Human endogenous retroviruses in cancer: Expression, regulation and function (Review)

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Abstract. Human endogenous retroviruses (HERVs) are the remnants of ancient retroviruses that infected human germline cells and became integrated into the human genome millions of years ago. Although most of these sequences are incomplete and silent, several potential pathological roles of HERVs have been observed in numerous diseases, such as multiple sclerosis and rheumatoid arthritis, and especially cancer, including breast cancer and pancreatic carcinoma. The present review investigates the expression signatures and complex regulatory mechanisms of HERVs in cancer. The long terminal repeats-driven transcriptional initiation of HERVs are regulated by transcription factors (such as Sp3) and epigenetic modifications (such as DNA methylation), and are influenced by environmental factors (such as ultraviolet radiation). In addition, this review focuses on the dual opposing effects of HERVs in cancer. HERVs can suppress cancer via immune activation; however, they can also promote cancer. HERV *env* gene serves a prime role in promoting carcinogenesis in certain malignant tumors, including breast cancer, pancreatic cancer, germ cell tumors, leukemia and Kaposi's sarcoma. Also, HERV ENV proteins can promote cancer via immune suppression. Targeting ENV proteins is a potential future antitumor treatment modality.

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1. Introduction

Human endogenous retroviruses (HERVs) are retroelements in the human genome (1). They originated from ancient retroviruses that infected our early ancestors' germ cells millions of years ago (2). According to initial sequencing and analysis of the human genome, HERV-like elements account for ~8.29% of the entire *Homo sapiens* genome (1). Most of the HERVs are incomplete and silent due to deletions and other loss-of-function mutations; however, there are still relatively intact open reading frames in a few HERV groups, such as HERV-K HML-2, which is the most active subtype of HERV-K and will be abbreviated to HK2 hereafter (3). Complete HERVs share 4 structure genes (*env*, *gag*, *pol* and *pro*), and they are flanked by long terminal repeats (LTRs) as their regulatory elements (4). The *env* gene encodes envelope proteins including transmembrane and surface envelope proteins responsible for fusion and receptor recognition (5). The *gag* gene encodes a polyprotein that can be digested by viral proteases, which belong to the aspartic protease family, into major structural capsid and nucleocapsid proteins (6). Capsid proteins form the core of the virion and nucleocapsid proteins participate in the packaging of viral particles (7). *Pol* encodes reverse transcriptase and integrase proteins involved in DNA synthesis and integration into the host genome (8). *Pro* encodes protease involved in processing viral polypeptides, such as GAG and POL proteins (6). Concerning LTRs, these regions contain *cis*-acting elements and promoters that regulate gene expression (Fig. 1).

HERVs are associated with numerous diseases, including nervous system diseases (such as multiple sclerosis) (9,10), infectious diseases (such as AIDS) (11,12), mental disorders and

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Abbreviations: Aza, 5-azacytidine; CSF1R, colony-stimulating factor 1 receptor; DCs, dendritic cells; DNMTi, DNA methylation inhibitor; EMT, epithelial to mesenchymal transition; HERVs, human endogenous retroviruses; HK2, HERV-K HML-2; ISU, immunosuppressive domain; KRAB-ZFP, Krüppel-associated box zinc finger proteins; KSHV, Kaposi's sarcoma-associated herpesvirus; LTRs, long terminal repeats; MITF, microphthalmia-associated transcription factor; PD-L1, programmed cell death ligand-1; PLZF, promyelocytic leukemia zinc finger

psychiatric diseases (such as schizophrenia) (13,14), obstetrical and gynecological diseases (such as preeclampsia) (15), endocrine diseases (such as diabetes) (16), dermatoses (such as lichen planus) (17,18), autoimmune diseases (such as rheumatoid arthritis) (19,20) and malignant tumors. Aberrant expression of HERVs is commonly observed in cancer, encouraging researchers to investigate possible roles of HERVs. Increasing evidence has demonstrated a close relationship between HERVs and cancer. For example, data indicate that the expression of HERVs is implicated in the stage of certain cancers, such as breast cancer and hepatocellular carcinoma (21,22). In addition, HERVs can activate multiple oncogenic signaling pathways (such as the Wnt/β-catenin signaling pathway) that promote the proliferation of cancer cells and suppress their differentiation and apoptosis (23,24). HERV ENV proteins have immunosuppressive properties, which also contributes to carcinogenesis (25); however, it has also been demonstrated that HERV ENV proteins can activate the immune system in cancer (26). In the present review expression signatures and regulatory mechanisms of HERVs in cancer, the effects of HERVs on carcinogenesis and their effects on cancer suppression via immune activation will be described.

2. Expression and regulatory mechanisms of HERVs in cancer

Expression signatures of HERVs in cancer. Compared with normal tissues, HERVs are extensively activated in diverse malignant tumors, including (27), prostate cancer (28), seminomas (29), bladder (30), ovarian (31), lung (32), hepatocellular cancer (22), leukemia (33), lymphoma (34), choriocarcinoma (35), colorectal carcinoma (36), soft tissue sarcoma (37) and Kaposi's sarcoma (38). HERV structural genes are expressed in different levels or as different variants, including full-length mRNAs (39), spliced mRNAs (34), non-coding RNAs (36), intact proteins (40) and truncated proteins (41). In cancer, activated intact proviral sequences can be transcribed into full-length mRNAs encoding intact proteins (39). RNA splicing, an important posttranscriptional regulation of gene expression, also serves a role in the expression of HERVs (34). Splice variants of *env*, such as *rec* and *np9*, can be translated into truncated proteins and may serve a role in carcinogenesis (41). The aforementioned point is discussed further in the section *Cancer-promoting effects and mechanisms of HERVs*. The efficiency of HERV mRNA splicing is associated with the orientation of transcription. For example, splicing events of HK2 mRNAs occur with sense orientation transcription, but cannot take place with an antisense orientation (42). In addition, proviral sequences can also be transcribed into non-coding RNAs through long non-coding RNA-associated transcription (39). These HERV transcripts, like transcripts of other genes in the human genome can be processed by the spliceosome and become spliced RNAs as long as they contain splice donor and receptor sites (43).

As the regulatory sequence of HERVs, HERV LTRs can also be transcribed into RNA (44). However, this type of RNA is usually transcribed by non-HERV genes, partly resulting from the random insertion of LTRs into these genes (44). Insertion of retroelements into genes may impact significant biological functions, such as cancer immunoregulation

through producing new proteins (45-48). For instance, the insertion of long interspersed nuclear elements into intron 4 of the *CD274* gene creates a soluble programmed death ligand-1 (PD-L1) protein, which is considered an antagonist of membrane-bound PD-L1 (45,46). Analogously, the ability of a few LTR-overlapping transcripts in generating cancer-specific antigenic polypeptides has been predicted through the unique protein-coding potential prediction and immunopeptidomics analyses (47,48). Studies on insertion of HERV LTRs into genes may provide a novel perspective of cancer immunotherapy (44-48). Although the identification and quantitation of these transcripts is difficult due to a plethora of LTR-overlapping transcripts, more possible roles of LTR-overlapping transcripts in cancer may be discovered in the future (49,50).

Regulatory mechanisms of the expression of HERVs in cancer. HERVs expression is influenced by their regulatory sequences and regulated by transcription factors and epigenetic modifications (51-53). In addition, environmental factors may also impact the transcription of HERVs in known or unknown ways (54,55).

Regulatory sequences. HERV proviral sequences are flanked by LTRs, which can act as promoters to drive the transcription of HERVs in cancer (4). The efficiency of HERVs expression is associated with the polymorphism of unique transcription factor binding sites (such as NBRE and CARF), which can be changed after integration into the human genome (51). Hence, the transcription activity of HERVs has differed in the course of human evolution due to the changing binding strength between transcription factors and their binding sites in the LTR regions (51,56). In addition, the promoter sequences of LTRs are unique among the HERV groups (57-59). For example, on investigation of expression of HERV-H in colorectal tumors, a 17 bp sequence in the LTR region that was significant for the transcription of HERV-H was discovered (57). The LTRs of HERV-K exhibit a TATA-less promoter and its transcriptional initiation is mediated by 3 GC boxes (58). For the HERV-W group in chromosome 7, a thymine at site 142 in the LTR region replaced with a cytosine drives a significant overexpression of syncytin-1 in urothelial cell carcinoma (59).

Transcription factors. Ubiquitous or specific transcription factors may regulate the expression of HERVs, such as SP1 and SP3, which are essential for the regulation of HERV-K LTRs (58,60). In the teratocarcinoma GH cell line and the melanoma Mel-C9 cell line, the HERV LTR region does not contain the TATA motif or a functional initiator. Using chromatin immunoprecipitation assays, Fuchs *et al* (58) identified the binding of SP1 and SP3 proteins to 3 GC boxes in the LTR region. This binding acts as an initiator to activate the LTR promoter (58). With the knockdown of SP1 or SP3 using RNA interference, the LTR promoter activity reduces by 30-50% (58). In addition, the YY1 protein can modulate the activities of HERV-K LTRs (52). Although SP1, SP3, and YY1 are ubiquitous, HERV-K LTRs are not always activated in different tumor cells (53). Epigenetic modifications may partly be the reason for this (54). However, specific transcription factors may also contribute to this. For example,
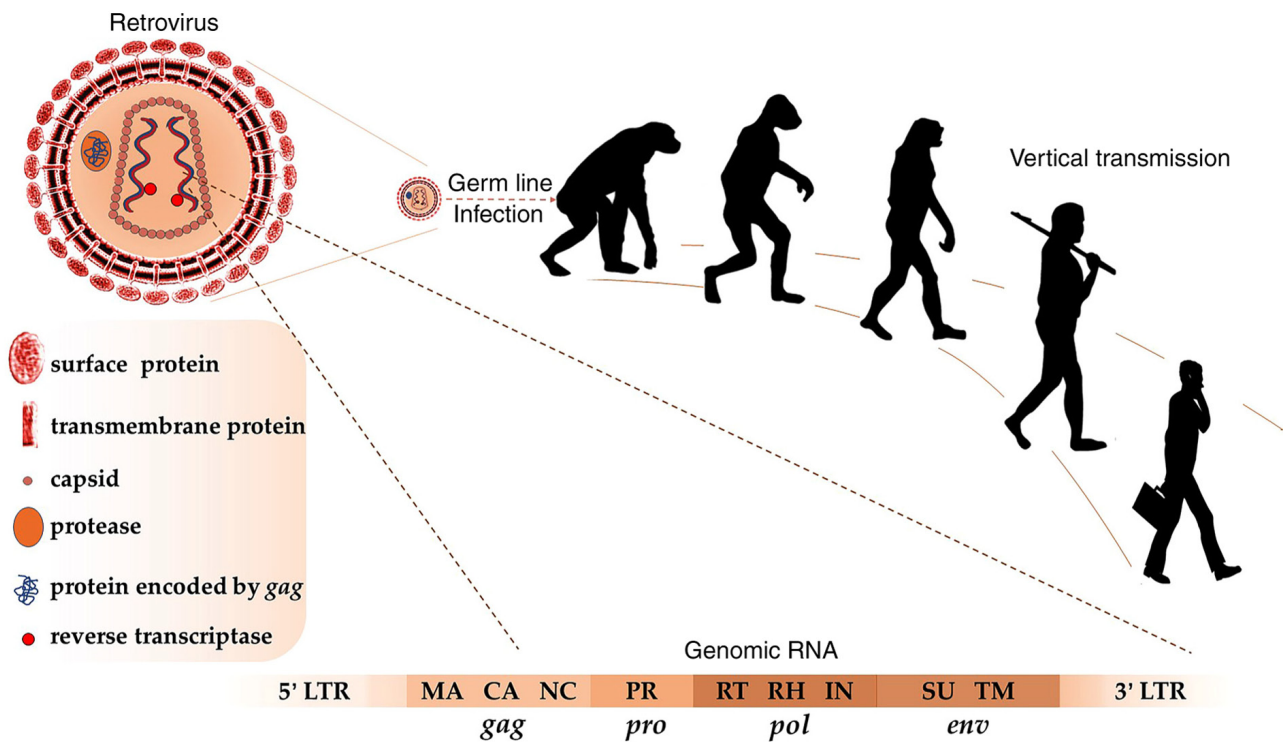
Figure 1. Retroviruses infected our early ancestors and HERVs were vertically transmitted in humans. A complete genomic HERV RNA sequence consists of *gag*, *pro*, *pol* and *env* genes, which are flanked by 5'- and 3'-LTR. MA, matrix; CA, capsid; NC, nucleocapsid; PR, protease; RT, reverse transcriptase; RH, RNase H; IN, integrase; SU, surface; TM, transmembrane; LTR, long terminal repeat; HERVs, human endogenous retroviruses.

the microphthalmia-associated transcription factor (MITF) can upregulate the expression of the HERV-K *env* gene in melanoma cells by interacting with a promoter composed of MITF binding motifs, 793TATA (TATA box-like sequence), and Inr 826 (transcriptional initiator site) (60). Additionally, activation of the *colony-stimulating factor 1 receptor (CSF1R)* gene in Hodgkin's lymphoma, which is activated by the canonical macrophage *CSF1R* promoter in healthy macrophage cells (61). NF-κB interacting with demethylated mammalian apparent LTR-retrotransposon LTRs replaces the canonical promoter to drive the *CSF1R* gene in Hodgkin's lymphoma (61). The aforementioned information indicates that several transcription factors and epigenetic modifications may be necessary for the activation of LTRs. There may be other specific transcription factors that can modulate the transcription of HERVs with epigenetic modifications (51).

Epigenetic modifications. Epigenetic modifications are essential regulatory mechanisms for the expression of HERVs in cancer (62). The activation of HERV LTRs can be regulated by 2 major epigenetic modifications: DNA methylation and histone modifications (63). The types of epigenetic modifications can partly represent the evolutionary age of the LTRs (53). Evolutionarily young LTRs are usually CpG-rich, and intermediate LTRs tend to be modified by histone methylation (53). Except for histone methylation, the alternative chromatin state may also regulate the transcription of HERVs via histone deacetylation modification (64).

CpG-rich promoters tend to indicate the inactive transcription of corresponding genes (63). The DNA demethylation of HERV LTRs may contribute to the increased

expression of HERVs. In the teratocarcinoma Tera-1 cell line, an association between the transcription level of HERV-K and methylation level of LTR has been observed (65). Also, the DNA hypomethylation of HERV-K and HERV-W has been discovered respectively, in urothelial carcinoma (66) and ovarian cancer (67). In particular, global hypomethylation of HERV-W has been observed in ovarian cancer (67). The aforementioned findings support the view that global hypomethylation of the genome in cancer leads to the desuppression and expression of HERVs. Such an expression of HERVs induced by hypomethylation is selective because several HERVs remain silent in cancer. For instance, 5-azacytidine (Aza) is an effective antitumor drug for hematologic tumors and also a type of DNA methylation inhibitor (DNTMi). It can upregulate some specific transcripts of HERVs in cancer, such as melanoma (68) and endometrial cancer (69). Analogously, several HERV-Ws are upregulated by Aza in neuroblastoma (70).

Histone acetylation and methylation can also impact the expression of HERVs. Histone acetylation can make local chromatin relax and genes located in the slack chromatin region are likely to be transcribed (64). On the contrary, histone deacetylation can make local chromatin tight and corresponding genes are relatively difficult to activate (64). For example, histone deacetylase inhibitors (such as Trichostatin A and Mocetinostat) exclusively induced the activity of the LTR12 promoter mediated by trimeric nuclear factor Y in diverse tumors (including testis cancer and lung carcinoma) (71). Notably, there may be an interplay between histone acetyltransferases and histone methyltransferases. For example, TIP60, a lysine acetyltransferase combined
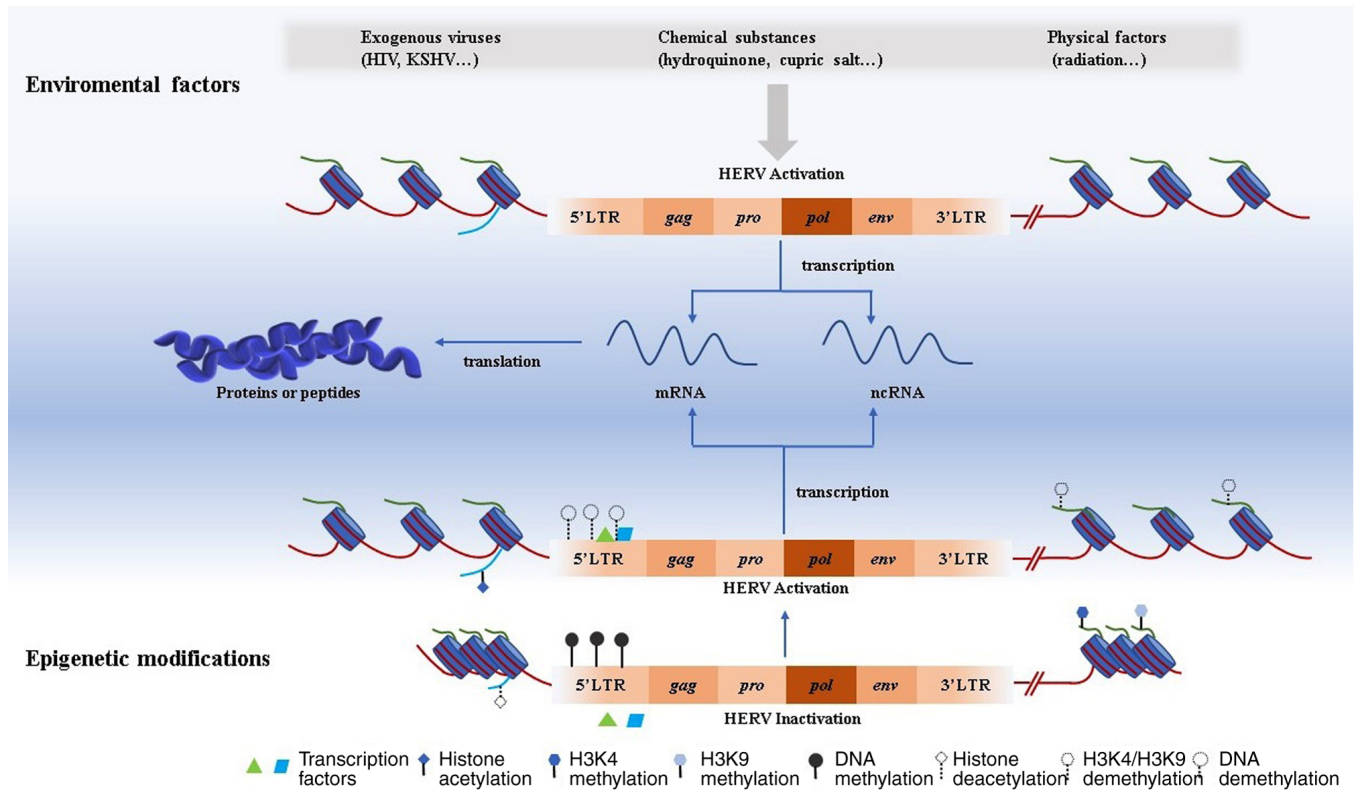
Figure 2. Activation of HERV is influenced by environmental factors and epigenetic modifications. Environmental factors including virus infection, chemical toxicity, and physical factors can impact HERV activation. Epigenetic modifications are crucial for HERV activation. DNA methylation, histone acetylation, or histone demethylation such as H3K4 and H3K9 demethylation can activate the expression of HERV. Transcription factors can contribute to HERV activation by combining with the promoter region in 5'LTR. Activated HERV can be transcribed into both ncRNA and mRNA. For the latter, it can be further translated into proteins or peptides. HERV, human endogenous retrovirus; LTR, long terminal repeat; HIV, human immunodeficiency virus; KSHV, Kaposi's sarcoma-associated herpesvirus; nc, non-coding.

with bromodomain 4 increases histone methylation and suppresses HERVs in colorectal cancer by the regulation of histone methyltransferases, such as SET domain, bifurcated 1 (SETDB1) and Su(*var*)3-9 homologue 1 proteins, and subsequently inhibits tumor growth (72).

For histone methylation, the relationship between histone methylation and gene activity is intricate (63). Distinct lysine sites of methylation and methylation types may have different impacts on the turning 'on' and 'off' of genes (63). LSD1, a histone H3K4 demethylase increased the transcripts of HERVs via the demethylation of LTRs in the MCF-7 cell line (73). G9Ai, a histone methyltransferase inhibitor which can inhibit the catalytic reaction of methylation of histone H3K9 also increases the transcripts of HERV-Fc1 in ovarian A2780 and CAOV3 cell lines (74). Lysine residue 9 of histone H3 has 3 methylation types, including mono-, di- and tri-methylation (75). G9A is one of the histone methyltransferases that can catalyze mono-methylation and demethylation (74). There are other histone methyltransferases, such as SETDB1 (catalyzing di- and trimethylation) (76) and GLP (catalyzing mono- and di-methylation) (77), involved in silencing HERVs. For instance, it was recently found that the former enzyme, SETDB1, controls the stability of T helper 2 cells through catalyzing the trimethylation of H3K9 in HERV regions, which act as enhancers for T helper 1 genes (78). These histone methylations are associated with crucial proteins called Krüppel-associated box zinc finger proteins (KRAB-ZFP,

also called KAP1 or TRIM28) (79). KRAB-ZFP contributes to histone and DNA methylation by combining with HERV LTRs leading to the HERVs becoming silent and regions of heterochromatin (79-81).

Environmental factors. Infection with tumor-associated viruses can regulate the expression of HERVs (38). The levels of HERVs were observed to be higher in HIV-infected patients compared with in healthy individuals (54). HIV-1 TAT protein serves an essential role in regulating almost 48 HERV-K proviruses in human peripheral blood lymphocytes (54). Two primary Kaposi's sarcoma-associated herpesvirus (KSHV)-encoded latent genes, the latency-associated nuclear antigen (*LANA*) and viral FADD-like interferon converting enzyme inhibitory protein (*vFLIP*) activate HERV-K transcription via the MAPK pathway and NF-κB pathway, respectively (38). Additionally, the human T-cell leukemia virus-1 TAX protein activates HERV LTRs, especially HERV-W8 LTRs (82). Infection by the Epstein-Barr virus has been demonstrated to be an activator for the transactivation of HERV-K18 in B lymphoblastoid cell lines (83). The mechanisms of HERV activation by viruses are relatively clear whereas those by physical and chemical factors remain uncertain (54,82,83). Hydroquinone (55), cupric ion (84), silver nanoparticles (85), and ultraviolet exposure (86), as well as hypoxia phenomenon (70) can influence the expression of HERVs in tumor cell lines. However, the underlying mechanism remains unknown (Fig. 2).

3. Dual opposing roles of HERVs in cancer

Cancer-promoting effects and mechanisms of HERVs. Carcinogenesis is a complex process that involves the inter-play between multiple genetic and environmental factors (87). Genetically, cellular proto-oncogene mutations like *RAS* or the loss of function of cancer suppressor genes like *p53* can cause various types of cancer (including breast cancer and colon carcinoma) (88,89). External factors, ionizing radiation, chemical carcinogens and viruses are also common cancerogenic substances (54,55,86). Persistent viral infection is crucial for the pathogenesis of some kinds of cancer (90). For example, viral hepatitis caused by the hepatitis B virus can ultimately lead to liver cancer, and cervix uteri infected by human papillomavirus can develop into cervical cancer (91,92). The fact that exogenous viruses can cause cancer has been extensively recognized (90). The present review will discuss the effects of endogenous retroviruses on cancer.

Immune surveillance is essential for hosts against tumors and HERV ENV proteins may facilitate carcinogenesis via immune suppression (93). Specific amino residues are necessary for the immunosuppressive effects of HERV ENV proteins, which means that not all HERV ENV proteins have this property (25). For instance, through immunogenicity and *in vivo* tumor-rejection assays, syncytin-2 was identified as an immunosuppressive protein and contributed to tumor growth, but syncytin-1 did not have these characteristics (25). Using sequence alignment and the introduction of a mutation in these 2 syncytins, it was found that K 14 (lysine residue) within the immunosuppressive domain (ISU) may be a crucial amino residue for the immunosuppressive property of syncytin-2 (25). In addition, there are quite a few HERV ENV proteins with immunosuppressive properties, such as HERV-H, ERV3, HERV-P(b) and HERV-V ENV proteins (25,94).

Nevertheless, how these proteins suppress host immunity remains unclear. Studies have demonstrated that HERV ENV proteins may suppress immunity via inhibiting the proliferation of T cells and alleviating lipopolysaccharide-induced immune activation (25,95). Predicted DNA sequences (~1,000) of ENV ISU have indicated that ENV proteins may serve a more significant role in immunosuppression (96). In a murine model (using BALB/c mice), tumor growth was repressed when mice acquired active or passive immunization against the HK2 ENV protein (97,98). Hence, targeting ENV proteins may be an effective future treatment for cancers with high expression levels of ENV proteins.

In general, the tumor-promoting effect of the ENV ISU domain may be a universal mechanism. In contrast, other mechanisms, such as activation of the ERK or Wnt signaling pathways, by which the *env* gene promotes tumorigenesis and cancer progression, vary by types of malignant tumors (24,40).

Breast cancer. Compared with other types of cancer, the association between HK2 and breast cancer has been studied more comprehensively (21,99). The expression of HK2 *env* transcripts and ENV proteins is increased in breast cancer (27), especially basal-like breast cancer subtype with *H-RAS* (wild-type) (21). In addition, antibodies against HK2 ENV proteins are detected in the blood of patients with early-stage breast cancer and the level of these antibodies in women

decreases with age (99). The aforementioned antibodies may have value in the diagnosis of breast cancer. The presence of HK2 ENV proteins in patients with early-stage breast cancer implies that they supposedly serve a role in breast cancer initiation. An observational study by Mastrangelo *et al* (100) also indicated the cancer-promoting effects of HK2 ENV proteins. Epidemiological data indicates that the yellow fever vaccine 17D, which elicits cross-reactive immunity against HK2 ENV halved the incidence of breast cancer in women ranging from 40-54 years (100). The effect of preventing breast cancer would be improved by the use of vaccines against HK2 (101). Hence, developing antitumor vaccines based on HK2 ENV to prevent breast cancer may be promising.

The possible mechanisms through which HERVs enhance the carcinogenesis of breast cancer have been gradually unveiled. The activation of the ERK pathway and the downregulation of p53 protein caused by increased HK2 ENV protein were notable findings (40). The aforementioned findings support the view that the HK2 ENV protein may promote the carcinogenesis of breast cancer (40). *p53* is a cancer suppressor gene whose mutation can induce a numerous cancers, such as lymphomas and sarcomas (102). Although a *RAS* gene mutation is rare in breast cancer, the RAS/ERK pathway is highly activated in most breast cancers (103). Hyperactivation of the RAS/ERK pathway in breast cancer is partly induced by the elevated HK2 ENV protein (103). In addition, the cytoplasmic tail of the transmembrane subunit of the ENV protein is required for hyperactivation of the RAS/ERK pathway (104). This activation is accompanied by a strong upregulation of several downstream genes, including *ETV4*, *ETV5*, and *EGR1*, which promote cellular transformation (104). In breast cancer, upregulation of the HK2 ENV protein suppresses p53 (40). The suppressed *p53* gene may be another contributor to the HERV-related carcinogenesis of breast cancer. Epithelial to mesenchymal transition (EMT) is an important mechanism of cancer metastasis. Increased HK2 ENV protein enhances the invasion and migration abilities of breast cancer promoting the process of EMT (104). The underlying mechanism for the aforementioned phenomenon remains unclear.

Cell-cell fusion mediated by HERV-W and HERV-FRD ENV protein in cancer is weakly evidenced (35,105). Both syncytin-1 and syncytin-2 serve a vital role in cytotrophoblast-cytotrophoblast fusion. Syncytin-1 is expressed and located at the cell membrane of breast cancer cells (105). It has been demonstrated that syncytin-1 promoted the fusion between breast cancer cells and endothelial cells by combining with ASCT-2, a cell membrane surface receptor for syncytin-1 (105). In line with this phenomenon in breast cancer, the fusion between choriocarcinoma cells and endothelial cells may be associated with syncytin-1 and HERV-Pb ENV (35). The fusion between endothelial cells and tumor cells mediated by HERV ENV may be observed in other kinds of cancer (106). There are two results of the fusion of healthy cells and cancer cells (107,108). One is the common scenario where the hybrid formed cell may be a normal cell due to the activation of tumor suppressors produced by the normal parental cell (107). The other is that the hybrid formed cell acquires more aggressive properties compared with the parental cancer cell, although this possibility is rare (108). The characteristic of the hybrid formed cell fused by these 2 types

of cancer cells and endothelial cells in research has not yet been identified (105). It may contribute to the invasion and metastasis of cancer if formed cells exhibit traits of cancer cells (108). Hence, this possible mechanism requires further explorations in carcinogenesis.

In addition to the ENV protein, non-coding RNA encoded by HERVs facilitates the progression of breast cancer, which may also be a contributing mechanism (109,110). ZMYND8 protein degradation promoting the proliferation and metastasis of triple-negative (estrogen receptor/progesterone receptor/HER2-negative) breast cancer is the second mechanism (110). ZMYND8 is a suppressor of metastasis-linked genes, such as *Slug*, *CD44*, *EGFR* and *VEGFR* (109). *TROJAN*, a HERV-derived long non-coding RNA is expressed exclusively in triple-negative breast cancer and promotes ZMYND8 degradation through ubiquitination (110). Hence, *TROJAN* may be a potential therapeutic target for triple-negative breast cancer.

Pancreatic cancer. Li *et al* (23) demonstrated the close relationship between pancreatic cancer and HK2 *env* gene. High expression of HK2 *env* is associated with stages IIB and III of pancreatic cancer (23). In addition, high expression of HK2 *env* is essential for cell proliferation, tumor growth, and metastasis (23). Similar to breast cancer, it has been observed that the RAS/ERK pathway and *p53* can also be regulated by HK2 *env* in pancreatic cancer (23). The distinction is that *RAS* mutation is common in pancreatic cancer and essential to its carcinogenesis (88). According to the best of our knowledge, the *RAS* mutation does not serve a primary etiological role in breast cancer (111). Based on this point, it seems possible that the increased expression of HK2 *env* may serve a more critical role in the carcinogenesis of pancreatic cancer compared with breast cancer. In addition to activating the RAS/ERK pathway, the expression of HK2 *env* may also promote the proliferation of pancreatic cancer cells via activating the JNK/MAPK pathway (23).

Germ cell tumors. Human germ cells are the gateway through which ancestor retroviruses invaded humans and retained HERVs (2). It is plausible that HERVs may make a massive difference to the survival or function of germ cells (2). In seminoma, it was reported that syncytin-1 and its splicing transcribed by HERV-W *env* were increased due to DNA demethylation of its promoter (112). This suggests that HERVs are also activated in germ cell tumors. HERVs can cause the pathological lesions of germ cells and may induce cancer. For example, the regulator of expression encoded by *corf* (REC) protein encoded by type II HK2 *env* can induce precancerous lesions of classic seminoma in transgenic mice (29). It is hard to identify whether the *rec* gene is an oncogene in humans. On the one hand, the increased expression of REC protein in patients with seminoma has not yet been reported, and precancerous lesions unlike carcinoma *in situ*, are reversible lesions by definition (29). The underlying mechanism of REC-induced precancerous lesions also remains elusive. On the other hand, proteins interacting with REC have been found and they could serve a dominant role in the REC-related carcinogenesis of germ cell tumors (113).

The *c-MYC* gene is a proto-oncogene and overexpressed in various types of cancer, including hepatocellular carcinoma and gastric adenocarcinoma (114,115). The REC protein can upregulate the expression of *c-MYC* via inhibiting promyelocytic leukemia zinc finger (PLZF), a tumor suppressor first found in promyelocytic leukemia and promote cell proliferation and survival (116). In addition, *c-MYC* modulated by the REC protein depends on 2 androgen receptor (AR) pathways (113). AR is an upstream activator of *c-MYC* (113). The REC protein can combine with AR and testicular zinc-finger protein and promote AR-induced transactivation (113). Another androgen receptor-dependent pathway is that REC binding with the human small glutamine-rich tetratricopeptide protein relieves the suppression of AR and activates the AR-dependent genes, such as *c-MYC* (117). Simultaneously, activated AR can promote the expression of the REC protein (117). The aforementioned findings indicate that the REC protein and AR may be mutually reinforcing (117). This 'vicious cycle' is proposed as the possible promotion of carcinogenesis (117).

Another protein encoded by type I HK2 env, nuclear protein of 9 kDa (NP9), can also suppress the PLZF protein and activate the c-MYC gene (116). In addition, NP9 can interact with the ligand of NUMB protein X, which is able to promote the degradation of NUMB protein (118). Decreased NUMB protein is usually required for increased NOTCH pathway activity (119). It is still unknown how NP9 affects carcinogenesis via the NOTCH pathway in germ cell tumors.

Leukemia. Upregulation of *Np9* mRNA expression was detected in patients with chronic lymphocytic leukemia compared with in healthy individuals (120). Chen *et al* (24) first identified NP9 as a potential oncogenic protein, providing new insights into the putative role that viruses play in human leukemias. Specifically, it was found that NP9 can increase the growth of human leukemia stem/progenitor cells by activating the β-catenin, ERK, AKT, and NOTCH1 pathways. Amongst these pathways, β-catenin has been reported as an essential protein for the survival and proliferation of CD34⁺ leukemia stem cells (121), and hyperactivation of the c-MYC/AKT pathway is sufficient to cause hematologic malignant tumors (122). Additionally, NP17 may be another oncogenic protein associated with refractory or relapsed leukemia (123).

Kaposi's sarcoma. Kaposi's sarcoma is a classic example that HERVs transactivated by exogenous virus infection can promote carcinogenesis (124). KSHV infection is an important cause of morbidity of Kaposi's sarcoma in patients with AIDS (125). The mechanism through which KSHV upregulates the expression of HERVs has been clarified as aforementioned. Increased NP9 induced by KSHV infection can enhance primary endothelial cell invasiveness via activating the CD147-A disintegrin and metalloproteinase with thrombospondin motifs 1 (ADAMTS1)/ADAMTS9 axis (38). In addition, the silencing of HK2 *env* can reduce the expression of vascular endothelial-derived growth factor, which is a prime pro-angiogenic cytokine in the invasion of primary endothelial cells triggered by KSHV (38).

Potential for the oncogenic role of HERVs. In summary, the aforementioned pathways and molecules are the possible mechanisms through which HERVs promote carcinogenesis. HK2 can promote carcinogenesis through a tiny number

Table I. Molecular evidence for the oncogenic effect of HERV-derived molecules.

First author, year	Tumor type	Group	Molecules	Molecular mechanism	Oncogenic effect	(Refs.)
Zhou et al, 2016; Lemaître et al, 2017	Breast cancer	HK2	ENV	Activated the MAPK pathway and regulating tumor-associated regulators including p53, TGF-β1, and MYC	Promoted cell proliferation, migration, and invasion	(40) (104)
Bjerregaard et al, 2006	Breast cancer	HERV-W	ENV (syncytin)	Syncytin-ASCT2-mediated fusion (ASCT2 is a receptor for syncytin)	Cancer-endothelial cell fusion	(105)
Jin et al, 2019	Breast cancer	HERV	TROJAN	Bound to ZMYND8 and then increased its degradation	Promoted tumor cell proliferation and metastasis	(110)
Argaw-Denboba et al, 2017	Melanoma	HK2	ENV	Unknown	Promoted cell phenotype-switching and stemness maintenance	(136)
Chen et al, 2013	Leukemia	HK2	ENV (NP9)	Activated the WNT, ERK, AKT, and NOTCH1 pathways	Promoted tumor growth	(24)
Denne et al, 2007	Germline cancer	HK2	ENV (NP9 and REC)	De-repressed c-MYC via inhibiting PLZF	Promoted cell proliferation	(116)
Li et al, 2017	Pancreatic cancer	HK2	ENV	Influenced signal transduction via the RAS-ERK-RSK pathway	Promoted cell proliferation, tumor, growth and metastasis	(23)
Dai et al, 2018	Kaposi's sarcoma	HK2	ENV (NP9)	Activated the CD147-ADAMTS1/ADAMTS9 axis	Promoted cell proliferation and invasion	(38)

ADAMTS1/9, A disintegrin and metalloproteinase with thrombospondin motifs 1/9; ASCT2, alanine serine cysteine transporter 2; ENV, envelope; HK2, HERV-K HML-2; MAPK, mitogen-activated protein kinase; NP9, nuclear-protein of 9 kDa; PLZF, promyelocytic leukemia zinc finger; REC, regulator of expression encoded by *corf*; ZMYND8, zinc finger MYND-type containing 8.

of molecules, including ENV, NP9 and REC protein which maybe therapeutic targets in specific cancers, in contrast with numerous HERV groups (38,104,116). Evaluating the individual contributions of these HERV molecules to carcinogenesis is difficult (29). Whether an aberrant expression of these HERV molecules occurs in the early stage of cancer or can be detected in cancer stem cells is vital for their roles in carcinogenesis. Anti-ENV antibodies are detected in patients with early stage breast cancer (99). NP9 protein can facilitate the growth of leukemia stem/progenitor cells as previously described. Numerous studies have indicated that these molecules are involved in significant molecular events in early carcinogenesis (Table I). More future studies should focus on this problem.

Cancer-suppressive effects and mechanisms of HERVs. Contrary to the immunosuppressive effects of HERVs, HERVs can activate immunity to inhibit cancer. An interesting phenomenon was observed: A higher immunogenic HERV level indicated increased immune infiltration, a more substantial fraction of CD8+ T cells and upregulation of the checkpoint pathway compared with a lower immunogenic ERV level in clear cell renal cell carcinoma (26). HERV products can activate innate immunity, cellular immunity, and humoral immunity in malignant tumors (26,31,126). These immunological mechanisms stimulated by HERVs may have an impact on tumorigenesis and cancer progression (127-130). Cytotoxic T cell-mediated immunity can be induced by the HERV ENV protein in a few types of cancer, such as ovarian (31), breast cancer (127) and colorectal carcinoma (128). The performance of HERV-K-specific T cells resembles a classical cellular immune response (31). The proliferation of T cells, IFN-γ products and HERV-K-specific cytotoxic T lymphocyte activity of lysing target cells is detected (31,129). A similar situation occurs in colorectal carcinoma, where the activators are HERV-H ENV peptides (128). The HERV ENV protein can regulate the stimulatory activity of dendritic cells (DCs) and target T cells (95). In this process, DCs and T cells conjugate formation is affected by the HERV ENV protein in an unknown way (95). Additionally, H17, a peptide originating from HERV-H can facilitate EMT and can enrich pluripotent

immunoregulatory CD271^{+} cells by activating the expression of CCL19, and serve a crucial role in the immune escape of tumor cells (130).

HERVs can stimulate B cell immunity and antibodies targeting HERV proteins are readily detectable in the blood of patients with tumors, such as breast cancer (126). Although it is hard to estimate the direct antitumor effect of these antibodies in patients, some antibody levels are associated with the tumor staging and predictors for prognosis, such as melanoma (131) and prostate tumors (132).

It has been clarified above that DNA and histone epigenetic modification can regulate the expression of HERVs. The activation of innate immunity of IFN-I signaling can be induced by epigenetic drugs, such as DNMTi and G9Ai, possibly mediated by increased HERV transcripts in ovarian cancer (74,133,134). An increase in CD8^{+} T cells accompanies this process (134). In addition, DNMTi can activate IFN-III signaling with increased HERV transcripts in colorectal cancer (134). The aforementioned epigenetic drugs can induce 'viral mimicry' effects by the possible production of HERV expression products that can lead to stimulation of the viral defense response and promotion of cell death (135). DNA hypomethylation of HERV LTRs, regardless of the tumor-intrinsic or pharmacologically-induced origin may result in antitumor effects (74,135).

4. Conclusion

Existing evidence indicates that HERVs are likely to promote carcinogenesis as accessory factors rather than causative factors (23,24,38,40,104,105,116,136). HERVs can activate multiple oncogenic signaling pathways and inhibit tumor suppressor genes. The effects of HERVs activating or suppressing immunity could reflect a profound interaction between host and ancient exogenous or endogenous retroviruses whose total effect may promote carcinogenesis. Based on existing knowledge, HK2 plays prime cancer-promoting effects among HERVs. HERV *env* gene provides a bridge between the HERVs and their roles in cancer. Besides, non-coding RNAs transcribed by HERVs, such as *TROJAN* are also worthy of attention. The regulatory mechanisms of HERVs are complex and partly vary by HERV groups. Nowadays, studies regarding HERVs are hampered due to a plethora of transcribed HERV RNAs in cancer and the lack of specific commercial antibodies (137). The emerging high-throughput RNA-sequencing technique may demonstrate a more explicit expression profile of HERVs and expand possible roles in cancer (137,138). Insufficient knowledge regarding HERV genes, their highly complex pattern of activation, and transcriptional and posttranscriptional regulation hinders the ability to study the mechanistic role of HERVs in numerous cancers. Efforts in exploring ways to interfere with HERVs during carcinogenesis are still at an early stage. Noteworthily, the aforementioned HERV molecules may be potential targets in the treatment of cancer. In addition, HERVs can induce immune responses of the host. Cancer prevention may be possible via the use of vaccines against HERVs, since HERVs serve crucial roles in early cancer as postulated. There are promising leads that will provide an improved understanding of how HERVs contribute to cancer (110,139,140). This knowledge of HERVs will lead to it being used in translational research and will guide researchers to develop vaccines against HERVs preventing cancers.

Availability of data and materials

Not applicable.

Authors' contributions

YG contributed to the conceptualization and visualization of the study, writing the original draft and reviewing and editing the manuscript. TC contributed to the conceptualization, reviewing and editing of the manuscript, supervision, project administration and funding acquisition. XFY contributed to reviewing and editing of the manuscript, supervision and project administration. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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