Abstract. The expression of genes is altered in various diseases and is responsible for the disease's initiation, progression and pathology. Several other genes, predominantly inactivated, may become activated in a given condition and contribute to the initiation and progression of the disease. Similarly, human endogenous viruses (HERVs) are an incomplete, non-productive and inactive viral sequence present in the heterochromatin of the human genome, and are often referred to as junk DNA. HERVs were inserted into the host genome millions of years ago. However, they were silenced due to multiple mutations and recombination that occurred over time. However, their expression is increased in cancers due to either epigenetic or transcriptional dysregulation. Some of the HERVs having intact open reading frames have been reported to express virus-like particles, functional peptides and proteins involved in tumorigenesis. To summarize, there is involvement of different HERVs in the initiation and progression of several cancers. The present review aims to provide concise information on HERV and its involvement in the initiation and progression of multiple types of cancer.

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1. Introduction

Human endogenous retroviruses (HERVs) are molecular remnants of exogenous retroviruses that infected the human germline millions of years ago (horizontal transmission). These genetic fragments are stably integrated into the human genome; they are called endogenous retroviruses (1). They are often considered ‘non-functional DNA’ and account for approximately 8.29% of the human genome (2). They are inherited from parents to the offspring like any other gene (vertical transmission) (Fig. 1). The retroviruses are known for their transforming potential in their animal host via reverse transcription, but the HERVs are typically silenced or non-productive due to the accumulation of mutations and therefore rendered inadequate to produce virions (3). However, due to epigenetic dysregulation, some HERVs can retain their potency, produce virus-like particles, and express some immunogenic protein products, such as Syncytins (4), Np9, and Rec (5). The production of these proteins by the retroelements affects the biological functions and cancer immunoregulation (2).

HERVs are not usually expressed in normal cells. Nevertheless, some of their gene products and viral components can be expressed in human cells as antigens in some instances. Their expression has been reported to have a dual impact on human physiology (2). They aid in human physiological functions like regulating pluripotency of embryonic stem cells (6), involvement in placental morphogenesis (7), modulating the innate immune response (8), and regulating gene expression (9). On the contrary, they are also involved in the pathogenesis of multiple sclerosis (10), rheumatoid arthritis (11), schizophrenia (12), AIDS (13), cellular senescence (14), and diabetes (15). HERVs have gained a significant attraction due to their association with various cancers and their progression (3). Due to their abnormal expression in multiple malignancies and their pleiotropic role in oncogenesis, extensive research has targeted HERV antigens for immunotherapy by triggering both innate and adaptive immune response.
response (16,17). Blocking the expression and function of HERVs in tumor cells via small interfering RNA (siRNA) or CRISPR (18), and anti-viral drugs (19) have also been studied by researchers (20). Since HERVs are extensively present in the human genome, their gene products can also be used as biomarkers to detect cancer progression (21).

### 2. Structure and classification of HERVs

HERVs have diverse structures ranging from solo LTR (long terminal repeats) to partially or fully intact open reading frames (ORFs) (22). The most active HERV group reported having a relatively intact ORF is the HERV-K HML-2 subtype (23). Classical HERVs structure contains the general components of the retroviruses, including protein-encoding sequences, GAG (gene-specific antigen), PRO (protease gene), POL (polymerase gene), and ENV (envelope gene) flanked by non-coding LTRs (Fig. 2) which are the regulatory region and can act as a promoter or an enhancer. Until now, 31 discrete groups of HERVs have been discovered. They can be categorized into three classes of retroviruses based on their similar phylogenetic origin to exogenous viruses (24) (Fig. 3).

### 3. Activation of HERVs

Generally, HERVs, having intact ORF, remain inactive due to CpG hypermethylation of their sequence which is catalyzed by DNA methylase-1 (25). However, they can be activated by multiple factors like exogenous viruses such as human immunodeficiency virus (HIV) (26), Kaposi sarcoma-associated herpesvirus (KSHV), also known as human herpesvirus 8 (HHV8) (27), Epstein-Barr virus (28), and human T-cell leukemia virus-1 (29). Also, epigenetic modifications (DNA demethylation, histone modification) (30), chemical substances (hydroquinone (31), phorbol-12-myristate-13-acetate (PMA) (32), phytohemagglutinin (PHA) (33), cupric salt (34)), physical factors (X-rays, UV-B) (35,36), and cytokines (37) (Fig. 4).

### 4. Role of HERV in cancer

Upon their activation, HERVs can be transcribed into full-length mRNA (38), spliced mRNA (39), and non-coding mRNA (40), resulting in either intact protein (41) or truncated protein (42). These HERV elements are found to be widely expressed in a variety of cancers like germ cell cancers (43), colorectal cancer (40), breast cancer (41), prostate cancer (44), ovarian cancer (45), lung cancer (46), melanoma (47), bladder cancer (48), lymphoma (39), hepatobiliary cancer (49), soft tissue sarcoma (50), Kaposi's sarcoma (27), seminomas (51), choriocarcinoma (52), and leukemia (53). Thus, these are potential biomarkers for cancers (49) and may have therapeutic potential if pursued to understand the causal relationship with individual disease types. However, according to the literature, HERVs have a dual opposing role in some cancer where they can either promote carcinogenesis or suppress it (2). Both the aspects of HERV mediated regulation will be discussed in the upcoming sections.

**Cancer promoting effects.** The Env proteins (Eg. syncytins, Np9, Rec) of different HERV groups HK2, HERV-W, HERV-V, HERV-H, and HERV-P (54) have been reported to suppress the immune system (55), affect the cell signaling pathways (56), and trigger cell-to-cell fusion (57) (Fig. 5). Thus, having an oncogenic effect. They may promote cell proliferation, growth, migration, invasion, metastasis, and stemness in different types of cancers, such as breast cancer (41), melanoma (58), leukemia (59), Kaposi's sarcoma (27), pancreatic cancer (60), etc. Therefore, HERV Env proteins are an exciting target for anti-cancer therapy. Few studies have shown that the expression of several HERV groups like HERV-W and HERV-K has contributed to cancer stemness or pluriPotency in colorectal cancer (19) and melanoma cells (58), respectively. However, the underlying mechanisms are yet to be studied. Further, non-allelic recombination of HERV sequences causes their translocation to different regions in the genome. Their new proximity may activate their expression resulting in the activation of specific oncogenes (61) or disruption of a tumor suppressor gene (62). Besides, HERV LTR can act as an alternative promoter that can regulate cellular gene expression, leading to abnormal gene expression, such as switching on of proto-oncogenes, and finally contributing to tumorigenesis (16) (Fig. 6). Also, HERV-E derivative exon E1B has been observed to downregulate the surface expression of CD5 on T-cell in the case of T-cell acute lymphoblastic leukemia (T-ALL), thereby, inhibiting its functions and causing uncontrolled proliferation of leukemic T-cells (63) (refer to section 5.5). Therefore, it can be concluded that HERV's have a major role in oncogenesis, especially the Env protein of different HERV subgroups (3,27,41,56-59,64-67) (Table I).

**Cancer suppressing effects.** On the contrary, HERVs can exert suppressive effects on cancer instead of promoting it. The HERV protein products have been reported to stimulate innate, humoral, and cellular immune responses against malignant tumors by acting as an antigen like PAMP (pathogen-associated molecular patterns) recognized by pattern recognition receptors (PRRs) of immune cells (68). This triggers an immune response and causes the pro-inflammatory signals to exert an anti-viral effect against the HERV antigens by treating them as exogenous infections (2,69). This phenomenon has been reported in the case of clear cell renal cell carcinoma (ccRCC), where the infiltration of CD8+ cytotoxic T-cells triggered by HERV-E antigen was increased in ccRCC patients with hematopoietic stem cell transfer, which negatively affected the cancer progression (70). It might happen due to viral mimicry, which causes the activation of the interferon signaling pathway to upregulate the antitumor immune responses (3,16,71) like HERV-W interacts explicitly with the TLR4 and CD14 receptors, inducing the production of IL-1β, IL-6, and TNF-α pro-inflammatory cytokines. These cytokines further activate the dendritic cells, resulting in a Th-1 response (72). Different HERV Env peptides activate specific cytotoxic T-cells and dendritic cells (DCs) in cancers like ovarian, breast, and colorectal cancer (2). Activation of B-cells and production of antibodies have been seen in the case of breast cancer (73). Hence, it can be concluded that triggering the viral mimicry pathway and targeting the HERV proteins/transcripts can be a potential anti-cancer therapy.

To summarize, HERVs, especially the HK-2 group (Env protein) (3,27,41,56-60,64-67), play an accessory role
in promoting carcinogenesis by suppressing the immune response by inhibiting tumor suppressor genes and activating
multiple oncogenic signaling pathways. However, the viral mimicry effect of the HERV antigens induces an anti-cancer response. Therefore, HERVs remain an attractive target for immunotherapy.

5. Expression of HERV in various cancer types

**Germ cell cancers.** Most germ cell tumors (GCTs) like teratocarcinoma, multiple GCTs, and testicular cancers are known to express HERV-K for a long time. Mueller et al (74) performed a study on different stages of GCTs and suggested that the expression of HERV-K is regulated by the epigenetic mechanisms occurring during different stages of cellular development, which also affects the neighboring cells. Its expression is linked with oncogenesis, migration, and resistance to chemotherapy and correlates with poor prognosis (42). A variety of HERV-K viral particles promote tumor development in multiple GCTs (75). Teratocarcinoma is known to be a classical model for the study of HERV-K. An increase in the Np9 accessory protein in teratocarcinoma has the oncogenic potential (42, 64). An increased expression of Gag protein in teratocarcinoma was also seen due to hypomethylation of the HERV-K sequence (76). Likewise, an increase in syncytin-1 transcribed by HERV-W Env was seen in seminoma patients, which may be involved in oncogenesis (2).

**Breast cancer.** HERV-K is the most reported and studied ERV in breast cancer. It is associated with tumor metastasis and invasion. It is also involved in cancer cell stemness and endothelial to mesenchymal transition (EMT) (41). HERV-K Env is involved in the carcinogenesis of breast cancer. The Env proteins downregulate the p53 cancer suppressor gene, causing the induction of cancer (77). They are also involved in the activation and upregulation of the RAS/ERK pathway, thus, causing the growth and proliferation of tumor cells (78). Anti-HERV-K Env antibody was able to inhibit tumor growth and induced breast cancer cells apoptosis, thus, showing an anti-tumor response (73). Increased HERV ENV, GAG mRNA, and RT (reverse transcriptase) expression in breast cancer are associated with poor prognosis (79, 80). Detection of Env proteins in the early stages suggests that they may be involved in initiating oncogenesis in breast cancer (81). Thus, understanding the downstream function of Env may offer a new therapeutic target besides improving our knowledge. Besides, using vaccines against HERV-K Env may prevent breast cancer (82). Also, HERV-K RT can be used as an early prognostic biomarker for breast cancer as its expression was found in patients who develop cancer (79).

Both HERV-FRD Env and HERV-W Env (syncytin-1 and syncytin-2) proteins are expressed in breast cancer cells, promoting cell-to-cell fusion between endothelial cells and breast cancer cells (65). Non-coding RNA encoded by HERVs also promotes cancer progression in breast cancer. ZMYND8 protein, which is involved in suppressing metastatic cancer genes (VEGFR, TROJAN, CD44, and Slug), is degraded by ubiquitination by long non-coding RNA derived from HERV (83, 84).
Skin cancers. HERV-K protein expression and HERV-K specific antibodies are found in different melanoma cell lines, assisting in cell-to-cell fusion. HERV-K proteins are immunogenic; therefore, antibodies are generated against them, resulting in increased antibody titer, which is correlated with poor prognosis. The \( \text{Env} \) protein maintains the tumor cell stemness and promotes phenotypic switching of tumorigenic cells, making them non-adherent and malignant (3,20). The overall expression of \( \text{Env} \), \( \text{Rec} \), \( \text{Np9} \) and \( \text{Gag} \) has been reported in melanoma patients (85,86). Similarly, HERV-H is also found in a cell line Hs294T of melanoma which promotes dedifferentiation of tumor cells and helps them escape the immune cells (87). Further, HERV-W \( \text{Env} \) is expressed in cutaneous T cell lymphoma (CTCL), which promotes cell fusion (88), similar to its function in mediating trophoblast fusion during placental development (89).

Prostate cancer. Until now, only HERV-K expression has been reported in the case of prostate cancer. HERV-K \( \text{Env} \) protein was upregulated in prostate cancer patients (90). Targeting the \( \text{Env} \) protein via CRISPR/Cas9 downregulated the proto-oncogene \( \text{SF2/ASF} \) and \( \text{RAS} \) pathway expression in prostate cancer cell lines (44). Likewise, HERV-K \( \text{Gag} \) protein expression was also upregulated in prostate cancer due to demethylation and androgen stimulation (91). \( \text{Gag} \) protein expressions are also associated with smoking, old age, and disease status, leading to more aggressive prostate cancer (90). Anti-HERV-K \( \text{Gag} \) antibody titer was increased in stage III and stage IV of cancer compared to stage I and II, promoting carcinogenesis and depicting worse survival (91). Both HERV mRNA and anti-HERV antibodies have been reported to be used as potential biomarkers in prostate cancer (90).
Blood cancers. The expression of HERVs has been reported in acute myeloid leukemia (AML) (12,92-94), acute lymphoblastic leukemia (ALL) (12,93,94), chronic myeloid leukemia (CML) (12), chronic lymphoblastic leukemia (CLL) (94), chronic mixed myeloid leukemia (CMML) (92), non-Hodgkin’s lymphoma (NHL) and other lymphomas (94-96), essential thrombopения (ET) (97), and myelodysplastic syndrome (MDS) (53) (Table II). The Np9 protein expressed by HERV-K activates a cascade of cellular signaling pathways (β-catenin, ERK, AKT, and Notch1), which helps in the proliferation of leukemia cells by increasing leukemia stem and progenitor cells (2,20). Expressions of HERV-K Env and Gag proteins are also reported in lymphoma patients. Likewise, HERV-W Env (syncytin-1) expression is found in leukemic patients and is a potential diagnostic marker (94). How these viral antigens influence oncogenesis is not clearly understood.

In a study performed by Rai et al (63) on T-ALL, exon E1B was observed to be regulating the surface expression of the CD5 gene on the T-cells. Exon E1B is a non-conventional exon of CD5 and a derivative of the HERV-E sequence. Exon E1B expression is seen to be upregulated in the case of T-ALL, while there is downregulation of conventional exon E1A. Due to the lack of leader peptide in the case of exon E1B, surface expression of CD5 is decreased and it is accumulated inside the cytoplasm. Consequently, the negative regulation function of CD5 is compromised, causing the uncontrolled proliferation of leukemic T-cells, thus, promoting carcinogenesis (63).

Kidney cancer. Cytotoxic T-cells were involved in the regression of kidney cancer in clear cell renal cell carcinoma (ccRCC) patients undergoing hematopoietic stem cell transfer. After the investigation, it was found that CT-RCC, a highly immunogenic antigen encoded by HERV-E, induces the activation of CD8+ T-cells and, therefore, triggers an immune response against the RCC cells. This led to tumor regression in-vitro and in-vivo (70). Further, it was found that an increase in the HERV-E expression was strongly correlated with the non-functional von Hippel Lindau (VHL) tumor suppressor gene. Absence of VHL protein induced the expression of HIF-2α, which regulated the expression of HERV-E due to the presence of hypoxia regulatory element (HRE) on the 5' LTR of HERV-E (98-100). A full-length protein of HERV-E, Env expression, was also selectively expressed in ccRCC patients, which can serve as a biomarker for ccRCC (101).

Kaposi's sarcoma. Kaposi's sarcoma is caused by the infection of human herpesvirus 8 (HHV8), also known as Kaposi's sarcoma-associated herpesvirus (KSHV), and is the leading cause of mortality in HIV infection (102). It is characterized by the most common AIDS-related malignancies, which still require effective treatment options. Kaposi's sarcoma is a classic example of activation of HERV through exogenous viral infection. KSHV infection was found to upregulate the production of HERV-K Np9 protein, which advanced the invasiveness of primary endothelial cells by the action of disintegrins and metalloproteinases, contributing to carcinogenesis increasing the morbidity among Kaposi's sarcoma patients (27).

Ovarian cancer. Various HERVs have been reported to be expressed in ovarian cancer. Both HERV-K Env and RT expressions were increased in ovarian cancer compared to adjacent healthy and benign tissues. HERV-K Env antigens triggered the proliferation and activation of specific cytotoxic T-cells and IFNy production. This led to the lysis of autologous tumor cells (45). Also, demethylation of ovarian cancer cells by DNA methyltransferase inhibitors (DNMTis) induces the production of double-stranded RNA (dsRNA) of HERV, which activates the viral defense pathway, enhancing the innate immune response and apoptosis (103).

Colorectal cancer. Various HERV expression in colorectal cancer (CRC) has been reported, including HERV-K,
HERV-K is involved in cell growth, proliferation, migration, and colonization (20). Expression of HERV-W is correlated with poor prognosis in syncytial cancer (104). HERV-H Env exerts an immune-modulatory effect (40). HERVs are also suggested to be used as a biomarker and clinical examination for better predicting CRC patient survival (105).

Pancreatic cancer. HERV-K Env plays a significant role in pancreatic cancer, whose high expression is associated with a poor prognosis. It promotes tumor cell proliferation, growth, and metastasis. In particular, HERV-K Env interferes with the signal transduction pathway RAS/ERK/RSK pathway and thus promotes carcinogenesis. HERV-K RT activity was also observed in pancreatic cancer tissues (60).

**6. Conclusion**

HERVs have been associated with cancer for a long time. Their abnormal level of expression has been found in a variety of cancers. Different groups of HERV are found to be overexpressed in different cancers. Multiple factors are responsible for
their activation like epigenetic dysregulation (30), exogenous infections (26–29), radiations (35,36), cytokines (37), chemical induction (31–34), etc. They encode highly immunogenic antigens whose expression can promote or inhibit cancer advancement by modulating the immune system. HERVs are correlated with tumor cell proliferation, migration, decreased apoptosis, endothelial to mesenchymal transition (EMT), and immune suppression, thus initiating and promoting oncogenesis (20). Since the expression of HERV is a natural phenomenon, each HERV protein must be characterized separately to elucidate its role in the pathogenesis of different cancer and other diseases. Future studies may shed light on the effect of vaccination against a specific epitope of HERV elements and monoclonal antibody (MAB) on the control and prevention of certain cancers. It suggests the need to develop an onco-immunotherapy approach for rapidly evolving cancer types.

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