Wallace, J. L., Deacon, R. M., Warren, K. and Power, C. (2004) Human endogenous retrovirus glycoprotein-mediated induction of redox reactants causes oligodendrocyte death and demyelination. Nat. Neurosci. 7, 1088–1095.

45 Yi, J. M., Kim, H. M. and Kim, H. S. (2004) Expression of the human endogenous retrovirus HERV-W family in various human tissues and cancer cells. J. Gen. Virol. 85, 1203–1210.

46 Yi, J. M., Kim, H. M. and Kim, H. S. (2006) Human endogenous retrovirus HERV-H family in human tissues and cancer cells: expression, identification, and phylogeny. Cancer Lett. 231, 228–239.

47 Wentzensen, N., Coy, J. F., Knaebel, H. P., Linnebacher, M., Wilz, B., Gebert, J. and von Knebel Doeberitz, M. (2007) Expression of an endogenous retroviral sequence from the HERV-H group in gastrointestinal cancers. Int. J. Cancer 121, 1417–1423.

48 Frank, O., Verbeke, C., Schwarz, N., Mayer, J., Fabarius, A., Hehlmann, R., Leib-Mosch, C. and Seifarth, W. (2008) Variable transcriptional activity of endogenous retroviruses in human breast cancer. J. Virol. 82, 1808–1818.

49 Hu, L., Hornung, D., Kurek, R., Ostman, H., Blomberg, J. and Bergqvist, A. (2006) Expression of human endogenous gammaretroviral sequences in endometriosis and ovarian cancer. AIDS Res. Hum. Retroviruses 22, 551–557.

50 Ono, M., Kawakami, M. and Ushikubo, H. (1987) Stimulation of expression of the human endogenous retrovirus genome by female steroid hormones in human breast cancer cell line T47D. J. Virol. 61, 2059–2062.

51 Tomita, N., Horii, A., Doi, S., Yokouchi, H., Ogawa, M., Mori, T. and Matsubara, K. (1990) Transcription of human endogenous retroviral long terminal repeat (LTR) sequence in a lung cancer cell line. Biochem. Biophys. Res. Commun. 166, 1–10.

52 Brodsky, I., Foley, B. and Gillespie, D. (1993) Expression of human endogenous retrovirus (HERV-K) in chronic myeloid leukemia. Leuk. Lymphoma 11 Suppl. 1, 119–123.

53 Depil, S., Roche, C., Dussart, P. and Prin, L. (2002) Expression of a human endogenous retrovirus, HERV-K, in the blood cells of leukemia patients. Leukemia 16, 254–259.

54 Armbruester, V., Sauter, M., Krautkraemer, E., Meese, E., Kleiman, A., Best, B., Roemer, K. and Mueller-Lantzsch, N. (2002) A novel gene from the human endogenous retrovirus K expressed in transformed cells. Clin. Cancer Res. 8, 1800–1807.

55 Wang-Johanning, F., Frost, A. R., Jian, B., Epp, L., Lu, D. W. and Johanning, G. L. (2003) Quantitation of HERV-K env gene expression and splicing in human breast cancer. Oncogene 22, 1528–1535.

56 Lindeskog, M. and Blomberg, J. (1997) Spliced human endogenous retroviral HERV-H env transcripts in T-cell leukaemia cell lines and normal leukocytes: alternative splicing pattern of HERV-H transcripts. J. Gen. Virol. 78, 2575–2585.

57 Herbst, H., Sauter, M. and Mueller-Lantzsch, N. (1996) Expression of human endogenous retrovirus K elements in germ cell and trophoblastic tumors. Am. J. Pathol. 149, 1727–1735.

58 Patzke, S., Lindeskog, M., Munthe, E. and Aasheim, H. C. (2002) Characterization of a novel human endogenous retrovirus, HERV-H/F, expressed in human leukemia cell lines. Virology 303, 164–173.

59 Katzourakis, A., and Tristem, M. (2005) Phylogeny of human endogenous and exogenous retroviruses. In: Retroviruses and Primate Genome Evolution, pp. 186–203, Sverdlov, E. D. (ed.), Eurekah.com.

60 de Parseval, N., Lazar, V., Casella, J. F., Benit, L. and Heidmann, T. (2003) Survey of human genes of retroviral origin: identification and transcriptome of the genes with coding capacity for complete envelope proteins. J. Virol. 77, 10414–10422.

61 Villesen, P., Aagaard, L., Wiuf, C. and Pedersen, F. S. (2004) Identification of endogenous retroviral reading frames in the human genome. Retrovirology 1, 32.

62 Seifarth, W., Frank, O., Zeilfelder, U., Spiess, B., Greenwood, A. D., Hehlmann, R. and Leib-Mosch, C. (2005) Comprehensive analysis of human endogenous retrovirus transcriptional activity in human tissues with a retrovirus-specific microarray. J. Virol. 79, 341–352.

63 Stauffer, Y., Theiler, G., Sperisen, P., Lebedev, Y. and Jongeneel, C. V. (2004) Digital expression profiles of human endogenous retroviral families in normal and cancerous tissues. Cancer Immun. 4, 2.

64 Schiavetti, F., Thonnard, J., Colau, D., Boon, T. and Coulie, P. G. (2002) A human endogenous retroviral sequence encoding an antigen recognized on melanoma by cytolytic T lymphocytes. Cancer Res. 62, 5510–5516.

65 Stoye, J. P. (1999) The pathogenic potential of endogenous retroviruses: a sceptical view. Trends Microbiol. 7, 430; author reply 431–432.

66 Löwer, R., Löwer, J. and Kurth, R. (1996) The viruses in all of us: characteristics and biological significance of human endogenous retrovirus sequences. Proc. Natl. Acad. Sci. USA 93, 5177–5184.

67 Medstrand, P. and Mager, D. L. (1998) Human-specific integrations of the HERV-K endogenous retrovirus family. J. Virol. 72, 9782–9787.

68 Barbulescu, M., Turner, G., Seaman, M. I., Deinard, A. S., Kidd, K. K. and Lenz, J. (1999) Many human endogenous retrovirus K (HERV-K) proviruses are unique to humans. Curr. Biol. 9, 861–868.

69 Turner, G., Barbulescu, M., Su, M., Jensen-Seaman, M. I., Kidd, K. K. and Lenz, J. (2001) Insertional polymorphisms of full-length endogenous retroviruses in humans. Curr. Biol. 11, 1531–1535.

70 Hughes, J. F. and Coffin, J. M. (2004) Human endogenous retrovirus K solo-LTR formation and insertional polymorphisms: implications for human and viral evolution. Proc. Natl. Acad. Sci. USA 101, 1668–1672.

71 Mayer, J., Sauter, M., Racz, A., Scherer, D., Mueller-Lantzsch, N. and Meese, E. (1999) An almost-intact human endogenous retrovirus K on human chromosome 7. Nat. Genet. 21, 257–258.

72 Mueller-Lantzsch, N., Sauter, M., Weiskircher, A., Kramer, K., Best, B., Buck, M. and Grasser, F. (1993) Human endogenous retroviral element K10 (HERV-K10) encodes a full-length gag homologous 73-kDa protein and a functional protease. AIDS Res. Hum. Retroviruses 9, 343–350.

73 Schommer, S., Sauter, M., Krausslich, H. G., Best, B. and Mueller-Lantzsch, N. (1996) Characterization of the human endogenous retrovirus K proteinase. J. Gen. Virol. 77, 375–379.
74 Boller, K., Konig, H., Sauter, M., Mueller-Lantzsch, N., Lower, R., Lower, J. and Kurth, R. (1993) Evidence that HERV-K is the endogenous retrovirus sequence that codes for the human teratocarcinoma-derived retrovirus HTDV. Virology 196, 349–353.
75 Boller, K., Schonfeld, K., Lischer, S., Fischer, N., Hoffmann, A., Kurth, R. and Tonjes, R. R. (2008) Human endogenous retrovirus HERV-K113 is capable of producing intact viral particles. J. Gen. Virol. 89, 567–572.
76 Tonjes, R. R., Boller, K., Limbach, C., Lugert, R. and Kurth, R. (1997) Characterization of human endogenous retrovirus type K virus-like particles generated from recombinant baculoviruses. Virology 233, 280–291.
77 Belshaw, R., Pereira, V., Katzourakis, A., Talbot, G., Paces, J., Burt, A. and Tristem, M. (2004) Long-term reinfection of the human genome by endogenous retroviruses. Proc. Natl. Acad. Sci. USA 101, 4894–4899.
78 Dewannieux, M., Harper, F., Richaud, A., Letzelter, C., Ribet, D., Pierron, G. and Heidmann, T. (2006) Identification of an infectious progenitor for the multiple-copy HERV-K human endogenous retroelements. Genome Res. 16, 1548–1556.
79 Beimforde, N., Hanke, K., Ammar, I., Kurth, R. and Bannert, N. (2008) Molecular cloning and functional characterization of the human endogenous retrovirus K113. Virology 371, 216–225.
80 Dewannieux, M., Blaise, S. and Heidmann, T. (2005) Identification of a functional envelope protein from the HERV-K family of human endogenous retroviruses. J. Virol. 79, 15573–15577.
81 Caporale, M., Cousens, C., Centorame, P., Pinoni, C., De las Heras, M. and Palmarini, M. (2006) Expression of the jaagsiekte sheep retrovirus envelope glycoprotein is sufficient to induce lung tumors in sheep. J. Virol. 80, 8030–8037.
82 Liu, S. L. and Miller, A. D. (2007) Oncogenic transformation by the jaagsiekte sheep retrovirus envelope protein. Oncogene 26, 789–801.
83 De las Heras, M., Barsky, S. H., Hasleton, P., Wagner, M., Larson, E., Egan, J., Ortin, A., Gimenez-Mas, J. A., Palmarini, M. and Sharp, J. M. (2000) Evidence for a protein related immunologically to the jaagsiekte sheep retrovirus in some human lung tumours. Eur. Respir. J. 16, 330–332.
84 De Las Heras, M., Murcia, P., Ortin, A., Azua, J., Borderias, L., Alvarez, R., Jimenez-Mas, J. A., Marchetti, A. and Palmarini, M. (2007) Jaagsiekte sheep retrovirus is not detected in human lung adenocarcinomas expressing antigens related to the Gag polyprotein of betaretroviruses. Cancer Lett. 258, 22–30.
85 Danilkovitch-Miagkova, A., Duh, F. M., Kuzmin, I., Angeloni, D., Liu, S. L., Miller, A. D. and Lerman, M. I. (2003) Hyaluronidase 2 negatively regulates RON receptor tyrosine kinase and mediates transformation of epithelial cells by jaagsiekte sheep retrovirus. Proc. Natl. Acad. Sci. USA 100, 4580–4585.
86 Haraguchi, S., Good, R. A., Cianciolo, G. J., Engelman, R. W. and Day, N. K. (1997) Immunosuppressive retroviral peptides: immunopathological implications for immunosuppressive influences of retroviral infections. J. Leukoc. Biol. 61, 654–666.
87 Cianciolo, G. J., Copeland, T. D., Oroszlan, S. and Snyderman, R. (1985) Inhibition of lymphocyte proliferation by a synthetic peptide homologous to retroviral envelope proteins. Science 230, 453–455.
88 Harris, J. R. (1998) Placental endogenous retrovirus (ERV): structural, functional, and evolutionary significance. Bioessays 20, 307–316.
89 Mangeney, M. and Heidmann, T. (1998) Tumor cells expressing a retroviral envelope escape immune rejection in vivo. Proc. Natl. Acad. Sci. USA 95, 14920–14925.
90 Blaise, S., Mangeney, M. and Heidmann, T. (2001) The envelope of Mason-Pfizer monkey virus has immunosuppressive properties. J. Gen. Virol. 82, 1597–1600.
91 Mangeney, M., de Parseval, N., Thomas, G. and Heidmann, T. (2001) The full-length envelope of an HERV-H human endogenous retrovirus has immunosuppressive properties. J. Gen. Virol. 82, 2515–2518.
92 Li, M., Huang, X., Zhu, Z. and Gorelik, E. (1999) Sequence and insertion sites of murine melanoma-associated retrovirus. J. Virol. 73, 9178–9186.
93 Mangeney, M., Pothlichet, J., Renard, M., Ducos, B. and Heidmann, T. (2005) Endogenous retrovirus expression is required for murine melanoma tumor growth in vivo. Cancer Res. 65, 2588–2591.
94 Dittmer, U., He, H., Messer, R. J., Schimmer, S., Olbrich, A. R., Ohlen, C., Greenberg, P. D., Stromnes, I. M., Iwashiro, M., Sakaguchi, S. et al. (2004) Functional impairment of CD8(+) T cells by regulatory T cells during persistent retroviral infection. Immunity 20, 293–303.
95 Cools, N., Ponsaerts, P., Van Tendeloo, V. F. and Berneman, Z. N. (2007) Regulatory T cells and human disease. Clin. Dev. Immunol. 2007, 89195.
96 Rasku, M. A., Clem, A. L., Telang, S., Taft, B., Gettings, K., Gragg, H., Cramer, D., Lear, S. C., McMasters, K. M., Miller, D. M. et al. (2008) Transient T cell depletion causes regression of melanoma metastases. J. Transl. Med. 6, 12.
97 Vence, L., Palucka, A. K., Fay, J. W., Ito, T., Liu, Y. J., Banchereau, J. and Ueno, H. (2007) Circulating tumor antigen-specific regulatory T cells in patients with metastatic melanoma. Proc. Natl. Acad. Sci. USA 104, 20884–20889.
98 Moyes, D., Griffiths, D. J. and Venables, P. J. (2007) Insertional polymorphisms: a new lease of life for endogenous retroviruses in human disease. Trends Genet. 23, 326–333.
99 Macfarlane, C. and Simmonds, P. (2004) Allelic variation of HERV-K(HML-2) endogenous retroviral elements in human populations. J. Mol. Evol. 59, 642–656.
100 Burmeister, T., Ebert, A. D., Pritze, W., Loddenkemper, C., Schwartz, S. and Thiel, E. (2004) Insertional polymorphisms of endogenous HERV-K113 and HERV-K115 retroviruses in breast cancer patients and age-matched controls. AIDS Res. Hum. Retroviruses 20, 1223–1229.
101 Moyes, D. L., Martin, A., Sawcer, S., Temperton, N., Worthington, J., Griffiths, D. J. and Venables, P. J. (2005) The distribution of the endogenous retroviruses HERV-K113 and HERV-K115 in health and disease. Genomics 86, 337–341.
102 Moyes, D. L., Goris, A., Ban, M., Compston, A., Griffiths, D. J., Sawcer, S. and Venables, P. J. (2008) HERV-K113 is not associated with multiple sclerosis in a large family-based study. AIDS Res. Hum. Retroviruses 24, 363–365.
103 de Parseval, N., Diop, G., Blaise, S., Helle, F., Vasilescu, A., Matsuda, F. and Heidmann, T. (2005) Comprehensive search for intra- and inter-specific sequence polymorphisms among coding envelope genes of retroviral origin found in the human genome: genes and pseudogenes. BMC Genomics 6, 117.
104 Götzinger, N., Sauter, M., Roemer, K. and Mueller-Lantzsch, N. (1996) Regulation of human endogenous retrovirus-K gag expression in teratocarcinoma cell lines and human tumours. J. Gen. Virol. 77, 2983–2990.
105 Lavie, L., Kitova, M., Maldener, E., Meese, E. and Mayer, J. (2005) CpG methylation directly regulates transcriptional activity of the human endogenous retrovirus family HERV-K(HML-2). J. Virol. 79, 876–883.
106 Sauter, M., Schommer, S., Kremmer, E., Remberger, K., Dolken, G., Lemm, I., Buck, M., Best, B., Neumann-Haefelin, D. and Mueller-Lantzsch, N. (1995) Human endogenous retrovirus K10: expression of Gag protein and detec-

tion of antibodies in patients with seminomas. J. Virol. 69, 414–421.
107 Boller, K., Janssen, O., Schuldes, H., Tonjes, R. R. and Kurth, R. (1997) Characterization of the antibody response specific for the human endogenous retrovirus HTDV/HERV-K. J. Virol. 71, 4581–4588.
108 Kleiman, A., Senyuta, N., Tryakin, A., Sauter, M., Karseladze, A., Tjulandin, S., Gurtsevitch, V. and Mueller-Lantzsch, N. (2004) HERV-K(HML-2) GAG/ENV antibodies as indicator for therapy effect in patients with germ cell tumors. Int. J. Cancer 110, 459–461.
109 Sauter, M., Roemer, K., Best, B., Afting, M., Schommer, S., Seitz, G., Hartmann, M. and Mueller-Lantzsch, N. (1996) Specificity of antibodies directed against Env protein of human endogenous retroviruses in patients with germ cell tumors. Cancer Res. 56, 4362–4365.
110 Buscher, K., Trefzer, U., Hofmann, M., Sterry, W., Kurth, R. and Denner, J. (2005) Expression of human endogenous retrovirus K in melanomas and melanoma cell lines. Cancer Res. 65, 4172–4180.
111 Ruprecht, K., Gronen, F., Sauter, M., Best, B., Rieckmann, P. and Mueller-Lantzsch, N. (2008) Lack of immune responses against multiple sclerosis-associated retrovirus/human endogenous retrovirus W in patients with multiple sclerosis. J. Neurovirol. 14, 143–151.
112 Rakoff-Nahoum, S., Kuebler, P. J., Heymann, J. J., Sheehy, M. E., Ortiz, G. M., Ogg, G. S., Barbour, J. D., Lenz, J., Steinfeld, A. D. and Nixon, D. F. (2006) Detection of T lymphocytes specific for human endogenous retrovirus K (HERV-K) in patients with seminoma. AIDS Res. Hum. Retroviruses 22, 52–56.
113 Takahashi, Y., Harashima, N., Kajigaya, S., Yokoyama, H., Cherkasova, E., McCoy, J. P., Hanada, K. I., Mena, O., Kurlander, R., Abdul, T. et al. (2008) Regression of human kidney cancer following allogeneic stem cell transplantation is associated with recognition of an HERV-E antigen by T cells. J. Clin. Invest. 118, 1099–1109.
114 Horwich, A., Shipley, J. and Huddart, R. (2006) Testicular germ-cell cancer. Lancet 367, 754–765.
115 Kalter, S. S., Helmke, R. J., Heberling, R. L., Panigel, M., Fowler, A. K., Strickland, J. E. and Hellmann, A. (1973) C-Type particles in normal human placentas. J. Natl. Cancer Inst. 50, 1081–1084.
116 Lower, R., Lower, J., Frank, H., Harzmann, R. and Kurth, R. (1984) Human teratocarcinomas cultured in vitro produce unique retrovirus-like viruses. J. Gen. Virol. 65, 887–898.
117 Bronson, D. L., Fraley, E. E., Fogh, J. and Kalter, S. S. (1979) Induction of retrovirus particles in human testicular tumor (Tera-1) cell cultures: an electron microscopic study. J. Natl. Cancer Inst. 63, 337–339.
118 Kurth, R., Löwer, R., Löwer, J., Harzmann, R., Pfeiffer, R., Schmidt, C. G., Fogh, J. and Frank, H. (1980) Oncovirus synthesis in human teratocarcinoma cultures and an increased anti-viral immune reactivity in corresponding patients. In: Viruses in Naturally Occurring Cancers, pp. 835–846, Essex, M., Todaro, G. J. and zur Hausen, H. (eds.), Cold Spring Harbor Laboratory Press, New York.
119 Lower, R., Boller, K., Hasenmaier, B., Korbmacher, C., Muller-Lantzsch, N., Lower, J. and Kurth, R. (1993) Identification of human endogenous retroviruses with complex mRNA expression and particle formation. Proc. Natl. Acad. Sci. USA 90, 4480–4484.
120 Bieda, K., Hoffmann, A. and Boller, K. (2001) Phenotypic heterogeneity of human endogenous retrovirus particles produced by teratocarcinoma cell lines. J. Gen. Virol. 82, 591–596.
121 Galli, U. M., Sauter, M., Lecher, B., Maurer, S., Herbst, H., Roemer, K. and Mueller-Lantzsch, N. (2005) Human endogenous retrovirus rec interferes with germ cell development in mice and may cause carcinoma in situ, the predecessor lesion of germ cell tumors. Oncogene 24, 3223–3228.
122 Boese, A., Sauter, M., Galli, U., Best, B., Herbst, H., Mayer, J., Kremmer, E., Roemer, K. and Mueller-Lantzsch, N. (2000) Human endogenous retrovirus protein cORF supports cell transformation and associates with the promyelocytic leukemia zinc finger protein. Oncogene 19, 4328–4336.
123 Denne, M., Sauter, M., Armbruester, V., Licht, J. D., Roemer, K. and Mueller-Lantzsch, N. (2007) Physical and functional interactions of human endogenous retrovirus proteins Np9 and rec with the promyelocytic leukemia zinc finger protein. J. Virol. 81, 5607–5616.
124 Costoya, J. A., Hobbs, R. M., Barna, M., Cattoretti, G., Manova, K., Sukhwani, M., Orwig, K. E., Wolgemuth, D. J. and Pandolfi, P. P. (2004) Essential role of PLZF in maintenance of spermatogonial stem cells. Nat. Genet. 36, 653–659.
125 Buaas, F. W., Kirsh, A. L., Sharma, M., McLean, D. J., Morris, J. L., Griswold, M. D., de Rooij, D. G. and Braun, R. E. (2004) PLZF is required in adult male germ cells for stem cell self-renewal. Nat. Genet. 36, 647–652.
126 Armbruester, V., Sauter, M., Roemer, K., Best, B., Hahn, S., Nty, A., Schmid, A., Philipp, S., Mueller, A. and Mueller-Lantzsch, N. (2004) Np9 protein of human endogenous retrovirus K interacts with ligand of numb protein, X. J. Virol. 78, 10310–10319.
127 Adamah, D. J., Gokhale, P. J., Eastwood, D. J., Rajpert De-Meyts, E., Goepel, J., Walsh, J. R., Moore, H. D. and Andrews, P. W. (2006) Dysfunction of the mitotic:meiotic switch as a potential cause of neoplastic conversion of primordial germ cells. Int. J. Androl. 29, 219–227.
128 Balda, B. R., Hehlmann, R., Cho, J. R. and Spiegelman, S. (1975) Oncornavirus-like particles in human skin cancers. Proc. Natl. Acad. Sci. USA 72, 3697–3700.
129 Birkmayer, G. D., Balda, B. R., Miller, F. and Braun-Falco, O. (1972) Virus-like particles in metastases of human malignant melanoma. Naturwissenschaften 59, 369–370.
130 Muster, T., Waltenberger, A., Grassauer, A., Hirschl, S., Caucig, P., Romirer, I., Fodinger, D., Seppele, H., Schanab, O., Magin-Lachmann, C. et al. (2003) An endogenous retrovirus derived from human melanoma cells. Cancer Res. 63, 8735–8741.
131 Buscher, K., Hahn, S., Hofmann, M., Trefzer, U., Ozel, M., Sterry, W., Lower, J., Lower, R., Kurth, R. and Denner, J. (2006) Expression of the human endogenous retrovirus-K transmembrane envelope, Rec and Np9 proteins in melanomas and melanoma cell lines. Melanoma Res. 16, 223–234.
132 Humer, J., Waltenberger, A., Grassauer, A., Kurz, M., Valencak, J., Rapberger, R., Hahn, S., Lower, R., Wolff, K., Bergmann, M. et al. (2006) Identification of a melanoma marker derived from melanoma-associated endogenous retroviruses. Cancer Res. 66, 1658–1663.
133 Hirschl, S., Schanab, O., Seppele, H., Waltenberger, A., Humer, J., Wolff, K., Pehamberger, H. and Muster, T. (2007) Sequence variability of retroviral particles derived from human melanoma cells melanoma-associated retrovirus. Virus Res. 123, 211–215.
134 Oricchio, E., Sciamanna, I., Beraldi, R., Tolstonog, G. V., Schumann, G. G. and Spadafora, C. (2007) Distinct roles for LINE-1 and HERV-K retroelements in cell proliferation, differentiation and tumor progression. Oncogene 26, 4226–4233.
135 Wang-Johanning, F., Liu, J., Rycaj, K., Huang, M., Tsai, K., Rosen, D. G., Chen, D. T., Lu, D. W., Barnhart, K. F. and Johanning, G. L. (2007) Expression of multiple human endogenous retrovirus surface envelope proteins in ovarian cancer. Int. J. Cancer 120, 81–90.
136 Mameli, G., Astone, V., Arru, G., Marconi, S., Lovato, L., Serra, C., Sotgiu, S., Bonetti, B. and Dolei, A. (2007) Brains and peripheral blood mononuclear cells of multiple sclerosis (MS) patients hyperexpress MS-associated retrovirus/HERV-W endogenous retrovirus, but not human herpesvirus 6. J. Gen. Virol. 88, 264–274.

137 Ruprecht, K., Obojes, K., Wengel, V., Gronen, F., Kim, K. S., Perron, H., Schneider-Schaulies, J. and Rieckmann, P. (2006) Regulation of human endogenous retrovirus W protein expression by herpes simplex virus type 1: implications for multiple sclerosis. J. Neurovirol. 12, 65–71.

138 Lower, R., Tonjes, R. R., Korbmacher, C., Kurth, R. and Lower, J. (1995) Identification of a Rev-related protein by analysis of spliced transcripts of the human endogenous retroviruses HTDV/HERV-K. J. Virol. 69, 141–149.

139 Mayer, J., Ehlhardt, S., Seifert, M., Sauter, M., Muller-Lantzsch, N., Mehraein, Y., Zang, K. D. and Meese, E. (2004) Human endogenous retrovirus HERV-K(HML-2) proviruses with Rec protein coding capacity and transcriptional activity. Virology 322, 190–198.

140 Magin, C., Lower, R. and Lower, J. (1999) cORF and RcRE, the Rev/Rex and RRE/RxRE homologues of the human endogenous retrovirus family HTDV/HERV-K. J. Virol. 73, 9496–9507.