Do the Human Endogenous Retroviruses Play a Role in Colon Cancer?

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ABSTRACT: Long terminal repeat retroelements comprise about 8% of the human genome and include the human endogenous retroviruses (HERVs). Earllier it was suspected that HERVs can become active and be involved in the process of transformation of cells, through several oncogenic mechanisms. Abnormal expression of HERVs proteins has been reported for various types of cancer, such as melanoma, breast, prostate, and germ cell cancer, in which encoded transcripts or proteins are overexpressed in the tumor tissues. However, less is known about the association between the HERVs and the colon cancer development. We review the state of the art for colon cancer with respect to the HERVs that can be considered as an open area of investigation, potentially leading to future innovative diagnostic and therapeutic approaches.

KEYWORDS: human endogenous retroviruses (HERV), colon cancer, HERV-H

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Introduction

Transposable elements (TEs) are DNA fragments capable of self-reproducing and changing their location in the host genome, ie, to transpose. These selfish repetitive elements proliferate either directly via their DNA copies (DNA transposons) or through RNA intermediates (retroelements), utilizing the reverse transcription method.[1] Sequencing of the human genome has highlighted that about 45% of the overall proportion of the genome is constituted of TEs and over 90% of these are retroelements. Transposons currently mobilizing on the human chromosomes include the long interspersed elements (LINEs), short interspersed elements (SINEs), and "SINE-R, variable of number of tandem repeats (VNTRs) and Alu (SVA) elements", all of which are in the "non-LTR retrotransposons" family. The only active mobile DNAs are the autonomous L1 retrotrasposon, belonging to the LINE family, and replicating by a simple "copy and paste" mechanism involving target-site primed reverse transcription.[2] Functional human-specific L1 insertion passed in the germline have negative effects on fitness, but continue to be a source of genetic diversity.[3] In addition to acting as insertional mutagens, retrotransposons can disrupt gene function and genomic integrity in many other ways, such as recombination-mediated gene rearrangements, genetic instability, transcriptional interference, alternative splicing, gene breaking, epigenetic effects, the generation of DNA double-strand breaks, and the expression of small noncoding RNAs.[4] All of these mechanisms are compatible with a tumorigenic potential of these elements.

Long terminal repeat (LTR) retroelements comprise about 8% of the human genome and can be divided into three groups: LTR-bounded elements, endogenous retroviruses (ERVs), and LTR retrotransposons.[5] ERVs have a similar genetic organization as exogenous retroviruses with two long LTRs encompassing the internal coding sequence of the three basic retroviral genes: group-specific antigen (*gag*), polymerase (*pol*), and envelope (*env*; Fig. 1). ERVs have been found in all vertebrates, including humans.[5,6]

Human endogenous retroviruses. Human endogenous retroviruses (HERVs) represent a "relic" of ancestral exogenous retroviral infection and entered primate genomes over 30 million years ago. Due to the requirement of a proviral stage in the retroviral life cycle, after the infection of the germ line, cells have preserved the HERVs as a "fossil record" of ancestral retroviral infections.[7] Afterward, each time an HERV-infected germ cell develops into offspring, it will transmit its provirus to every cell of the offspring that expands in the population, ultimately achieving fixation, or that becomes extinct by random events or selection pressure.[5] The life cycle of the HERVs comprises reverse transcription of the viral genomic RNA, followed by the integration of a nascent DNA copy into the genomic DNA of the host cell.[8,9] Retroviral genomic RNA differs from genomic copy by the absence of LTRs, which are built during the reverse transcription, a multistep complex

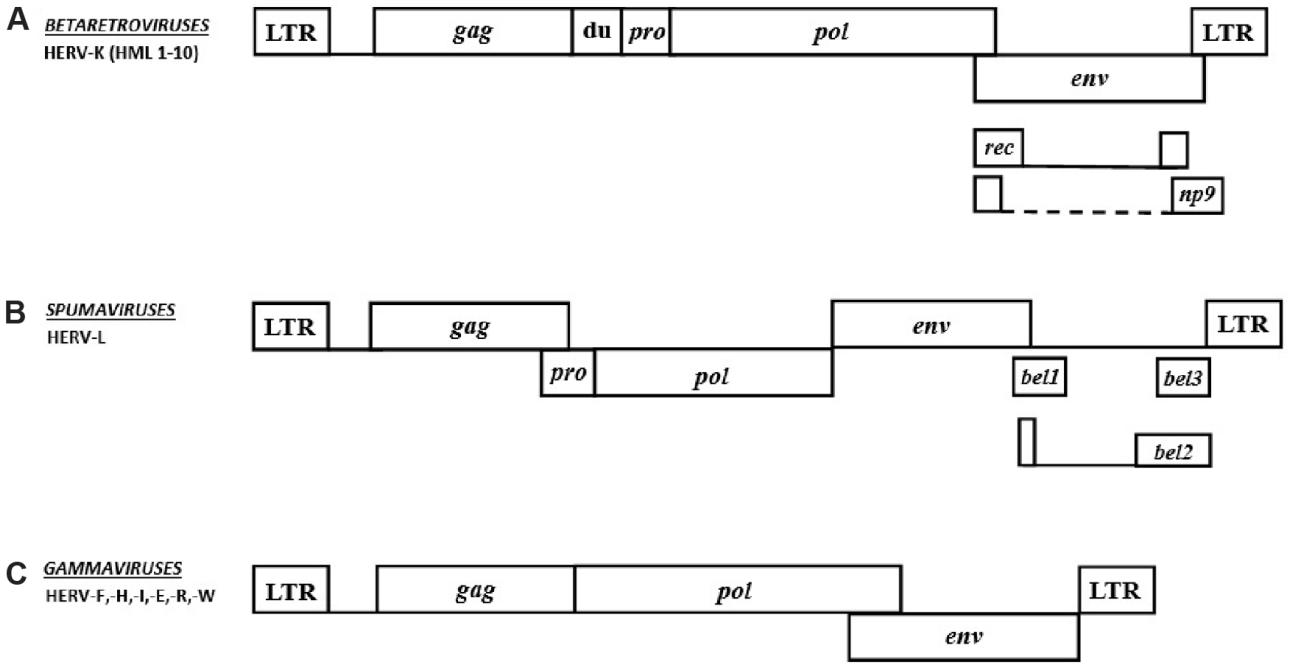

Figure 1. Schematic genome organization of three different classes of HERVs (drawings are not to scale).
Abbreviations: HERV, human endogenous retrovirus; LTR, long terminal repeat, consisting of the U3, R, and U5 regions; *gag*, group-specific antigen; du, dUTPase; *pro*, protease; *pol*, polymerase (reverse transcriptase and integrase); *env*, envelope; *bel 1–3* encode small additional proteins. *rec* and *np9* are two accessory proteins arising from *env* splice variants.

process including several template-switching events.[10] Since the first integration, most HERVs have been severely damaged in their original genetic structure from the accumulation of mutations, insertions, and deletions up to the total excision, through homologous recombination, of the internal coding region between the two flanking LTRs.[6] Furthermore, even if not transcribed or translated, LTRs can change the transcriptional regulation of neighboring genes by supplying new promoters and definitely contribute to genomic plasticity during primate evolution. This indicates that they could be used as genetic markers for understanding evolutionary history.[11] To date, no HERV has been shown to replicate and produce infectious viruses except for the human species-specific HERV-K (HML2), which still retains some proteins coding potential. In this regard, there was a report of retrovirus-like particles secreted in a steroid-dependent manner by human mammary carcinoma cell line by Faff et al.[12,13]

Important issues of HERVs research are their nomenclature and classification. Historically, names of HERVs are linked to the different approaches and methodologies applied for their identification, leading to a puzzle of names difficult to be interpreted and translated.[6] Although till now there is no standard nomenclature for HERVs, a classification based on sequences homology to different genera of exogenous retroviruses has generally been adopted.[5,6] HERVs are divided into three classes based on phylogenetic analysis: HERVs sequences broadly clustering with gamma and epsilon retroviruses have been termed "Class I", those clustering with beta retroviruses as "Class II", and those having the greatest homology to spumaviruses as "Class III" (Fig. 1).[5,14] Well-defined groups within the different classes are named "families", that generally represent single invasion followed by a copy number expansion within the host's genome.[15] Traditionally, HERVs families have been named after the amino acid carried by the tRNA, complementary to the primer binding site of the HERVs genome (for instance, HERV-K, HERV-H, HERV-W, HERV-R, and so on). To date, a total of 40 families have been defined.[16,17] The ubiquitous nature of HERVs and the plurality of their molecular functions stress the importance of organizing and maintaining related databases.[18] A complete database of HERVs does not exist, but two complete reviews by Vargiu et al[6] and Suntsova et al[18] described the identification and classification of 3173 HERVs proviral sequences in the human genome, summarizing the most important HERVs database and the story of their implementation.

Role of HERVs in the Human Genome: The Positive Side

The integration of ERVs in mammalian genomes has been known since 1970s.[19] The presence of multiple copies, as well as the possible sharing of protein products among distinct groups of retroelements, underlines the marked potential of distinct endogenous retroelement loci to interact with one another, proposing HERVs as elements that facilitate the regulatory network evolution.[20,21] The potential of HERVs to induce an immune response depends on their expression but may be

further influenced by the combination of interacting retroelements that are expressed in certain cell types.[20] Endogenous retroelements can elicit the innate and the adaptive immune responses against their own products, but they also have great potential to influence the immune reactivity against unrelated immune challenges.[20] A recent paper has explored the influence of TEs on interferon gamma (INFγ)-inducible regulatory networks, in particular on the transcription of innate immunity factors, defined as INFγ-stimulated genes.[21] Employing ChIP and RNA sequencing, the authors observed that HERVs could represent the sources of novel binding sites for INFγ-inducible transcription factors, suggesting a potentially widespread role for HERVs in the regulation of the human INFγ response.[21] Few studies have investigated the relation between HERVs and the Toll-like receptors (TLRs). A member of the HERV-W family, that has been associated with multiple sclerosis, has been shown to interact with TLRs and stimulate the production of pro-inflammatory cytokines.[22,23] These data suggest that HERVs products also have the ability to activate pro-inflammatory signaling pathways and interact with some components of the innate immune response.

The expression of some HERVs is indeed thought to be beneficial to the host. Well-studied examples are the cases of the HERV-W and HERV-FRD *env* genes, which encode for the proteins syncytin-1 and syncytin-2, respectively, required for the placenta formation. They allow the fusion of the cells to form the syncytiotrophoblasts and contribute to the immune tolerance of the fetus.[24] Another example is given by the insertion of the HERV-K LTR, creating an enhancer element for the human gene *PRODH* that has a strong implication in higher nervous activity in hippocampus.[25] Again, ERVL TLR promotes the transcription of the human gene *b3GAL-T5* that concurs to the synthesis of type 1 carbohydrate chains, expecially in colon.[26] HERV LTR was also shown to regulate the transcription of the *BIRC1* gene that encodes a neuronal apoptosis inhibitory factor.[27]

HERVs and Oncogenic Mechanisms: The Negative Side

Since a couple of decades ago, it has been suspected that HERVs, generally recognized as silent sequences, can become active and play some physiological roles, influencing the development of chronic diseases such as diabetes, multiple sclerosis, cancer, and autoimmunity or immune suppression-related diseases.[28–31] The role of HERVs in cancer is most likely limited to retrovirus-driven gene expression and does not involve their insertional activity.[18] In particular, multiple copy numbers of HERVs elements can create new functional exons, alternative splicing products, and microRNAs via integration and adaptation events.[11,30] Many oncogenic mechanisms have been attributed to HERVs to explain their complex role in the development of cancer. Mullins and Linnebacher proposed that the hypothesized oncogenic mechanisms employed by HERVs included: (a) the general or specific (re)activation of HERVs sequences due to hypomethylation,[32–34] (b) the expression of HERVs encoded oncogenes,[35] (c) the inactivation of tumor suppressor genes by de novo insertion or translocation of retroelements within the genome,[35] (d) the regulation of nearby (proto-) oncogenes or growth factors by the regulatory sequences of LTRs,[36,37] and (e) the ability of the *env* proteins to induce cell fusions, which may contribute to the tumor progression or metastasizing processes.[38,39] The main mechanisms are briefly described here below and in Figure 2.

Methylation. Methylation is a crucial event involved in the modification of heavy metals, regulation of gene expression and protein function, and RNA processing. In healthy somatic and mature germ cells, HERVs sequences are generally hypermethylated and transcriptionally silenced by epigenetic mechanisms. Two recent studies by Chiappinelli et al[40] and Roulois et al corroborated the assumption of an epigenetic deregulation in cancer, showing that the DNA methylation in humans silenced the HERVs sequences, and other viral sequences in the human genome. The antitumor DNA-demethylating agents were shown to act by inducing transcription of endogenous double-stranded RNAs that activate the viral recognition and the interferon response pathway, reducing the proliferation of colorectal cancer cells.[41] In parallel, several studies also reported that LTR activation was controlled by DNA methylation, and the implication of this mechanism seemed to be involved in various types of cancer.[42–44] Gimenez et al[42] observed that hypomethylation of the promoter domain of the HERVs U3 element was a prerequisite for the increased

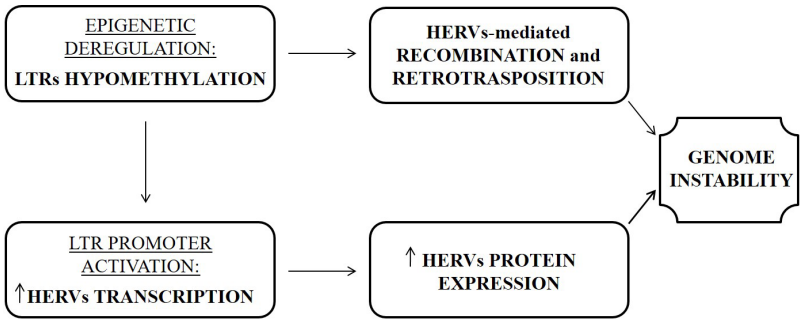

Figure 2. Oncogenic mechanisms employed by HERVs and leading to genome instability and transformation of cells.

expression in tumor tissues compared to normal tissues. Colon cancer cells, treated with DNA methylation and histone deacetylase inhibitors, altered the expression pattern of HERV-H in several colon cancer cells, suggesting that the hypomethylation context affected the expression of HERV-H elements in colon cancer cells.[43] Moreover, increased HERV-K expression in melanomas may be due to increased promoter activity and demethylation of the 5′ LTR.[44] Thus, overexpression of the HERVs sequences in cell lines is correlated with the demethylation of LTR.

LTR promoter activation. Genetic instability is one of the key features associated with cancer rising and progression. As mobile elements, the HERVs LTRs possess the features to affect the human genome stability. It was shown that the LTR activation was involved in nontumor diseases, such as rheumatoid arthritis, diabetes, and schizophrenia, and also in different types of tumors.[45–48] In particular, the detection of RNA transcripts of HERVs was described in various human tissues and cancer cells.[49,50] LTRs are usually oriented opposite to the transcription direction of the corresponding host genes and the antisense transcripts affect the partner gene functions, by modifying both the transcriptional and post-transcriptional regulation processes.[51–58] LTRs may also promote cellular transformation by *cis* activation of downstream oncogenes, and the repetitive nature of HERVs provides an ideal substrate for nonallelic homologous DNA recombination, which results in a variety of germline chromosomal rearrangements, leading to human genetic disorders and cellular transformation of somatic cells.[59–61] LTRs were reported to be involved in tumorigenesis via three principal mechanisms: insertional mutagenesis, recombination, and polymorphisms. HERVs LTR could carry functional enhancers, promoters, polyadenylation signals, and splice sites that regulate the transcription in human cells.[18] LTRs are perfect docking sites for DNA binding factors, which are critically involved in cancer development.[59] For example, one of the three p53 binding sites was identified within the LTRs of two HERVs families in human cancer cell lines which, when bound by p53, could activate the transcription of some downstream genes.[62] Finally, HERVs LTRs could also be reactivated by exogenous factors such as cytokines, radiation, proteins of other retroviruses and, as previously described, by methylation.[63–67]

Proteins expression. Despite the fact that HERVs sequences accumulated replication-inactivating mutations during the evolutionary process, many retroviral sequences still contain intact open reading frame (ORF) that produce retroviral proteins.[68,69] These ERV-derived proteins may serve as a source for antigens for the immune system; may contribute to the selection process of T- or B-cells antigen-receptor repertoire; and may stimulate the activation of the immune reactivity to the ERVs products in mice and non-human primates, suggesting that the immunological tolerance to ERV-derived proteins is not complete.[70–72] Furthermore, HERVs proteins may retain biological functions that contribute to cellular transformation.[38] To this regard, HERV's encoded proteins have been detected in a variety of human cancers.[35] Although certain HERV proteins were shown to be expressed in healthy nontransformed tissues, some others showed exclusive expression in tumors.[35,73] For instance, the expression of two accessory proteins, named Rec and Np9, *env* splice variants, has been described in several tumors, including leukemia, and breast cancer. Rec is a functional homolog of the HIV Rev and HTLV1 Rex proteins, while Np9 is a nuclear protein encoded by HERV-K. Analysis of serum from breast, prostate, ovarian, testicular cancer, leukemia, and melanoma showed the presence of antibodies directed against HERVs Gag and Env.[74–88] A large population (>85%) of breast cancer patients expressed HERV-K Env protein, while HERV-K Gag protein was shown to be expressed in germ cell tumors (Table 1).[76,89] Furthermore, Cherkasova et al[90] reported a unique HERV-E envelope peptide presented on the surface of von Hippel-Lindau deficient clear cell renal cell carcinomas, offering potentially useful tumor-restricted targets for T-cell-based immunotherapy of kidney cancer.

In light of these scientific evidences, it can be suggested that failures and errors in single somatic cell's efficiency to control HERVs activity potentially result in genome damage and may thus contribute to the formation of cancer.[39]

HERVs and Colon Cancer

Abnormal expression of HERVs is well known in cancer and reported in literature for different types of cancer, such as melanoma, testicular cancer, kidney cancer, breast cancer, and prostate cancer, in which encoded transcripts or proteins are overexpressed in patients and related to poor prognosis.[91–97] Nevertheless, characteristic patterns of HERVs expression are often seen in various tumors and can be considered as possible biomarkers of malignization, offering a unique approach to immunotherapy.[58] The major contribution of HERV sequences to the evolution of the species presumably depends on their LTRs that can trigger chromosomal breaks through recombination events and serve as natural or alternative promoters/enhancers, capable of modulating transcription.[98,99] A major consequence of the abundance of LTR regulatory elements within the human genome is that permissive HERVs reactivations are often associated with pathological context including cancer.[100]

The overall data present in literature regarding an association between HERVs expression pattern and colon cancer are rather in accordance and the majority of the scientific reports is focused on HERV-H family and colon cancer.[101,102]

HERV-H and colon cancer. The history of the possible association between HERVs and colon cancer dates back to 1986, when Moshier et al reported the expression of human endogenous A-type retrovirus *pol* sequences in human colon tumor and the surrounding mucosa.[103] After that, the following paper focused on the HERVs expression in colon cancer and normal tissues was published by Stauffer et al.[102] They analyzed

Table 1. Expression of HERVs proteins and/or transcripts in human cancers.

TUMOR TYPE	HERV TYPE	DETECTION (GENE)	REFERENCES
Melanoma	HERV-K	Pr (*gag, pol, env, rec*)	130
	HERV-K	Pr (*gag, env*)	78, 131, 132
	HERV-K	RNA, Pr (*gag, env, rec*)	133
	HERV-K	RNA, Pr (*env, rec, np9*)	134
	HERV-K	RNA, Pr (*env*)	96
Breast	HERV-K	Pr (*gag*)	77
	HERV-K, E, F, W, T.I, FRD	RNA (*pol*)	135–138
	HERV-K	RNA (*env*)	139–143
	HERV-K	RNA, Pr (*gag, pol, env*)	144
		RNA (*gag*)	145
Leukemia/ lymphoma	HERV-K	RNA, Pr (*gag, pol, env*)	139
	HERV-K, H	Pr (*gag*)	77
	HERV-K	RNA (*gag*)	78, 146
	HERV-K	RNA (*pol, env*)	143, 147–149
	HERV-K	RNA (LTR)	150
	HERV-E	RNA (*gag, pol, env*)	151
	HERV-H	RNA (*gag, env*)	152
Astrocytoma	HERV-K	Pr (*env*)	153
Prostate	HERV-K	RNA, Pr (*gag*)	78
	HERV-E, R	RNA (*env*)	154
Lung	HERV-K	Pr (*gag*)	78
	HERV-E	RNA (LTR)	155
	HERV-R	RNA (*env*)	156
Pancreatic	HERV-K	RNA (*env*)	157
	HERV-H	RNA (*gag*)	76
Gastro-intestinal	HERV-K	Pr (*gag*)	78
	HERV-K	RNA (*env*)	143
	HERV-H	RNA (*gag*)	76, 105, 106
Ovarian	HERV-K	RNA, Pr (*gag*)	78
	HERV-K, E, R	RNA, Pr (*gag*)	74, 75
	HERV-E	RNA (–)	158
	HERV-K	Pr (*gag*)	159
	HERV-H	RNA (LTR)	160
Endometrial	HERV-W	Pr (*env*)	161
Testicular/ germ cell	HERV-K	Pr (*gag, env*)	77, 92, 159, 162–164
	HERV-K	RNA (*gag*)	165, 166
	HERV-K, H	RNA (LTR)	160, 167
	HERV-K	Pr (*gag*)	77

the digital expression patterns of the HERV-K, -W, -H, and -E families in several normal and cancerous tissues. A total of 31 proviral members of the HERV-K family and one representative each for the other HERVs families were used as probes to search human expressed sequence tags (ESTs). HERV-H was the only family expressed in cancers of the intestine, bone marrow, bladder, and cervix and was more expressed than the other families in cancers of the stomach, colon, and prostate. The rate of EST expression is summarized in Table 2. The association between HERV-H and colon cancer has also been reported in a Chinese paper, which studied the deletion of a part of the *env* gene in the HERV-H provirus and named the deleted virus HERV-H-X. The missing part in the *env* region of HERV-H-X corresponded to the *env* ORFs in the other known HERV-H strains (env62, env60, and env59). HERV-H-X was detected only in colon cancer tissues, while *env* ORFs were detected in both cancer and normal tissues. Additionally, the authors showed that the expression of HERV-H-X was upregulated in colon cancer tissues by 24.9-folds than that in normal tissues ($P < 0.01$). However, this study was conducted in only eight pairs of tumor and normal tissues.[104] The full-length transcript sequence of the HERV-H-X was published by Liang et al,[105] who also indicated that HERV-H-X was upregulated in colon tumor samples, while *env* ORFs were transcribed in colon tumor and normal samples in an irregular pattern, suggesting that the involvement of HERV-H-X and *env*-intact HERV-H in colon cancer might be different.

Table 2. EST-based expression profiles of HERV-H, -K, -W, and -E in colon cancer tissues.[102]

CANCEROUS TISSUE	HERV-H RATIO*	HERV-K RATIO*	HERV-W RATIO*	HERV-E RATIO*
Testis	129.85	106.24	–	11.80
Intestine	27.50	–	–	–
Colon	**20.96**	**4.73**	**0.68**	**1.35**
Stomach	20.45	13.01	–	1.86
Bladder	18.35	–	–	6.12
Prostate	15.14	1.51	–	1.51
Bone marrow	10.92	–	–	–
Cervix	9.98	–	–	–
Bone	8.99	–	–	–
Endocrine glands	5.35	–	–	5.35
Head and neck	5.25	5.25	–	–
Lung	5.19	–	–	–
Ovary	4.54	6.05	–	1.51
Breast	4.35	6.96	–	2.61
Lymph node	3.69	3.69	–	–
Pancreas	1.69	3.38	–	–
Skin	1.13	3.38	–	–
Brain	0.55	2.20	–	–

Note: *HERVs ratio: EST counts/total number of ESTs in tissues $\times 10^5$.

Using massively parallel signature sequencing techniques, Alves et al[106] identified many genes and several HERVs sequences that seemed to be differentially expressed in colon cancer samples when compared to normal tissues. The testing of a subset of these candidate genes by semiquantitative Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) analysis showed the differential expression of a specific *gag* transcript from the HERV-H located on chromosome 22 (Xp22.3) in the majority of primary and metastatic colon cancer samples analyzed, as well as in the adenoma. In contrast, no detectable expression was found in any normal tissues tested, except bladder. In particular, the candidate genes were tested in 25 primary colon cancer samples, 6 colon cancer metastases to the liver, and 1 sample of tubule-villous colon adenoma. The amplified cDNA from the *gag* region was sequenced and an amber mutation was found 280 bp downstream from the initiation codon, in all the analyzed samples. The authors concluded that this protein may serve as a target for antitumor therapy. In a large-scale analysis of 139 colon cancer samples and adjacent normal tissue pairs, Pérot et al,[100] about 10 years later, confirmed the expression of the HERV-H Xp22.3 transcripts in half of the analyzed tumor samples (70/139). Furthermore, HERV-H expression patterns were assessed with regard to clinical parameters and molecular features of the cancer, showing a strong correlation between HERV-H expression and the microsatellite instable (MSI) tumor as well as lymph node invasion of the tumor cells. The authors showed great enthusiasm for the results, affirming that "HERV-H sequences in addition to tumor-specific mutations may represent clinically relevant, truly CRC specific markers for diagnostic, prognostic, and therapeutic purposes". Due to the immunosuppressive property of the envelope protein of HERV-H, many studies focused on the *env*-related transcripts and their association with colorectal cancer.[101,107–109] Liang et al[105,110,111] reported in different studies that there were many spliced noncoding RNAs transcribed from HERV-H elements, both in normal and cancerous colon tissues, as well as colon cancer cell lines. They observed that the expression pattern of the spliced noncoding transcripts from HERV-H was not clear and that the overall expression of HERV-H elements in colon cancer was complex and different between tumor samples and adjacent normal samples. Finally, they reported that all the active HERV-H elements found in their study were structurally incomplete, with six fragments commonly deleted, which were distributed through the *gag*, *pol*, and *env*-regions, but, even so, some of them (40%) retained putative ORFs (Fig. 3).[43] Although they observed no difference in the RT-PCR products between tumor and adjacent normal samples, the total numbers and loci of active HERV-H elements were significantly different. In particular, seven HERV-H elements were found to be transcriptionally active in the adjacent normal colon samples and 14 elements were found to be active in the tested colon tumor tissues.[43]

Multiplex degenerate PCR assays also indicated that HERV-H was increased in colon tumor tissues, but not in other types of tumors.[101]

The possible mechanism driving to the HERV upregulation in colon cancer has been studied and hypothesized by several authors. Increase in HERVs transcription in cancer cells has been linked to the liberation of HERV LTRs from epigenetic controls via demethylation in agreement with data showing that 5%–8% of repetitive elements demonstrate cancer-related DNA methylation patterns.[112,113] Liang et al[43] and Wentzensen et al[76] showed that the expression of HERV-H Xp22.3 was correlated with the demethylation of the 5′ LTR and its promoter activity, strongly supporting the hypothesis that changes in the methylation status were tumor specific.

Pérot et al[100] added many other information to the hypothetic function of HERV-H in colon cancer. They found an increased HERV-H expression in MSI colon cancer and speculated that HERV-H ORF interrupted by inactivating mutations might be restored by MSI-induced frameshift mutations. Thus, a better prognosis was connected with MSI. HERV-H reactivation was also shown to be correlated with lymph node infiltration and consequently with the aggressiveness of the tumor, even if no correlation with the presence or absence of metastasis was observed. It seems that HERV-H expression may induce epithelial–mesenchymal transition in the early phases of metastasization, and then may not play any role in the subsequent phases of tumor progression. Cancer immunoevasion may be another mechanism employed in order to facilitate the transformation of cells. It has been shown that HERV-H expression increased in tumor cells undergoing

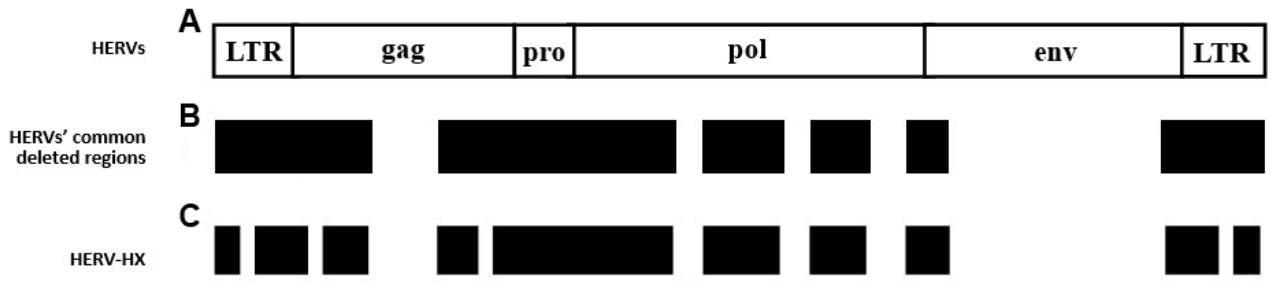

Figure 3. (**A**) Genomic structure of HERVs. (**B**) Scheme of the commonly deleted regions (white box) of the active HERV-H-X elements in colon cancer. (**C**) Genomic structure of the HERV-HX.[105]

epithelial–mesenchymal transition and that the HERV-H-derived H17 peptide further amplified these events. The HERV-H-derived H17 peptide induced the production of CCL19, which has been connected with immune dysfunction and which in turn recruited and expanded a population of pluripotent immunoregulatory CD271$^+$ cells, which included mesenchymal stem cells and myeloid-derived suppressor cells. Notably, changes in the expression of HERV-H or CCL19, or depletion of CD271$^+$ cells, improved immune responses in vitro and in vivo accompanied by tumor regression (Table 3).[114]

Other HERVs and colon cancer. If on one hand there are many data concerning colon cancer and HERV-H family association, very few studies are focused on all the other HERVs families. Only one scientific study analyzed the expression of HERV-R *env* protein product in colon cancer, using a tissue microarray containing 55 colon cancer, normal colon, and metastatic colon cancer tissues. The results were confirmed using five colon cancer samples and the surrounding normal colon fresh tissues by Western blot analysis. The expression of HERV-R *env* resulted significantly increased in the colon cancer tissues, and also when compared with the surrounding normal tissue of the same patient. In particular, the expression of HERV-R *env* protein was significantly upregulated in both primary and metastasized colon cancer with no change between the two types of tumor. Additionally, the expression of HERV-R *env* protein resulted increased in all stages of colon cancer with no differences according to cancer stage.[115]

Negative results of association between HERV-W and colon cancer were observed by Kim et al,[116] who examined the expression pattern of the HERV-W *env* gene using a real-time RT-PCR system and did not find any significant difference among the colon tumor and the normal adjacent tissues. Interestingly, the same authors, one year later, found high levels of expression of HERV-P in colon cancer tissues, as compared to the adjacent normal tissues (Table 3).[117]

Table 3. Expression of HERVs proteins and/or transcripts in colon cancers.

TUMOR TYPE	HERV TYPE	DETECTION (GENE)	REFERENCES
Colon cancer	HERV-H	RNA (*gag, env, pol, LTR*)	100
	HERV-H	RNA (*pol*)	101
	HERV-H	RNA (EST)	102
	HERV-H	RNA (*pol*)	103
	HERV-H	RNA (*env*)	104
	HERV-H	RNA (*env*)	107
	HERV-H	RNA (*env*)	105
	HERV-H	RNA (*gag*)	106
	HERV-H	Pr (*env*)	108
	HERV-H	RNA (*env*)	109
	HERV-H	RNA (*env*)	110
	HERV-H	RNA (*env*)	111
	HERV-H	RNA (*gag, env, pol*)	43
	HERV-H	Pr, RNA (*gag, env, pol*, rec)	113
	HERV-H	RNA (*gag, env, pol*)	76
	HERV-R	Pr (*env*)	115
	HERV-W	RNA (*env*)	116
	HERV-P	RNA (*env*)	117

Abbreviation: EST, expressed sequence tag.

Immunotherapy of HERVs-Positive Colon Cancer

Although the overexpression of HERV-H and HERV-R was found to be correlated with the development of colon carcinoma, any relationship to chemotherapy resistance or tumor aggressiveness has not been described. Regarding this topic, a study by Mullins and Linnebacher[118] reported that stimulation of peripheral T-cells with retroviral peptides presented by autologous antigen-presenting cells clearly resulted in sustained proliferation of predominantly CD8$^+$ T-cells, suggesting that HERV-H *env* gene may be suited as colon cancer-specific tumor-associated antigen. This scenario can provide the rationale for inclusion of HERV-H Xp22.3 into clinical vaccination protocols. A study conducted by Díaz-Carballo et al[119] demonstrated that the expression of various HERVs proteins was not only detectable in colon cancer cells but might also have therapeutic implications for the patients, especially in chemorefractory tumors. In particular, several retroviral transcripts resulted overexpressed in HCT8 colon cancer cell line up to three times in chemotherapy refractory HCT8 cells, suggesting a relationship to chemoresistance. The group hypothesized that the chemotherapy resistance might be a result of the interaction between retroviral proteins with cell membrane structure, promoting cell fusion and generation of multinucleated giant cancer cells, representing an alternative membrane-mediated defense mechanism. This evidence proposed the HERVs' overexpression as a tool for monitoring the therapy resistance.[119] Bronte et al[120] reported that the inoculation of a DNA plasmid encoding mouse gp70 or p15E (two products of the *env* gene of an endogenous murine leukemia virus) elicited the T-lymphocyte response and resulted in partial protection of the mouse tumors possessing these antigens. Furthermore, systemic administration of agonistic anti-CD40 monoclonal antibodies increased the therapeutic potential of the DNA vaccine uniquely when administrated during the tumor rejection phase. This effect was observed to be associated with the increase of ERV-specific CD8$^+$ T-lymphocytes count. These data taken together suggest that HERVs overexpression, with particular regard to HERV-H, might help to further improve existing tests for the detection of precancerous colorectal lesions. The issue/entity specificity of HERV-H expression may also provide a diagnostic tool for tumor and metastases of unknown origin.[100]

Role of LINE-1 Hypomethylation in Colon Cancer

The methylation status of LINE-1 was first demonstrated in cancer cell lines in 1993.[121] Thererafter, LINE-1 hypomethylation was detected in several tumors, such as bladder, gastric, and head and neck cancers, and its association with poor prognosis of the disease was demonstrated.[122,123] Concerning the colon cancer patients, several studies investigated the potential value of LINE-1 hypomethylation and they were recently analyzed and summarized in a meta-analysis by Tang et al.[124] The authors confirmed the previous observation that the methylation level of LINE-1 repeats declines in colon cancer and consequently it may be considered as a potential prognostic biomarker of the risk of colon cancer. One of the highly cited studies regarding the role of the hypomethylation of LINE-1 in colon cancer studied two different populations, comprising more than 170,000 patients. The authors showed that LINE-1 hypomethylation was associated with a statistically significant increase in colon cancer-specific mortality and in overall mortality.[125] The same group, more recently, confirmed this association and further observed that LINE-1 hypomethylation association with higher colorectal cancer-specific mortality was stronger in proximal colon cancers than in distal colon cancers or rectal cancers.[126] Again, it was shown that the association of LINE-1 hypomethylation with inferior survival was stronger in colon cancer patients with high microsatellite instability than in patients with microsatellite stability, confirming that tumor LINE-1 methylation level may be a useful prognostic biomarker to identify the aggressivity of the cancer.[127]

Conclusions

Colorectal cancer is one of the most common malignancies throughout the world, with more than 140,000 new cases every year in United States.[128] Over the last decade, the expression of HERVs sequences and their potentially immunogenic proteins have been detected in different colon cancer tissues and in several other tumor types, indicating a possible role of HERVs as tumor promotes. However, a clear pathogenic role for HERVs remains difficult to be proven conclusively, particularly due to the complexity of HERVs structure and biology, such as their repetitive nature and abundance among the human genome. The hypothesis of HERVs-mediated oncogenesis is so far based on the evidences that they contribute to genomic instability of the cells, through epigenetic changes and activation of the LTR sequences, upregulation of the proteins' expression, and probably retrotransposition and recombination. Additionally, the immunosuppressive properties of the *env* proteins should not be forgotten, since they lead to the induction of immune tolerance at the maternofetal barrier via a physiological expression in the placenta,[129] but also to the suppression of an antitumoral immune response through aberrant expression in cancers.

Specifically regarding colon cancer, the expression of the only HERV-H family has been associated essentially with colon cancer, but, to date, the identification of individual reactivated HERV-H loci remains poor. One unique HERV-H locus on Xp22.3 has been repeatedly described to be upregulated in colon cancer. The lack of an association between HERVs and colon cancer may also be due to the lack of studies, focused on this topic. Consequently, this should be considered a widely open area of investigation, especially taking into account the strong potentialities of the HERV-H family to produce proteins, that could be good candidates as biomarkers of diseases, or, more interesting, as tumor-associated antigens, target of therapeutic approaches.

Author Contributions

Conceived and designed the experiments: SD, MB. Analyzed the data: SD, MB, PF. Wrote the first draft of the manuscript: LS. Contributed to the writing of the manuscript: SD, SV. Agree with manuscript results and conclusions: SD, SV, LS, MB, PF. Jointly developed the structure and arguments for the paper: MB, LS, SD. Made critical revisions and approved final version: PF, SD. All authors reviewed and approved of the final manuscript.

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