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Review Article

Molecular Phylogenetic Analysis of Human Endogenous Retroviruses with Associated Malignancies

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Abstract

Human Endogenous Retroviruses (HERVs) are remnants of ancient retroviral infections with similarities to exogenous retroviruses and make up 8% of the entire human genome. HERVs are found to play a role in carcinogenesis. We sought to analyze 37HERVs in relation to 16 types of cancers using phylogenetic analysis. HERVs nucleotide sequences were aligned and corrected manually. A neighbor-joining method was used to create a phylogenetic tree using CLUSTAL X2 version 2.1.0.0 algorithm. Two thousand (2000) replications were employed for bootstrap probabilities in creating the tree. The results obtained were systematically analyzed as they relate to different types of cancer. The phylogenetic analysis shows three main branches of HERVs. The first main branch was made up of HERV-H, HER-F, HERV-S71-related, ERV9, MSRV, HERV-K1.1, HERV-E, HERV-R, HERV-I, RTLVH, HERV-S and HRES-1. The second main branch was composed of HERV-T, HERV-P, HERV-FRD, HERV-KHTDV, and HERV-W. The third main branch contains majorly HERV-Ks family, HERV-L, HERV-P-T47D and XMRV. The HERV-Ks family is the most homologous among all the HERVs and also ubiquitous in terms of cancer tissues expression. The youngest sub-class of the HERV-Ks - HERV-K 133, and HERV-KHML 1.1, together with the widely debated XMRV were nested in the same group and present about 60% similarity. HERV-F and HERV-H present 70% similarity. HERV-S-71-Related, ERV9 and MSRV showed 100% similarity. Testing for HERVs, which phylogenetically present high sequence homology with each other, may help further research in the use of HERVs as an agent of immunotherapeutic target in cancer management.

INTRODUCTION

HERVs are remnants of ancient retroviral infections with similarities to exogenous retroviruses that were believed to have been vertically transmitted to humans about 1 to 40 million years ago or even earlier [1-8]. About 8% of the entire human genome is made up of HERV sequences [9]. HERVs have been reported to be actively present in human placental tissues and also implicated in tumorigenesis [10-12]. The HERVs also serve several important functions [12] that range from formation of placental syncytiotrophoblasts and immune defense mechanism [13,14] to a role in gene transcription [3,14]. Several HERVs such as HERV-K contain viral enzyme Polymerase (pol), the group specific antigen (gag) and glycoprotein of the viron envelope (env) flanked by 5' and 3' Long Terminal Repeats (LTRs) [2,4] of which the exogenous retroviruses also have.

The LTRs contain direct Repeat (R) together with the U5 and U3 regions and regulate viral gene transcription. The *gag*gene encodes for structural proteins of which the Matrix (MA), Capsid (CA) and Nucleocapsid (NC) proteins are its product, and participate in viral assembly during budding. The

pol gene encodes for the Reverse Transcriptase Enzyme (RT), Ribonuclease H (RNase H) and Integrase (IN) enzymes for protein cleavage, viral replication and integration. The *env* gene codes for viral envelop Surface (SU) proteins and Transmembrane (TM) protein components produced and modified through proteolytic cleavage and glycosylation respectively which help in viral fusion.

Accumulated mutations, multiple termination sequences or re-combinational silencing of internal coding regions render many HERVs non-replicative [16,8]. However, clear underlying pathways in which HERVs result to carcinogenesis have not been well defined. The current data suggest that HERVs most likely is implicated in different type of cancer through multifactorial pathway.

MATERIALS AND METHODS

Nucleotide sequence mining

References and accession numbers of published thirty seven most reported HERVs nucleotide sequences were used in this study as deposited in the GenBank database [17]. Full details of the accession numbers and method of identification of the HERVs sequences used were clearly highlighted in Table 1a-d.

Phylogenetic analyses

Phylogenetic analysis employs nucleotide or protein sequences to classify organism based on their developmental relationships. The evolutionary tree obtained from phylogenetic analysis is referred to as cladogram. The phylogenetic relationships within 37 HERVs subgroup were studied using selected known human endogenous retroviruses whose sequences were mined from the NCBI Genome Browser [17]. The phylogenetic analysis was conducted by building a tree irrespective of the env, gag, pol nucleotide sequence of the HERVS because we discovered these have negligible effect on creating the tree most especially since we are not considering the differences in age of the HERVs. HERVs sequences without appropriate linkage to a published article "with the exception of Xenotropic Murine Leukaemia Virus-Related Virus (XMRV)" were excluded from the analysis. In creating the phylogenetic tree, nucleotide sequences were aligned and corrected manually. All positions with an alignment gap in at-least one sequence were excluded from the sequence comparison. The neighbor-joining method [48] was used to create the phylogenetic tree using CLUSTAL X2 version 2.1.0.0 algorithm [49]. Two thousand replication was employed for bootstrap probabilities in every tree. A TREEVIEW program [50] was used to visualize the phylogenetic tree. Each of the trees obtained was mapped to the malignant tumour implicated in colour coding format using paint.inc - Microsoft Windows [51].

RESULT

From the result of the phylogenetic analysis, a majority of the HERV-Ks studied were found to be nested in the same class and present a similarity of about 100% (Figure 2). The HERV-Ks were found to be expressionally ubiquitous in different cancer tissues and cell lines (Table 2, 3c). Table 2 show the topology of HERVs as grouped by the phylogenetic analysis into 3 divisions. The table also highlighted those of HERVs that have been associated to certain malignancies. A phylogenetic tree showing HERV-F and HERV-H with about 70% similarity on the same clad was obtained (Figure 2). Interestingly, HERV-S-71-Related, ERV9 (HERV. pHE.1) and MSRV were phylogenetically confirmed to have about 100% similarity. Perhaps, MSRV and ERV9 conserved motifs were reported to be similar with 2 amino acids shorter than other HERV types [53].

HERV-K1.1 and HERV-E with HERV R (ERV-3) have more than 70% similarity and were co-located on the same clad. The HERV-I, RTLV-H, HERV-S and HRES-1 (HERV-18) present about 50% similarity on the same clad (Figure 2). HERV-T, HERV-P, HERV FRD (ERV-FRD), HERV-K-HTDV and the HERV-W were found to be nested on the same clad with about 90% similarity. Several cancer types were showed to have relationship with HERVs protein expression as tabulated in table 2.

Table 1a: Summary of studies on identification of HERVs with molecular methods and samples of identification employed.

Type of HERVs	Accession number	Methods	Samples used	Comments
HERV-F pol (Yi and Kim. [18])	AB120696	RT-PCR and Sequencing	Different human tissues, and cancer cell lines	HERV-F family over expressed
HERV-H (Shiraishi *et al.* [19])	1902980	*In situ* Hybridization and PCR	Small cell lung cancer tissues, and yeast cells	HERV-H is close to the GRPR locus on X chromosome
HERV-S pol (Yi *et al.* [20])	AB162188	PCR and Western blot	Blood samples and cell lines	HERV-S was integrated about 43 Million years ago
HERV-S71-related gag and pol (Haltmeier *et al.* [21])	U12969	PCR and Southern blot	Blood and tissue samples.	*Pol* genes are not prerequisite for insertion mutagenesis of HERVs
ERV9 (Mantia *et al.* [22])	X57147	Northern blot	NT2/D1 cell line	ERV-9 sequences identified
MSRV pol (Perron *et al.* [23])	AF009668	RT-PCR	Human blood and CSF from	MSRV was related to, but distinct from ERV9
HERV K1.1 (Zsíros *et al.* [24])	HSU87589	RT-PCR	Bone marrow, blood and tissue cells	Strong selection may be operating on HERV-K RT gene segments.
HERV-E env (Yi and Kim. [25])	AB098322	PCR	Blood samples	HERV-E over expressed.
ERV3 pol-env-3'LTR (Cohen *et al.* [26])	M12140	DNA sequencing	Human foetal tissues	ERV may encode other retroviral proteins-like polypeptides.

Table 1b: Summary of studies on identification of HERVs with molecular methods and samples of identification employed.

Type of HERVs and study	Accession number	Methods	Samples used	Comments
HERV-I *pol* (Forsman *et al.* [27])	AY836237	RT-PCR	Human tissues, blood and saliva	HERV-I over expressed
RTVL-H (Johansen *et al.* [28])	M27826	Northern-blot	Blood samples and placenta.	RTVL-H subtypes were over expressed.
HRES-1 (Perl *et al.* [29])	X16660	Southern and Northern blots	Human blood , *E.coli*, and Bacteriophage	HRES-1/1 was active in melanoma, and lymphoid cells.
HERV-T pol (Forsman *et al.*, [27])	AY836234	PCR	Blood, tissue and saliva samples	HERV- T and I were over expressed
HERV-P env (Yi *et al.* [30])	AB240045	RT-PCR	human tissues and cell lines	HERV-P *gag* gene over expressed
ERV FRD *pol* (Seifarth *et al.* [31])	U27240	RT-PCR and Southern blot	T47-D cells	T47-D cell line may be generated by complementation of HERVs
HERV-K/HTDV (Casau *et al.* [32])	AF148679	RT-PCR, Southern blot, and IF	Human tissue samples nad c cell lines.	Germ line expression aid expansion, Somatic repression limit insertional mutagenesis
HERV-W env (Strausberg *et al.* [33])	BC137381	Genetic and Phylogenetic analysis.	Tissue samples and cell lines.	More than 9, 000 human and mouse gene were sequenced and verified.
HERV-K(HML-7.107) pol (Muradrasoli *et al.* [34])	AY615723	Q-PCR	Human blood and tissue fluids	Brain, adrenal gland and testis contain beta retrovirus-like sequences

Table 1c: Summary of studies on identification of HERVs with molecular methods and samples of identification employed.

Type of HERVs	Accession number	Methods	Samples used	Comments
HERV-KC4 (Tassabehji *et al.* [35])	X80240	Southern blot and DNA sequencing	Somatic and Primate samples	HERV-KC4 contributes to interlocus and interallelic length heterogeneity of C4 genes.
HERV-K18 env (Conrad *et al.*, [36]; Stauffer *et al.* [37])	AF012336	PCR, immune-cytochemical stain.	Serological sample from IDDM patients	MHC class II–dependent SAG via IFN- by HERVs explains environmental factors links to genetically susceptible IDDM
HERV-K4 complete (Romano *et al.* [38])	DQ112097	Genetics and phylogenetic analysis	Human and chimpanzee genomes	76 complete ERV-K elements identified.
HERV-K11 complete (Romano *et al.* [38])	DQ112099	Genetic and phylogenetic analysis	Human and chimpanzee genomes	54 HERV-K element identified
HERV-K8 complete (Romano *et al.* [39])	DQ112094	Genetic and phylogenetic analysis	Human and chimpanzee genomes	54 HERV-K element identified
HERV-K5 complete (Romano *et al.* [38])	DQ112093	Genetic and phylogenetic analysis	Human and chimpanzee genomes	HERVs were involved in chromosomal rearrangements.
HERV-K complete sequence (Tönjes *et al.* [39])	Y18890	PCR	λ bacteriophage, Plasmids, COS7, D17, GH cell lines	Human genome may not contain all intact HERV-K proviral copy
HERV-K10 complete (Ono *et al.* [40])	M14123	Genetic and phylogenetic analysis	Human foetal liver cells	HERV-K env gene expressed
HERV-K2 (HML-2.HOM) complete (Mayer *et al.* [41])	AF074086	PCR and FISH	Serological samples from Europe, Africa, and Asia	Chromosome 7p22 contains complete HERV-K *gag* and *env*. This is the most intact of all HERVs.

Table 1d: Summary of studies on identification of HERVs with molecular methods and samples of identification employed.

Type of HERVs	Accession number	Methods	Samples used	Comments
HERV-K 108 complete Barbulescu *et al.* [42])	AF164614	PCR, and other genetic analysis	Human and primate samples	HERV-K OFR and the cis-like sequence were crucial for proviral replication
HERV-K12 complete (Romano *et al.* [38])	DQ112143	Genetic and phylogenetic analysis	Human and chimpanzee genomes specimen	54 HERV-K element identified
HERV-K7 complete (Romano *et al.* [38])	DQ112131	Genetic and phylogenetic analysis	Human and chimpanzee genomes	54 HERV-K element identified
HERV-K6 complete (Romano *et al.* [38])	DQ112120	Genetic and phylogenetic analysis	Human and chimpanzee genomes	54 HERV-K element identified
HERV L complete (Cordonnier *et al.* [43])	X89211	Southern blot, RT-PCR and phylogenetic analysis	Human placenta tissues	HERV-L with *pol* gene was associated to foamy retroviruses

HERV-K 3.1 gag (Contreras-Galindo *et al.* [44])	DQ157715	RT-PCR	Blood samples	HERV-K (HML-2) were over expressed in HIV patients
HERV-P-T47D (Seifarth *et al.* [45])	AF087913	Genetic and phylogenetic analyses	T47D cell line	2 types exist. Expressed in liver and kidney.
HERV-K113 3'LTR (Turner *et al.* [46])	AF387849	PCR	Human DNA samples	HERV-K113 lacks may re-infect humans
HERVK/HML 1.1 pol Medstrand, and Bloomberg [47].	U35102	PCR and northern blot	Human serological and tissue samples	HML-1, 2, 3, 4, 5 and 6 were present
XMRV gag (Genbank [17])	JN99019	-	-	-

Table 2: The topology of HERVs as grouped by the phylogenetic analysis into 3 division and associated cancers.

DIVISION 1	DIVISION 2	DIVISION 3
Group 1: HERV-F[B] HERV-H[M,P,X,V,L,C] **Group 2:** HERV-S-71-Related ERV9 (HERV. pHE.1) MSRV **Group 3:** HERV-K1.1[Y,U,V,K,L,B,C] HERV-E[Y] HERV R (ERV-3)[Y,U,V,L] **GROUP 4:** HERV-I[B] RTLV-H HERV-S HRES-1 (HERV-18)	**Group 1:** HERV-T[B] HERV-P[L,C] HERV FRD (ERV-FRD)[B] **GROUP 2:** HERV-K-HTDV[B,C,V,P] HERV-W	**GROUP 1:** HERV-K (HML-7) **GROUP 2:** HERV-KC4 **GROUP 3:** HERV-K-18 HERV-K4 HERV-K11 **GROUP 4:** HERV-K8 HERV-K-5 HERV-K (complete) HERV-10 HERV-K (HML-2)[B,M,P,O,J,N,U,X,V,b,C,H] HERV-K108 **Group 5:** HERV-K12 HERV-K7 HERV-K6 **Group 6:** HERV-L HERV-K1.3 HERV-P-T47D **Group 7:** HERV-K113 HERV-K (HML-1.1) XMRV[Y]

[B]Breast cancer, [P]Testicular cancer, [Y]Prostate cancer, [J]Gastric cancer, [O]Cervical cancer, [N]Leukemia/Lymphoma, [M]Melanoma, [X]Pancreatic cancer, [U]Lung Cancer, [V]Endometrial cancer, [K]Kidney cancer, [L]Liver cancer, [b]Bladder cancer, [C]Colorectal cancer, Stomach cancer[S], Head and neck Cancer[h]

DISCUSSION

In this study, phylogenetic analysis was used in assessing the relationship between major HERVs and cancer types. Notably, chemical carcinogens [54] such as 5-aza-deoxycytidine – pyrimidine-5 substitute [55] radiations [54] such as ultraviolet-B radiation, [56,57] and smoking [58] exogenous viruses [54] such as human immunodeficiency virus, [59,60] herpes simplex virus-1, [61,62] human cytomegalovirus, [63] Epstein-Barr virus and human papillomavirus [64] mitogens such as phytohaemagglutinin and phorbol-12-myristate-13-acetate [65-67] cytokines such as interleukin-1α, interleukin -1β, tumour necrosis factor –α, and IFN-γ [67-69] hormones such as steroid hormones [70,71] vitamins such as retinoic acid [72,73] and bacterial infections such as *Toxoplasma gondii* [74] may switch-on the HERVs genetic components leading to, but not limited to carcinogenesis as schematically represented in Figure 1. Table 2 show a phylogenetic presentation of HERVs into 3 divisions. The arrow used in the table 2 was aimed to indicate those HERV-Ks that phylogenetically presented high homology with each other and to also indicate the considerable ubiquitous expression of HERV-Ks family in cancers. This phylogenetic classification might help further future research in not cancer alone but, several other neurodegenerative disorder, multiple sclerosis and rheumatoid arthritis. Interestingly, about 12 cancers where noted to be implicated with HERV-Ks family over expression. Perhaps, HERV-KHML2, HERV-K10 and HERV-K12 are the most common types of HERV-Ks family members much often implicated in disease causation. Flockerzi *et al.,* have reported the identification of 23 transcriptionally active HERV-K (HML-2) proviruses [52].

The study showed that HERV-F and HERV-H were found to be nested in the large class I HERV clade with 70% similarity thus closely related. This finding agrees with the results of Kjellman *et al.* which showed that there was a close similarity between HERV-F and HERV-H [75]. The HERV-F (XA34) was reported to be located on human chromosome 7q31.1–q31.3 [76] and over expressed in breast cancer [6,18] (Table 3a). HERV-H

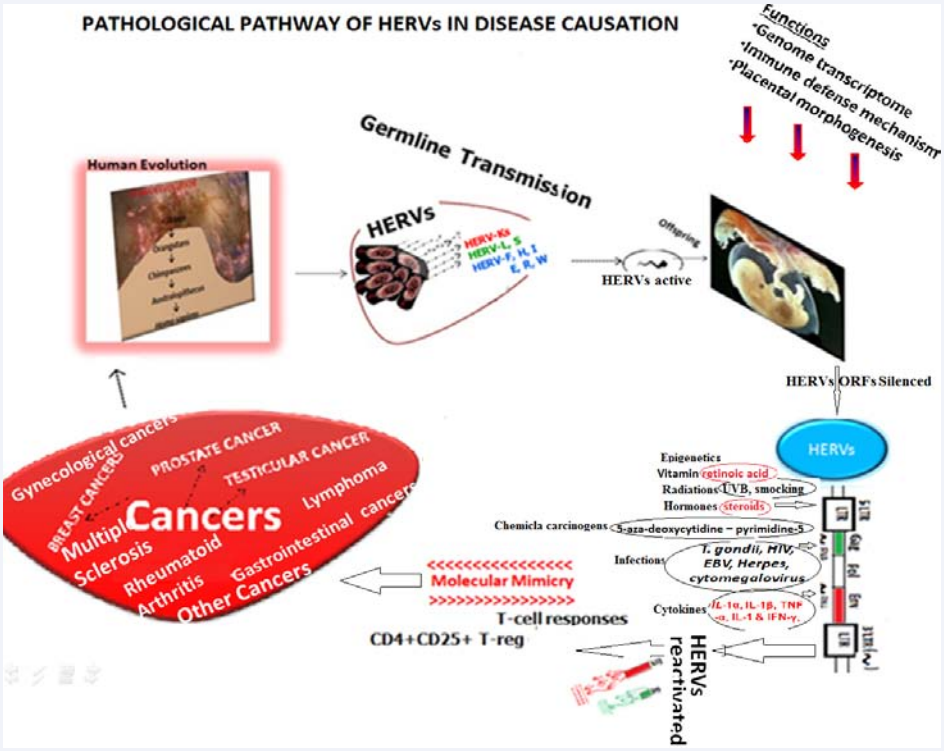

Figure 1 The human endogenous retroviruses that include but, not limited to the "super group" (HERV-Ks), HERV-L, HERV-F, HERV-I, HERV-E, HERV-W were vertically transmitted in to humans through the germ line millions of years ago. The HERVs serve several beneficial roles that include genome transcription to protection against maternal immune response [5]. HERVs contain canonical structure of single open reading frame (ORF) made up of *pol, gag*, and *env* genes flanked by 5' and 3' long terminal repeats (LTR)[2] like most exogenous retroviruses. Perhaps, radiations, hormonal changes, vitamins, infections, and other environmental agents may reactivate these silenced HERVs components towards the causation of disease as highlighted in the discussion. Env proteins of HERVs have the capacity for modulating the expression of several cytokines, which in turn act on T lymphocytes and negatively skew the immunological activities [102]. Thus, several malignancies such as breast, prostate, cervical, colorectal, testicular cancers, lymphomas and malignant melanoma were noted to be associated with over expressed of the HERV transcript proteins (Table 3).

was reported to be highly abundant in human genome base on pol similarity [77] and was integrated in humans around 30 million years ago [78,79]. The HERV-H was also reported to be over expressed and located on X chromosome in lung, [19,80] testicular [80] colorectal [81-84] gastric, [81] pancreatic [81] liver [80] and endometrial cancers [85] (Table 3a). The over expression of HERV-H in endometrial cancer was related to immunosuppressive [86] role possibly through the env gene. The expressions of HERV-F in human cancer tissues were less frequently reported as compared to HERV-H. However, the close phylogenetic similarity of HERV-H and HERV-F with up to 70% similarity (Figure 2)may implies that HERV-F might also be expressed in all the human cancer tissues implicated by HERV-H. The same principle may apply to HERV-H expression in breast cancer since HERV-F was reported to be implicated.

The phylogenetic analysis showed that HERV-S71-relatedwith accession number U12969 [21] has close similarity with ERV-9 or HERV pHE.1 (accession number X57147) [22] and MSRV (accession number AF009668) [23] with both creating a single phylogenetic group with up to 100% similarity as showed in Figure II. This apparently agrees with the report of Perron *et al.,* which indicated that there was a similarity between ERV-9 and MSRV [23]. The HERV-S71-1 (envT) was reported to be expressed in endometrial cancer tissues [85] (Table 3a). However, the phylogenetic analysis contradictorily showed that nucleotide sequence of multiple sclerosis-associated retrovirus [(MSRV) accession number AF009668] was not in a phylogenetic manner closely related to HERV-W (accession number BC137381), as previously cited by Perron *et al* [23]. However, the possibility that the two sequences used might be from different source cannot be ruled out. In essence, each one of the HERVs may serve the function of the other especially when they can be used for immunological target in several diseases. Also, from the same class of HERVs in Figure 1, HERV-K1.1 with accession number HSU87589 [24] was co-nested on the same group with the widely reported HERV-E (accession number AB098322) [25] and ERV-3/HERV-R (accession number M12140) [26]. The HERV-E and ERV-3 presented with about 90% similarity whereasHERV-K1.1 and HERV-E/R showed about 75% sequence similarity (Figure 2).The nucleotide codon position of the HERV-K1.1 was reported to show a strong selection, which may be operating on HERV-K RT gene segments [24].

The ERV3/HERV-R was also reported to have the capability of encoding other retroviral proteins-like polypeptides [26]. ERV3 have been found to be expressed in different cancers. In this context it is interesting that Kewitz and Stage found a down regulation of ERV3 in proliferating cancer cells [130]. The ERV-3/HERV-R whose integration was dated back to about 42

Table 3a: HERVs Implicated in malignancies and method of identification employed.

HERV Type	Study	Methods	Cancer type	Sample(s) used	Comments	Refs.
HERV-F	Frank, *et al.*, 2008	RT-PCR and qRT-PCR	Breast cancer	Human breast cancer tissues and T47D cell line cell lines.	HERV-T and HERV-FRD were also present	[6]
HERV-H	Ahn and Kim, 2009	RT-PCR	Lung and testicular Cancers	Lung, testicular and several other cancer tissues.	HERV-H transcript over expressed.	[80]
HERV-H	Wentzensen *et al.*, 2007	RT-PCR and northern blot.	Colon, gastric and pancreatic cancers.	Human tissue samples and tissue cell lines and several cancer tissues and cell lines.	HERV-H on Xp22.3 was over expressed in colon cancer tissues but not in SW480 and HT29.	[81]
HERV-H (gag)	Alves *et al.*, 2008; Liang et al., 2009; Liang, *et al.*, 2012	RT-PCR	Colorectal cancer	Human colon cancer tissue and SW480, SW620, LS174T, RKO, and HT29 colon cancer cell lines.	HERV-H *gag* transcript protein over expressed	[82,83,84]
HERV-H and HERV-S71-1	Strissel *et al.,* 2012	RT-PCR, qPCR, and IHC.	Endometrial cancer	38 endometrial Cancer tissue samples and cancer cell lines.	HERV-H and HERV-S71-1 over expressed	[85]
ERV-3 (env)	Wang-Johanning *et al.*, 2003; Ahn and Kim, 2009; Andersson *et al.,* 1998; Wang-Johanning *et al.*, 2006	PCR, RNA *in situ* hybridization, Northern blot and ELISA.	Prostate, liver and lung , endometrial and ovarian cancers	Prostate, liver, lung, endometrial and several other cancer tissues samples. DU145, LNCap, PC3, and GP6F2 cell lines.	HERV-R over expressed and can be of important immunotherapeutic targets.	[80,85,,89,90]
HERV-E (env)	Wang-Johanning *et al.*, 2006	RT-PCR, flow cytometry, IHC, IF, and ELISA	Ovarian cancer	Ovarian cancer tissues and SKOV3, OVCA 430, OVCA 433, OVCA 420, OVCAR3, DOV 13 and OVCA 429 cancer cell lines. NOE 114, NOE 116, NOE 113 and NOE 119, T29, T72 and T80 normal ovarian cell lines.	HERV-K env proteins present	[90]

Table 3b: HERVs Implicated in malignancies and method of identification employed.

HERV Type	Study	Methods	Cancer type	Sample(s) used	Comments	Refs.
HERV-E (env)	Frank, *et al.*, 2008; Yi and Kim, 2007; Wang-Johanning *et al.*, 2003	PCR, RNA *in situ* hybridization, and Northern blot , phylogenetic analysis	Breast, prostate, liver, lung, ovary, leukaemia, skin, lymphoma, kidney, bladder, brain, colon, oesophagus, pancrease, stomach and cervical cancers.	Human breast cancer BT-474, MCF7 and T47D; DU145, LNCap, PC3, and GP6F2; HepG2; A549; OVCAR-3; Jurkat; LOX-IMVI; 2F7 and U-937; UO-31; RT4; PFSK-1; HCT-116; TE-1; MIA-PaCa-2; AZ521; and C-33A cancer cell lines. Several other tissue samples.	HERV-E env transcripts are over expressed and can be used in immunotherapeutic target.	[6, 88,92]
HERV-E (env)	Takahashi *et al.*, 2008; Strissel *et al.*, 2012	RT-PCR, flow cytometry and IHC	Kidney and endometrial cancers.	Several human endometrial and kidney tissue samples and blood samples	HERV-E (envE) over expressed	[14,85]
HERV- W (env)	Frank, *et al.*, 2008; Strissel *et al.*, 2012; Menendez, *et al.*, 2004; Gimenez, *et al.*, 2010	RT-PCR and qRT-PCR, southern blot and IHC	Breast, cancer, endometrial, ovarian, and testicular cancers.	Human tissue cells and tissue cell lines.	HERV-W (syncytin -1) over expressed	[6,85,96,97]
HERV-T and HERV-FRD.	Frank, *et al.*, 2008	RT-PCR and qRT-PCR	Breast cancer	Human tissue cells and tissue cell lines.	HERV-T and HERV-FRD present	[6]
HERV-P (*env* and *gag*)	Yi *et al.* 2007; Ahn and Kim, 2009	RT-PCR	Liver, colon, oesophagus, stomach, brain, ovary, kidney, T- cell, prostate, and skin cancer cell lines	Several human cancer cell lines.	HERV-P env and gag genes were over expressed.	[30,80]

million years ago [87] is also widely reported to be expressed in prostate, [88] liver, lung, [80,89] ovarian [90] and endometrial cancers [86] (Table 3a). The HERV-E was widely reported to be over expressed in breast, [6,88,92] and prostate [91,92] cancers (Table 3b).The over expression of HERV-E was also noted in melanoma, lymphoma, leukemia, gastric, stomach, colorectal, cervical, liver, lung and bladder cancers [92] (Table 3b). The HERV-E env protein was also reported to be over expressed in ovarian, [90,92] endometrial [85] and kidney [92,14] cancers (Table 3b). Perhaps, HERV-R/ERV-3 may also be expressed in most malignant tissues implicated by HERV-E. Taking in to consideration the nucleotide sequence identity of HERV-E and ERV-3/HERV-R, which was up to 90% (Figure 2) and the wide nature of expression of HERV-E in different cancer types, it can be suggested that the HERV-E and ERV-3/HERV-R proteins may be interchangeably employed within each other as agent of immunotherapeutic target in different cancer types.

Nucleotide sequences comparison of HERV-I (accession numberAY836237) [27] RTVL-H with (accession number M27826), [28] HERV-S (accession number AB162188) [20] and HRES-1/HERV-18 with (accession numberX16660) [29] showed them phylogenetically nested in the same group with similarity of about 50%. The HERV-S was said to have integrated into the primate genomes approximately 43 Million years ago [20]. The HERV-I, RTVL-H and HRES-1/HERV-18 were respectively reported to be active in human brain and testicular tissues, human placenta, and melanoma and lymphoid cells [27-29].

The phylogenetic sequence analysis grouped HERV-T (accession number AY836234) [27], HERV-P (accession number AB240045) [92], HERV-FRD (accession number U27240), [31] HERV-K/HTDV (accession number AF148679), and HERV-W (accession number BC137381) in the same class (Figure 2). There sult also grouped HERV-T and HERV-P in the same sub-class (Figure 2). HERV-T, HERV-P, HML5 and HERV-S were noted to be among the families of HERVs with about 60 copies thus regarded as small-to-medium-sized HERVs member [93]. HERV-W that was reported to play role in placental morphogenesis [94] and is the only HERV type, which was reported to be functionally copied by those genomic elements that lack regulatory LTRs [95]. The HERV-W was reported be over expressed in breast [6], endometrial [85], ovarian [96] and testicular [97] cancers (Table 3b). HERV-T and HERV-FRD were both reported to be over expressed in breast cancer [6]. HERV-P*env* gene was reported to be over expressed in liver and colorectal cancers [80] (Table 3b). In addition, the *gag* gene of HERV-P was reported to be over expressed in colon, esophagus, stomach, brain, ovary, kidney, T cell, prostate, and skin cancer cell lines [30] (Table 3b). The high sequence similarity between HERV-W, HERV-K/HTDV, HERV-P, HERV-FRD and HERV-T as indicated by the study may imply possible exchangeable role of the HERVs in terms of expression and immunotherapeutic target in several cancer types.

Nucleotide sequence comparison of different HERV types as in Figure 2 showed that the HERV-K18 (AF012336), HERV-K4 (DQ112097), HERV-K11 (DQ112099), HERV-K8 (DQ112094),

Table 3c: HERVs Implicated in malignancies and method of identification employed.

HERV Type	Study	Methods	Cancer type	Sample(s) used	Comments	Refs.
HERV-K (*env*)	Frank, *et al.*, 2008; Ono *et al.,* 1987; Wang-Johanning *et al.*, 2001; Wang-Johanning *et al.*, 2006; Ejthadi *et al.,* 2005; Contreras-Galindo *et al.*, 2008; Zhao *et al.,* 2011; Wang-Johanning *et al.,* 2012; Agoni *et al.*, 2013;	Southern, northern, and western blots. PCR, insitu hybridisation, IHC, EM, immunoflorescence, ELISA, flow cytometry and phylogenetic analysis.	Breast cancer	T47D, BT-20, ZR-75-1, MCF-7, SKBr-3, MDA-MB-231, MDA-MB-453, BT-474, T47D, Tera I, Tera II, BT-474, BT549, SKBR3, ZR-75-1 breast cancer cell lines. Several cancer tissues, blood samples with MCF-10A and MCF-10AT non-malignant breast cell lines.	HERV-K (HML-2, 3, 4, 6) envelop proteins are over expressed. The over expressed may be related to T-cell responses stimulated by dentritic cell.	[6, 70, 90, 98, 99, 100, 101, 102, 103]
HERV-K (*gag*)	Ishida*et al.,* 2008; Agoni*et al.*, 2013	RT-PCR, qRT-PCR, Western blot, H/E and IHC	Leukaemia, melanoma, prostate, lung, and ovarian cancers.	Human tissues and cell lines	HERV-K gag protein expression may be important in immunotherapeutic target.	[103,104]
HERV-K (env)	Wang-Johanning *et al.*, 2006; Ahn and Kim, 2009; Roelofs *et al.*, 1998; Goedert *et al.,* 1999; Kaufmann *et al.*, 2010	RT-PCR, Flow cytometry, IHC, Immunoflorescence, and ELISA	Ovarian and testicular cancers	Human Ovarian cancer tissues, cell lines and 293T testicular cancer cell line.	HERV-K (HERV-K10) or HERV-K env over expressed.	[80,90.105,106, 107]
HERV-K	Gattoni-celli *et al.*, 1996; Agoni*et al.*, 2013	Southern blot, RT-PCR and qRT-PCR	Head and neck, and colorectal cancers	HCT and $Caco_2$ cell lines	HERV-K protein was expressed	[103, 108]
HERV-K HML2	Contreras-Galindo *et al.*, 2008; Wildschutte et al., 2014	RT-PCR, western blot, EM, and in-silico methods	Lymphoma	Breast cancer blood/tissue samples from 18 and 50 subjects respectively	HERV-K (HML2) is over expressed.	[100, 129]
HERV-K (env and rec)	Büscher *et al.*, 2005	RT-PCR, western blot, IHC, and IF.	Melanoma	Several cancer cell lines.	Full-length mRNA env, and rec protein of HERV-K present	[110]

Table 3d: HERVs Implicated in malignancies and method of identification employed.

HERV Type	Study	Methods	Cancer type	Sample(s) used	Comments	Refs.
HERV-K	Agoni *et al.,* 2013	RT-PCR and qRT-PCR	Cervical cancer	Several cancer cell lines	HERV-K transcript protein present	[103]
HERV-K (MEL)	Schmitz-Winnenthal *et al.,* 2007	RT-PCR	Pancreatic Cancer	130 pancreatic cancer tissue samples and 23 controls	HERV-K-MEL protein present, may be of immumnotherapeutic target	[111]
HERV-K HML2 (sLTR)	Kahyo *et al.,* 2013	RT-PCR	Lung cancer	Human lung cancer tissue samples	HERV-K (HML-2) sLTR on19q12 and 1p13.2 present	[124]
HERV-K (env)	Wang-Johanning *et al.,* 2006; Florl *et al.,* 1999	RT-PCR, Southern blot, Flow cytom-etry, IHC, Immuno-florescence, and ELISA	Ovarian and bladder Cancers	Several human tissue samples and cell lines. Part of the cell lines include Tera-1, NCCIT, and GH, Pa 1, AV3, JAR, Molt 4 T-cell and rhabdomyosarcoma A204 cell lines	HERV-K env over expressed. The HERV –K type 1 and 2 in urothelial carcinoma was linked to DNA hypomethylation	[90, 112]
XMRV	Urisman *et al.,* 2006; Schlaberg *et al.,* 2009; Arnold *et al.,* 2010; Dong *et al.,* 2007; Kim *et al.*, 2008; Cingöz *et al.,* 2012	PCR, Western blot, H/E, IHC, FISH, IF, EM, Florescence in situ hybridisation	Prostate cancer	Several prostatic FFPE tissues, peripheral blood DNA and 293T cell line	XMRV was Over-expressed in prostate cancer specimens.	[113-117]
XMRV	Hohn, *et al.*, 2009; Aloia *et al.,* 2010; Yang, *et al.,* 2010; Lee *et al.,* 2012; Stieler *et al.,* 2012	PCR, IHC, EM, ELISA western blot and Single nucleotide polymorphism technique	Prostate cancer	Several FFPE prostate cancer tissues and CWR22 22Rv1 and 293T cell lines	No XMRV over-expression in prostate cancer both at DNA and the RNA level.	[118-122]

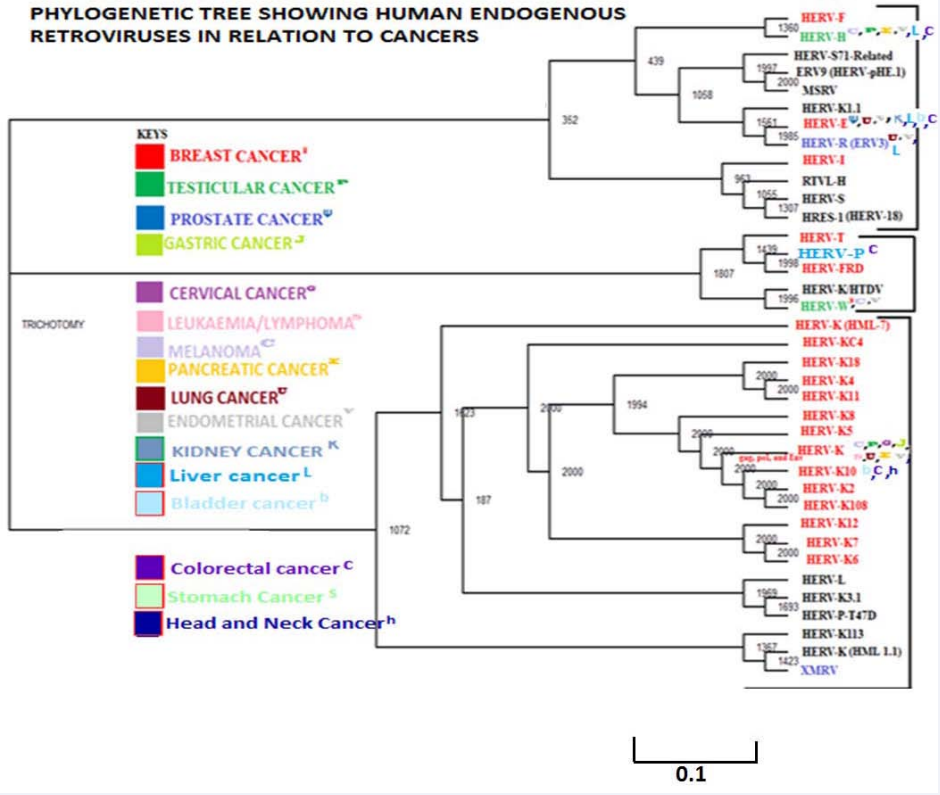

Figure 2 Phylogenetic tree of human endogenous retroviruses involved in cancers. HERV-F and HERV-H present about 70% similarity; HERV-S-71-Related and ERV9 (HERV. pHE.1) with MSRV have about 100% similarity; HERV-K1.1 and HERV-E with HERV R (ERV-3) present with more than 70% similarity; HERV-I, RTLV-H, HERV-S and HRES-1 (HERV-18) show about 50% similarity. HERV-T, HERV-P, HERV FRD (ERV-FRD), HERV-K-HTDV and the HERV-W were found to be nested on the same clad with about 90% similarity. With the exclusion of HERV-K/HTDV, HERV-K1.1, HERV-K113, HML.1.1, and HERV-K3.1 all other fourteen types of have HERV-Ks were on the same class with similarity of 100%.

HERV-K5 (DQ112093), HERV-K [full-length(Y18890)], HERV-K10 (M14123), HERV-K2 (AF074086), HERV-K 108 (AF164614),HERV-K12 (DQ112143),HERV-K7 (DQ112131) and HERV-K6 (DQ112120) phylogenetically possess sequence similarity of about 100% (Figure 2). Several reports documented the expression of HML-1, 2, 3, 4, 5, 6 in human genome [47]. Increased level of HERV-K (HML-2) in a population of breast cancer cells strengthened hypothesis recent insertion of the retrovirus [129]. HERV-K4, HERV-K11, HERV-K8, HERV-K5, HERV-K12, HERV-K7, and HERV-K 6 were also reported to be expressed in human and chimpanzee genomes [38]. The HERV-K HML 7 and HERV-KC4 were found nested independently each as a class of its own in the HERV-K family. Report have implicated that the integration of HERV-KC4 was the major contributor to interlocus and inter allelic length heterogeneity of C4 genes [35]. The sequence analysis indicated that the "HERV-Ks super family" is the most homologous among all the HERVs and also ubiquitous in terms of expression in cancer tissues.

The youngest sub-class of HERV-Ks; HERV-K113 (AF387849),HERVK/HML 1.1(U35102) and the most widely debated Xenotropic Murine Leukemia Virus-related-virus [(XMRV) JN990139] were showed nested in the same group of HERV-K super-family with sequence similarity of about 60% (Figure 2).It has been reported that HERV-K113 was composed of full-length open reading frames for viral proteins, and lacks non-synonymous substitutions in amino acid motifs, thus serves as a good infectious type of HERVs [46]. Also, in the super-family, was the presence of HERV-L (X89211), HERV-K clone 3.1 *gag* (DQ157715) and HERV-P-T47D (AF087913) with more than 70% nucleotide sequence similarity. The HERV-K/HML-7.107 (AY615723) and HERV-KC4 (X80240) were each independently contested with the HERV-Ks super-family (Figure 2). HERV-K transcript proteins were widely reported to be significantly expressed in Breast [6, 70, 98,99,101-103] prostate [103,104] testicular [80,105-107] and colorectal [108] cancers (Table 3c). The over expression of HERV-K transcript protein in colorectal cancer was related to a role in lymph node metastasis, tumor staging and size, and decreased survival rate of colorectal cancer patients [101]. The HERV-K transcript proteins were also reported to be over expressed in leukemia [104] lymphoma [100], head and neck, [103] gastric [109] and cervical [103] cancers (Table 3c). Over expression of HERV-K was reported significantly in melanoma [104,110] pancreatic, [111] lung, [104] ovarian [90,104] and bladder [112] cancers (Table 3d). Perhaps, HERV-K Rec protein was reported to be capable of unblocking oncogenic transcription factors [107]. The XMRV was controversially reported to be implicated in prostate cancer. There are research reports that argue for [113-117] or against [118-122] the over expression of XMRV in prostate cancer (Table 3d). A greater number of evidence in term of molecular techniques and samples used apparently indicated no significant relationship between XMRV and prostate cancer. Convincingly, it is now accepted that XMRV has no association with human prostate cancer [126-128].

Relationship between Human Endogenous retroviruses and exogenous retroviruses

As earlier reported, Human Endogenous Retro Viruses (HERVs) have been implicated in the causation of some cancers notably breast, prostate, testicular and gastric cancers. Other cancers like cervical, Leukemia's and lymphomas, lung and colorectal cancers are also linked to HERVs over expression. About 8% of the human genome is made up of these endogenous retrovirus sequencies [9] which could be either beneficial or oncogenic. while, exogenous retroviruses that contributes about12% of human cancers worldwide [131] are implicated in the causation of kaposi sarcomanon-Hodgkin lymphoma and cervical cancer In Human Immunodeficiency Virus (HIV) infection; Burkitt's, Hodgkin's and Non-Hodgkin's lymphomas and nasopharyngeal carcinoma in Epstein-Barr Virus (EBV) infection; hepatocellular cancer in Hepatitis C and B virus (HCV and HBV) infections; cervical, vaginal, vulvar, penile, and oral carcinomas in high-risk Human Papilloma Viruses (HPVs) infections. See Mesri et al., [132] and Gopal et al., [133] for review. The exogenous retroviruses are different from the endogenous retroviruses in that their genomic sequences are active and ex-vivo while those of the endogenous retroviruses are quiescent. The genomic sequences of the endogenous retroviruses can be activated through a lot of factors like chemical carcinogens, bacterial infections, and most importantly interaction between the sequences and exogenous retroviruses. They retroviruses may interact with each other through recombination, pseudo typing, trans-activation and phenotypic mixing. Thus, the exogenous virus may activate the quiescent cellular genetic components of endogenous retrovirus making them replication proficient. Even though, their association may sometime not lead to replication as typified by the endogenous retrovirus envelop glycoproteins blocking infection from the exogenous virus with a similar glycoprotein called Human apolipo protein B mRNA-editing enzyme catalytic polypeptide-like (APOBEC3) protein family which is shown to protect against HIV-1 infection [134] possibly via endogenous retrovirus trans-activation and retrotransposition. However, the APOBEC3 protein family over expression is also linked to cancer causation [135]. Perhaps, the implication of HERV-K in breast cancer may not be unrelated to APOBEC3 activity [136].

CONCLUSION

In conclusion, it should be noted however that virtually all humans might have been vertically infected with particular class of viruses termed "human endogenous retroviruses HERVs" through the germ line mutations especially after the Pan troglodytes lineage evolutionarily diverged [125]. Certain types of these HERVs contain canonical structure of single ORF made up of *pol, gag*, and *env* genes flanked by 5' and 3' LTR [2], in similarity to most exogenous retroviruses such as HIV viruses. However, several HERVs lack infectious capacity due to silencing of the LTRs. Perhaps, radiations, hormonal changes, vitamins, infections, and other environmental agents may reactivate these silenced HERVs components. The LTR also contains terminal inverted repeats; polyadenylation signal and TATAA box [40]. These HERVs play important roles that range from genome transcription to protection against maternal immune response. Interestingly, HERV-K-MEL antigen encoded by *env* gene was noted to be made up of protective amino acid sequence against melanoma and also closely related to antigenic components expressed in BCG, vaccinia virus and the yellow fever virus vaccines [8]. HERV-K transcript protein was noted as s contributor

to lymph node metastasis, tumor staging and size, and decreased survival rate of colorectal cancer patients [101]. Over expression of HERV-K env proteins in breast cancer have encouraged the development of anti-HERV-K monoclonal antibody which was able to inhibit breast cancer growth [102]. The over expression of different types HERVs such as HERV-H in endometrial cancer was also linked to immune suppression. Perhaps, this phylogenetic analysis result have interestingly demonstrated a new promising means that may need to be further investigated towards simplification of research on human endogenous retrovirus in malignancies and other diseases. Putting in mind the report, which indicated that immunization against HERVs can induce antitumor immunity [123], and other information demystified by this study, it can be inferred that testing for HERVs which present high sequence homology with each other may help further research in the use of HERVs as an agent of immunotherapeutic target in cancer management. Conceivably, future research in this area also needs to take into consideration the various factors that will employ different HERVs as immunological targets towards cancer management/prevention.

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