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REVIEW

K-type human endogenous retroviral elements in human melanoma

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Abstract: Human endogenous retroviral elements (HERVs) are thought to be germline-integrated genetic remnants of exogenous retroviral infections. HERVs comprise approximately 5%–8% of the human genome. Although all HERV genomes are highly defective, some, especially the K type (HERV-K), have the potential to be expressed and have biological activities. HERV-K expression has been detected in human melanomas. There are also reports on the regulation and potential activities of HERV-K in melanoma cells. Although a causal link between the activation of HERV-K and melanoma development has yet to be determined, existing data support the further research efforts in this area. In this review, we summarize the published studies on the expression, regulation, and activity of HERV-K in human melanoma.

Keywords: HERV, HERV-K, melanoma development

Introduction

Human endogenous retroviral elements (HERVs) comprise approximately 5%–8% of the human genome.[1–3] It is hypothesized that HERVs were once exogenous retroviruses that became incorporated into the human germline, which resulted in a vertical transfer of HERV DNA elements into the human genome, and thus HERVs became endogenous to the human genome.[5] A complete HERV element has common sequences shared by other well-known retroviruses such as the human immunodeficiency virus, the human T lymphotropic virus, the mouse mammary tumor virus (MMTV), and the murine leukemia virus.[6] These well-studied viruses can exist as proviruses in the host genome, and commonly contain open reading frames of group-specific antigen (*gag*), polymerase (*pol*), and envelope (*env*) that are flanked by non-coding regulatory sequences, namely long terminal repeats (LTRs).[1–3,5] Similarly, HERVs can have homologous sequences of *GAG, POL, ENV, and LTR*. With open reading frames, the *GAG*, *POL*, and *ENV* sequences may be transcribed and translated to the corresponding proteins, similar to retroviruses. Thus, *GAG* encodes core and structural proteins; *POL* encodes reverse transcriptase (RT), protease, and integrase; and *ENV* encodes envelop proteins (Figure 1).

HERVs are degenerated, defective, and non-infectious due to the accumulated mutations and deletions within their DNA sequences. Without an established function, they are often referred to as part of the "junk DNA" of the human genome. Although expression of HERVs is limited due to their degenerated genetic sequences, some HERVs retain relatively complete genetic sequences, capable of encoding functional proteins,[3] while others play *cis*-regulatory roles in gene transcription.[7,8] If expressed in active form, as in retroviruses, RT and integrase may generate new copies of HERV

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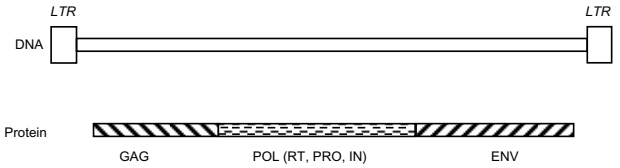

Figure 1 HERV genome organization.
Notes: Similar to exogenous retroviruses, a complete HERV sequence is composed of group-specific antigen (*GAG*), polymerase (*POL*), and envelope (*ENV*) genes flanked by two long terminal repeats (*LTR*s). An intact HERV genome is approximately 9,700 nucleotides long. Although most HERVs are degenerated with disruptive open reading frames, a few have retained intact genes, and the corresponding proteins can thus be expressed. It has been reported that some HERV-Ks have open reading frames that can encode functional proteins to make viral-like particles. As in wild-type retroviral sequences, *GAG* codes for the GAG protein that provides the basic physical infrastructure of retroviral-like particles; *POL* codes for enzyme reverse transcriptase (RT), protease (PRO), and integrase (IN); *ENV* codes for proteins embedded in the envelope which could enable the viral-like particles to attach to and fuse with target cells. Flanking functional genes are *LTR* regions that play important roles in initiating DNA synthesis and its integration, as well as regulating transcription of HERV-K genes.
Abbreviations: HERV, human endogenous retroviral element; HERV-K, K-type human endogenous retroviral element.

DNA from messenger RNA and insert them back into the human genome. Protease might cause proteolytic cleavages to make mature GAG and POL proteins, and ENV might mediate cellular entry/exit of viral-like particles.[5,9]

HERVs can be classified into more than 20 families based on the transfer RNA specificity of the primer binding site used to initiate reverse transcription; thus, HERV-K would bind lysine and HERV-W, tryptophan, if they were replicating viruses.[10] HERVs have also been broadly grouped into three classes based on phylogenetic studies with exogenous retroviruses.[11] Class I HERVs are closer to gamma retroviruses, which include HERV-H and HERV-W; class II are closer to beta retroviruses and alpha retroviruses, and include HERV-K; class III are closer to spuma retroviruses, which include HERV-L and HERV-S.[11] Class I and class III HERVs are older and less evolved viruses, compared to class II HERV-K, which has the most complete sequence and, therefore, most likely to be biologically active in the human genome.[12] An alternative name for HERV-K is MMTV-like HML, due to HERV-K's sequence homology with MMTV.[13,14] Based on sequence homology, HERV-Ks are further classified into ten families, HML-1 to HML-10.[15] Unlike most HERVs that harbor defective mutations, HERV-Ks have retained intact genes, can code for functional proteins, and may form viral-like particles.[6,15–17]

Studies have shown that HERV DNA can be expressed and may play important roles in the physiological and pathological processes of human cells. For example, through millions of years of evolution, HERVs have become indispensable parts of the human genome, as demonstrated by syncytin-1 (ERVW-1), encoded by the *ENV* gene of HERV-W. Syncytin-1 mediates intercellular fusion of trophoblast cells to form syncytiotrophoblasts and prevents maternal immune attack against the developing embryo, thereby facilitating embryo implantation.[2,3] HERV LTR sequences have integrated into human coding sequences, for example, as parts of splicing variants of methylthioadenosine phosphorylase gene (*MTAP*). Mutations in the LTR sequences in *MTAP* cause diaphyseal medullary stenosis with malignant fibrous histiocytoma.[18] HERV-K proteins and virus-like particles have been detected not only in the placenta,[19] but also in cancer cells, including teratocarcinoma,[20] breast cancer,[21] and melanoma.[22] Up-regulation of HERV expression has been implicated in the pathogenesis of malignancy, inflammation, and autoimmune diseases.[3,23]

Melanoma is one of the most prevalent malignancies and has a very bleak prognosis. The rates of melanoma have been rising for the past 30 years. The American Cancer Society estimate that about 76,100 new cases of melanomas will be diagnosed, and about 9,710 melanoma patients are expected to die in the United States in 2014.[24] Melanoma arises from melanocytes. The majority of melanomas arise in the skin; other sites of origin include oral and anogenital mucosal surfaces, esophagus, meninges, and eye. The natural history of melanomas involve multiple stages, often start with precursor lesions, progress from the primary site to regional or sentinel lymph nodes, and metastasize to distant sites. Melanomagenesis is driven by multiple genetic and epigenetic changes and presents multiple cancer hallmarks.[25–27] Melanoma is characterized by early metastasis and is resistant to therapies.[28,29] A better understanding of melanoma biology is necessary for the diagnosis and treatment of this devastating disease. It has been described that the abnormal expression of HERV-K seems to precipitate the pathological process leading to melanoma and also contributes to the morphological and functional cellular modifications implicated in melanoma maintenance and progression. In this review, we summarize the published literature on HERV-K expression, regulation, and potential activity in human melanoma.

Expression of HERV-K elements in human melanoma

The expression of HERV-K in human melanoma was first discovered during the characterization of melanoma antigens, which are families of antigens specifically recognized on human melanoma cells by cytolytic T lymphocytes.[30,31]

A short reading frame of the HERV-K *ENV* gene, namely *HERV-K-MEL*, encodes a melanoma antigen peptide on the autologous melanoma tumor cells.[32] It was subsequently shown that human melanoma cells can produce retrovirus-like particles that exhibit RT activity and comprise mature GAG and ENV proteins.[22]

HERV-Ks exist in the human genome in multiple copies, generated through reinfection or intracellular formation of new copies following initial germline invasion.[1–3] A recent study analyzed more than 1,400 HML-2 cDNA sequences that are transcribed in the human melanoma cell lines and melanoma tissues, and identified 23 HERV-K loci distributed on at least eleven chromosomes.[33] This study underscores the complexity of HERV-K sequences, which is challenging when trying to assign specific loci for expressed HERV-K sequences. We also found that careful DNase I digestion is necessary to remove "contaminating" cellular DNA in order to accurately evaluate HERV-K RNA expression.[34] As retroviral elements, HERV-K sequences do not have introns which are otherwise present in nearly all eukaryotic genes. The human genome harbors tens if not hundreds of HERV-K loci with homologous sequences. Without removal of genomic DNA (gDNA), HERV-K gDNA copies may be amplified, detected, and interpreted as HERV-K cDNA. This represents a technical challenge for the detection and quantification of human gene transcripts when using primers and probes that cannot distinguish gDNA from cDNA.

Regulation of HERV-K expression in melanoma cells

In general, HERV-K expression is suppressed. When expressed, there are differences in expression between cell and tissue types (Figure 2), which indicates that the expression is somehow regulated. However, the precise mechanism leading to the expression of HERV-K has yet to be clearly elucidated. Both endogenous and exogenous factors that can regulate the expression of HERV-K in melanoma cells have been identified (Table 1). Like other human genes, HERV-K transcription is regulated by *cis*-acting promoter and enhancer elements and *trans*-acting transcription factors.[35] It has been shown that HERV-K LTR sequences contain promoter and enhancer elements that interact with melanocytic lineage-specific transcription factor microphthalmia-associated transcription factor (MITF),[35] which may be necessary for HERV-K expression in melanoma cells. MITF is essential for the development of retinal pigmented epithelium and neural crest-derived melanocytes. MITF also induces genes that are essential for melanin synthesis and melanosome formation, and genes that support cell cycle progression and cell survival. MITF gene is classified as a lineage-specific oncogene based on its gene

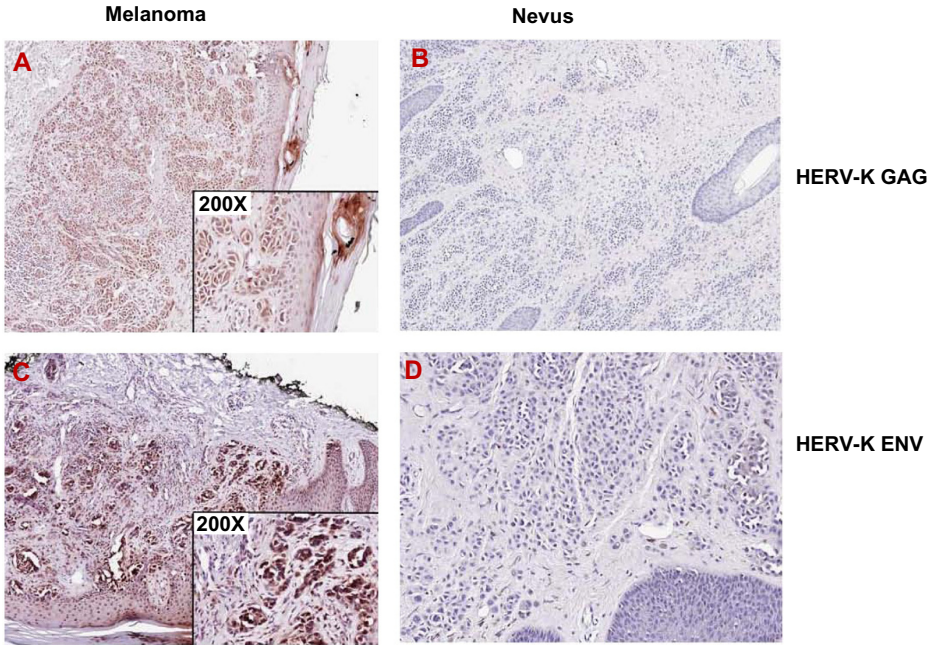

Figure 2 Expression of HERV-K GAG and ENV in melanoma.
Notes: HERV-K GAG (**A** and **B**) and ENV (**C** and **D**) are strongly expressed in melanoma (**A** and **C**, 100X and 200X), but not in nevus (**B** and **C**, 100X). Formalin-fixed and paraffin-embedded microscopic sections of human melanoma (**A** and **C**) and nevus (**B** and **D**) were analyzed by immunohistochemical staining (IHC) using HERV-K GAG (**A** and **B**), and ENV (**C** and **D**) specific antibodies. IHC was performed as previously described.[4] Adapted from Li Z, Sheng T, Wan X, et al, *Cancer Invest*, 2010;28(10):1031–1037. Copyright © 2010, Informa Healthcare. Adapted with permission of Informa Healthcare.[4]
Abbreviations: HERV-K, K-type human endogenous retroviral element; GAG, group-specific antigen; ENV, envelope protein.

Table 1 Factors that regulate the expression of HERV-K in melanoma cells

Factors	Effects	References
cis DNA elements	Promoter and enhancer elements in LTR. Positive or negative regulation depends on sequence motifs and interacting transcription factors	35
Transcription factors	MITF-M serves as transcription activator of HERV-K	35
DNA methylation	Suppresses HERV-K transcription	37
MEK-ERK pathway	HERV-K expression positively correlates with MEK-ERK pathway activation	4
p16-CDK4-RB pathway	HERV-K expression negatively correlates with p16 expression and CDK4 inhibition	4
UV radiation (UVB, UVC)	UV radiation can activate or repress HERV-K expression	33,43,44

Abbreviations: HERV-K, K-type human endogenous retroviral element; LTR, long terminal repeat; MITF-M, M-isoform microphthalmia-associated transcription factor; UV, ultraviolet; UVB, ultraviolet B; UVC, ultraviolet C.

amplification and growth promoting function in melanoma. MITF gives rise to various isoforms, MITF-A, -B, -C, -H, and -M, depending of the N-terminal sequences. Only the MITF-M isoform undergoes melanocyte/melanoma-specific transcription in response to the alpha-melanocyte-stimulating hormone signaling.[36] Katoh et al[35] studied the HERV-K transcription controlling mechanism by determining the initiator (Inr) sites and enhancer sequences in the LTR, and found that an arrangement of (MITF motifs)-(TATA box)-(Inr) functions as the core enhancer/promoter inducible by MITF-M. The study suggests that MITF-M may be a prerequisite for the pigmented-cell-lineage-specific function of HERV-K LTR, leading to a high level of expression in malignant melanomas.[35] Like other human genes, HERV-K promoter and enhancer elements are regulated by CpG DNA methylation.[37]

Li et al[4] have recently reported that the expression of HERV-K *GAG* and *ENV* correlates with p16 loss and ERK activation in melanoma specimens, and that CDK4 and MEK inhibitors can suppress HERV-K expression in cultured melanoma cells. This study suggests that HERV-K is downstream of p16-CDK4-RB and MEK-ERK pathways, which is significant given the critical importance of these pathways in melanoma biology and as proven and potential targets for melanoma treatment.[38–40]

Exposure to ultraviolet (UV) radiation (UVR) is a major environmental risk factor contributing to skin cancers, including melanoma.[25,41] Sources of UV include natural sunlight and artificial lights such as tanning and germicidal lamps and lasers. UV radiation is divided into three categories, based on its wavelength: UVA (315–400 nm), UVB (280–315 nm), and UVC (100–280 nm).[41] Cutaneous absorption of both UVA and UVB can reach the dermis, whereas UVC is limited to the epidermis. Therefore, all the three wavelengths of UVR can reach melanocytes that are located in the basal layers of the epidermis.[41,42] UV radiation can cause mutations by direct interaction with DNA or indirectly through the generation of reactive oxygen species.[25,41] Expression of HERV-K sequences in melanocytes and melanoma cells can be regulated by UVR.[33,43,44] Reiche et al[43] showed that after exposure to UVC, the expression of HERV-K was up-regulated in normal human epidermal melanocytes, but down-regulated or remained unchanged in melanoma cell lines. This study is consistent with a role, if any, of UVC and HERV-K in the initiation stage of melanoma development. Schanab et al[44] reported that irradiation with UVB resulted in a significant transcriptional activation of HERV-K *POL* and *ENV* genes, and induced the production of retroviral-like particles. Since the positive effects were not observed in benign melanocytes, this study supports a role of UVB in the malignant progression of melanoma cells. On the other hand, Schmitt et al[33] found that the overall HERV-K *GAG* gene transcripts were reduced both in melanocytes and melanoma cell lines after UVB radiation, and there were comparable responses in cultured melanoma and melanocytes. Interestingly, the study demonstrated that different HERV-K loci do not necessarily have comparable responses to UVB radiation, pointing to locus-specific regulation of HERV-K. These studies underscore the complex regulation of HERV-K by UV radiation; and the fact that HERV-K expression is dependent on factors such as the types and strength of UV radiation, melanocyte versus melanoma cells, and the presence of specific HERV-K loci and HERV-K genes.

Potential activities of HERV-K in melanoma

Although the activity of HERV-K in melanoma is not completely understood, clues have been revealed through a number of studies (Table 2). HERV-K has been reported to encode antigen epitope HERV-K-MEL, which can activate cell-mediated immunity, and serves as a melanoma vaccine candidate.[32,45] Conversely, in immunocompetent mice, the inhibition of murine melanoma-associated retrovirus (MelARV) resulted in a reduction of melanoma aggressiveness and rejection of

Table 2 HERV-K activities in melanoma cells

Activities	Phenotypes	HERV-K genes	References
Encode antigen (HERV-K-MEL) that activates cell-mediated immunity	HERV-K-MEL is recognized by cytotoxic T-lymphocytes (CTLs) on the autologous tumor cells of melanoma patient; potential vaccine candidate	*ENV*	32,45,63
Encode antigen that inhibits cell-mediated immunity	Active regulatory T cells that mediate subversion of immunosurveillance	Murine melanoma-associated retrovirus (MelARV)	46*
Encode antigens that induce humoral immunity	Production of antibodies that correlate with worse prognosis	Immunodominant epitopes on GAG and ENV proteins	49–51
Cause insertional mutagenesis by retro-transposition	Insertion in the introns of integrin α2 (*itgα2*) and docking protein 5 (*dok5*) gene, causes changes in gene expression, cell motility, and metastasis of B16 melanoma cells	Murine melanoma-associated retrovirus (MelARV)	48*
Promote malignant growth in vitro	Promote proliferation and colony formation, and inhibit morphological differentiation	*POL*, *GAG*, and *ENV*	52–54
Promote malignant growth in vivo	Promote tumor growth and formation of metastasis		46–48

Note: *Studies of murine endogenous retrovirus (MelARV).
Abbreviations: HERV-K, K-type human endogenous retroviral element; HERV-K-MEL, the HERV-K envelope antigen; ENV, envelope protein; GAG, group-specific antigen; POL, polymerase protein.

melanoma cells inoculated in nude mice, suggesting that MelARV acts to evade the immune system.[46–48] It has also been reported that, compared to healthy individuals, sera from patients with melanoma were much more likely to harbor HERV-K, ENV, and GAG antibodies.[49–51] The antibody concentration varied from 16%–22% depending on the study, but was negligible in healthy controls and correlated with worse prognosis.[49–51] These studies show that HERV-K may have a positive or negative effect on cell-mediated and humoral immune systems.

Retrotransposition-mediated mutagenesis has been reported in murine melanoma cells, which promotes tumor cell mobility, metastasis, and in vivo growth.[48] Another function of HERV-K in melanoma, proposed by Huang et al,[52] is the blockage of HERV-K by RNA interference and monoclonal antibodies in melanoma cells, resulting in a marked reduction in colonic growth of melanoma cells in vitro.[52] The down-regulation of the HERV-K reverse transcriptase by RNA interference or pharmacological inhibitors resulted in a lower mitotic rate and induced morphological differentiation,[52–54] and slowed down melanoma growth and formation of metastasis in vivo.[46–48]

Conclusion

In summary, the human genome projects have identified approximately 20,000 protein-coding genes and 55,000 non-coding loci with evidence of gene transcription.[55–57] Significant numbers of both protein-coding and non-coding loci contain mobile genetic elements consisting of DNA transposons and retro-elements.[58–60] HERVs are a type of retro-element, with HERV-K being the more complete with open reading frames that have been expressed and have biological activities in melanoma and other human cells. Expression of HERV-K has been detected in melanoma cells and is increasingly implicated in melanoma pathogenesis. Recently, HERV-K has been studied as a molecular biomarker for melanoma and other malignancies, and as a vaccine candidate for cancer treatment.[61–63] Further studies are warranted to examine and validate the activation and function of HERV-K in melanoma and other cancers, which should help improve the diagnosis and treatment of melanoma and other human diseases.

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Disclosure

The authors report no conflicts of interest in this work.

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