Analysing the HERV-K env, np9, rec and gag expression in cervical tissues

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Abstract

Cervical cancer is considered to be the fourth common cancer. It is assumed that numerous risk factors, especially infectious ones, can have a detrimental effect on cervical cancer. In this study, we evaluated the expression of Herv-K env, np9, rec and gag in cervical tissues. After RNA extraction and cDNA sensitizing of 12 cervical cancer tissues and CIN3, 51 CIN1,2 and 18 normal ones, Herv-K env, np9, rec and gag were assessed using quantitative real-time PCR analysis. There was a decrease in the level of HERV-K env expression in cervical cancer and CIN 1-3 in compression with normal tissues. Cervical cancer and CIN3 indicated the most increase in expression. Meanwhile, we observed an increase in gag and rec expression in CIN 1,2; although cervical cancer and CIN 3 had a decrease in rec and gag expression, we did not report any changes in np expression. In conclusion, given the relationship between HERV-associated genes and cervical cancer, our study suggests that these genes can be useful for cancer diagnosis. However, further investigations are needed to provide a better perspective about the effectiveness of these genes in the diagnostic strategies of gastrointestinal cancer. These results are just an observation that could open a wider investigation to test the correlation between the expression of these genes and cervical cancer.
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Introduction

Cancer is one of the main health problems all around the world. Among different types of cancer, cervical cancer can have a detrimental effect on health care system as statistics reported approximately 200, 000 deaths annually due to this type of cancer [1]. Cervical cancer is considered to be the fourth common cancer. More than 75, 000 new cases of invasive cervical carcinoma were estimated in 2002 [2,3].

It is assumed that numerous risk factors, including radiation, environmental pollution, alcohol consumption, smoking and even infectious diseases, are able to accelerate the progression of different kinds of cancers [4–7]. Human endogenous retrovirus [HERV] is one of these risk factors, which can create dilemmas in different parts of the world as this virus is likely to trigger cell division by encoding protein causing tumour immune escape; therefore, cancer can be observed [8]. This virus has entered into human DNA since 30–40 million years ago, and is able to transmit vertically into the host's genome [9]. With a notice of this distinguishing features, more than 8% of our genome comprise the HERV genome. There are three different important families of HERV, including class I [gammaretrovirus- and epsilonretrovirus-like], class II [betaretrovirus-like] and class III [spumaretrovirus-like] [10–13]. As the genome of this virus is intact, some subfamilies of HERV are able to synthetise retrovirus-like particles [14]. The regulation of HERV structural genes, including HERV-K np9, rec, env and gag, depends on long terminal repeats [LTR] sequence, and an increase or decrease in the level of these genes can result in autoimmune diseases and different types of cancers [15]. As the impact of infectious risk factor, especially HERV-K, is the nub of health problem, in this study, we attempted to evaluate the expression of HERV-K np9, rec, env and gag in cervical tissues.

Material and methods

Sample

This study was approved by the Shahid Beheshti University of Medical Sciences, IR.SBMU.MSP.REC.1399.24 [Grant no 20789]. We collected cervical cancer tissues, cervical intra-epithelial neoplasia [CIN] and healthy tissues of patients in Mahdieh hospital between 2018 and 2020 in Tehran, Iran. We categorized our samples into three groups, including the normal cervix, CIN1,2 and CIN3, cervical cancer. Normal adjacent cervical samples were collected from patients who participated in the hospital for routine screening. All tissues were stored in minor 20 degrees in RNA latter [Qiagen GmbH, Hilden, Germany]. Three expert pathologists confirmed the stage of tissues, and some of the clinical characteristics were summarized in Table .1. The samples of those patients who were treated either by chemotherapy or radiation were excluded from this study. Moreover, patients were not under antibiotic/anti-inflammatory treatments.

RNA extraction

To extract the RNA of different cervical tissues, we digested our samples in 1 ml RNX-plus solution reagent [Cinnagen, Tehran, Iran] in a homogenizer. Actually, protein was removed after adding chloroform and centrifuging, and RNA was precipitated with the isopropanol. Finally, we diluted the RNA in 50 ul of DEPC-treated water. The total RNA was run in agarose gel electrophoresis. Indeed, we confirmed the purity of RNA, observing the 5S, 18S and 28S bands. In this study, DNaseI (Invitrogen, Carlsbad, CA, USA) was employed to remove DNA contamination [16,17].

cDNA synthesis

In the final volume of 20 μl reaction containing 1 ul random hexamer, 9 ul master mixes and 10 ul of RNA samples, and in 40 min at 50 °C and 10 min at 95 °C in Bio Intellectica PCR by Bio fact cDNA kit [Daejeon, South Korea], cDNA was synthetised and diluted two times in sterile water.

Quantitative real-time PCR

The expression of HERV-K np9, rec, env and gag were evaluated by 6000 [Corbett life sciences, Sydney, Australia]. In a final 20 ul volume, we combined 10 ul BIOFACT™ 2 × real-time PCR master mix [for SYBR Green I; BIOFACT, South Korea], 6 ul sterile water, 1 ul forward 10 pmol, 1ul reverse primer 10 pmol and 2 ul cDNA, and incubated in one cycle at 95 °C for 10 min, 40 cycles at 95 °C for 30s; 55 °C for 30s, 72 °C for 30s. The melt curve was between 60 °C and 95 °C.

TABLE 1. The clinical characteristics of 12 cervical cancer patients

Age	
>50	58.3 %
<50	41.7 %
FIGO stage	
IA2	16.6 %
IB1	83.4 %
Histology	
Squamous cell carcinoma	66.6 %
Adenocarcinoma	25 %
adenosquamous cell carcinoma	8.4 %
Tumour size	
<2	66.6 %
>2	33.4 %
Lymph node metastasis	10 %

GAPDH housekeeping gene was used as an internal control, and based on $2^{-\Delta\Delta ct}$ expression formula, the values for the relative quantification were calculated. The list of primers was summarized in Table 2 [16,17].

Statistical analysis

To analyse the results of gene expression, we used Graph-PadPrism software. Experimental data are expressed by mean ± standard deviation of three independent assays. Statistical significance was calculated using ANOVA tests. *P*-value less than [$P < 0.05$] was used for the differences.

Result

Sample

We collected 12 cervical cancer tissues and CIN3, 51 CIN1,2 [25 CIN1 and 26 CIN2] and 18 normal ones from patients in Mahdieh hospital, Tehran, Iran. To assess the correlation between the expression of HERV-K env, gag, rec, np and cervical malignancy, we extracted the RNA of all tissues, and RQ-PCR illustrated the HERV-K env, gag, rec, np expression.

Decrease in env mRNA expression in cervical cancer and CIN 1-3

Our results indicated a decrease in the level of HERV-K env expression in cervical cancer and CIN 1-3 in compression

TABLE 2. Nucleotide sequences of primers used for real-time RT-PCR

Gene	Forward primer (5′-3′)	Reverse primer (5′-3′)
GAPDH	ATGTTCGTCATGGGTGTGAA	GGTGCTAAGCAGTTGGTGGT
rec	ATCGAGCACCGTTGACT CACAAGA	GGTACACCTGCAGACAC CATTGAT
np9	AGATGTCTGCAGGTGTACCCA	CTCTTGCTTTTCCCCACATTTC
gag	AGCAGGTCAGGTGACCGTAAC	GGTGCCATAGCATTGTCTCCT

with normal tissues. Cervical cancer and CIN3 indicated the most increase in expression, but none of these figures was meaningful [Fig. 1].

Rec and gag expression in cervical tissues
The figures for rec and gag expressions were similar. We observed an increase in the gag and rec expression in CIN 1,2, although cervical cancer and CIN 3 had a decrease in rec and gag expression. All these changes were not meaningful [Figs. 2 and 3].

No changes of np expression in cervical tissues
There were small differences in all three groups of tissues. Actually, we did not report any changes in np expression [Fig. 4].

Discussion

Cervical cancer is the second type of cancer among women. Different risk factors are supposed to have a dire consequence in the progression of different types of cancers, such as cervical cancer [18–21]. Early detection, especially cervical cancer screening [CCS] in people suffering from this disease, can be very useful [18]. To detect cervical cancer in early stages, many used and reported some molecules as biomarkers. In different studies, it is cleared that HERV expression can be changed in some types of cancers, but the effect and the expressions of HERV gag, env, rec and np in cervical cancer are not confirmed.
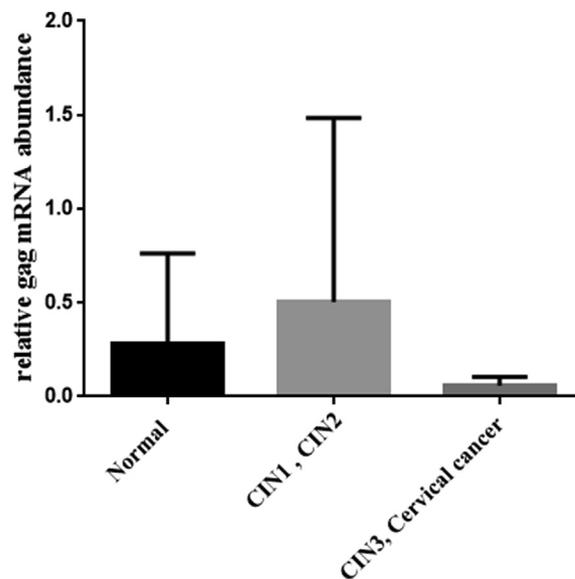

FIG. 2. gag expression in cervical tissues.

In this study, we collected 12 cervical cancer tissues and CIN3, 51 CIN1,2 and 18 normal ones. After extracting RNA of all tissues, we evaluated the expression of HERV gag, env, np and rec expressions. Our results confirmed that there was a decrease in the level of env expression in cervical cancer and CIN 1-3 in compression with normal tissues. Also, we observed an increase in the gag and rec expression in CIN 1,2, although cervical cancer and CIN 3 had a decrease in rec and gag expression. [the decrease and increase of different genes expressions are not statistically significant.] However, in all of
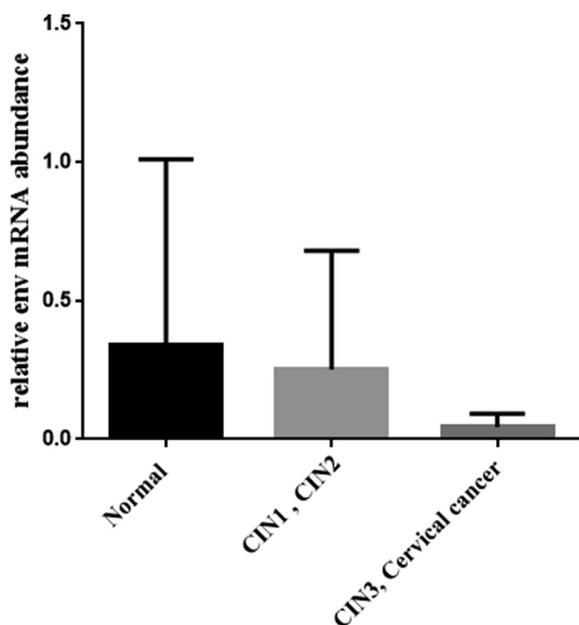

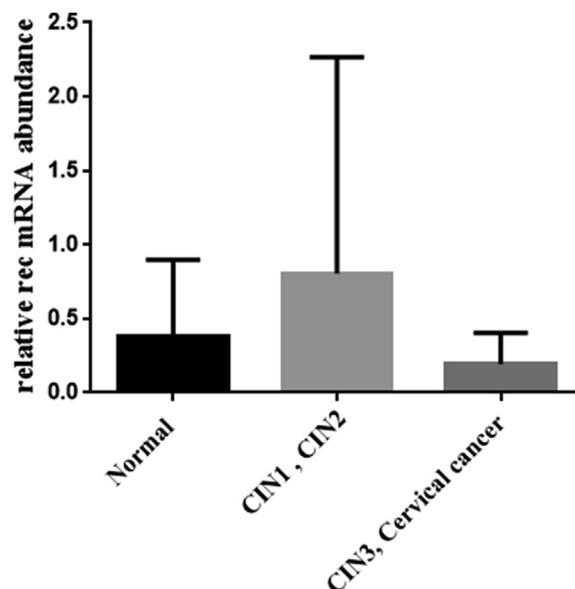FIG. 1. Decrease in env mRNA expression in cervical cancer and CIN 1-3.

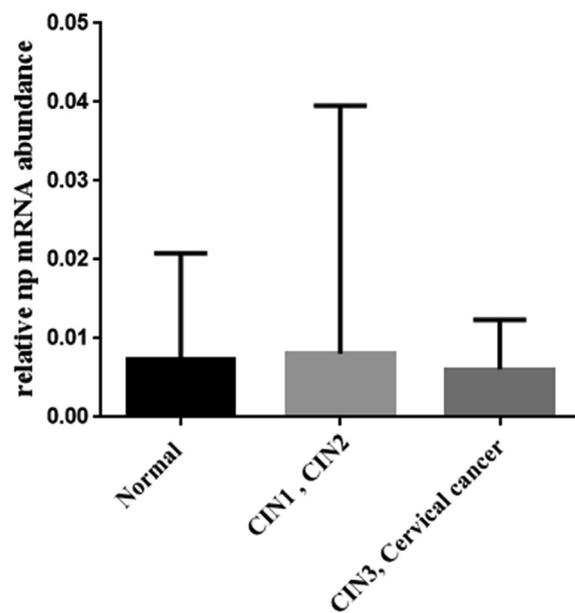FIG. 3. rec expression in cervical tissues.

FIG. 4. No changes of np expression in cervical tissues.

tissues, we did not report any changes in np expression. The limitation of this study was the low number of samples.

Few studies focused on HERV-K expression in cervical and reproductive organs; for example, it is reported HERV-K env protein was lower in normal tissues compared to ovarian cancer ones [22]. In tumours with low malignant potential and low grade, there was an increase in the expression of HERV-K. Furthermore, anti-ERV3 [30%], anti-HERV-E [40%] and anti-HERV-K [55%] were detectable in cancerous tissues. Among 254 samples, anti-HERV-K specific antibody by flow cytometric analysis indicated that this virus expresses a higher amount of HERV-K env mRNA in ovarian epithelial tumours than in normal ovarian tissues [23]. It is reported, HERV-K env protein in normal tissues was low but higher in bronchus submucosal gland, salivary gland acini, pancreas acini, testis seminiferous tubule, uterine cervix epithelium, ovary stroma, skin epidermis, and heart [24]. HERV-R env and KAP1 proteins were lower in normal tissues than ovarian cancerous ones, but HERV-K env did not illustrate a meaningful change in cancerous tissues [25]. Patients suffering from advanced-stage ovarian clear cell carcinoma, decrease in HERV-K, HERV-E, and LINE-1 were observed [P = 0.0179, P = 0.0021, and P = 0.0307, respectively], and the more methylated [hypomethylated] HERV-K existed, the less chance of survival [P = 0.006] can be seen [26].

Also, in other kinds of cancers HERV-K was detected. Johanning et al. reported that breast cell lines [MDA-MB-231, MCF-7, SKBR3, MDA-MB-453, T47D and ZR-75-1] had a higher expression of HERV-K env in comparison with non-malignant ones [MCF-10A and MCF-10AT] [27]. With some signalling pathways, such as WNT, ERK, Akt and Notch1, np9 had a relationship with breast cancer [28,29]. HERV-K was expressed higher in Hepatocellular Carcinoma than adjacent normal ones. [p < 0.01] Also, this virus was related with cirrhosis [p < 0.05], tumour differentiation [p < 0.01], and TNM stage [p < 0.01]. Patients with a high expression of HERV-K had a poorer overall survival [30]. Hu and et al. illustrated that normal cervix tissues have a higher level of HERV-E and HERV-W RNA compared with cancerous ones [31]. However, HERV-W was higher in cancerous ovarian tissues in the study of Menendez [32].

In conclusion, given the relationship between HERV-associated genes and cervical cancer, our study suggests that these genes can be useful for a cancer diagnosis; however, further investigations are needed to provide a better perspective about the effectiveness of these genes in the diagnostic strategies of gastrointestinal cancer. [These results are just an observation that could open a wider investigation to test the correlation between the expression of these genes and cervical cancer.]

Consent for publication

The consent for publications was obtained from all patients.

Authors' contributions

E.F., A.M. and SH.T designed the study and performed the molecular experiments. H.G performed the statistical Analyses. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

This study has been conducted in the Department of the School of Medicine, Shahid Beheshti University of Medical Sciences.

Transparency declaration

The authors declare no conflicts of interests.

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Availability of data and materials

Please contact author for data requests.

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References

[1] Pisani P, Bray F, Parkin DM. Estimates of the world-wide prevalence of cancer for 25 sites in the adult population. Int J Cancer 2002 Jan 1;97(1):72–81.

[2] Lai TO, Boon SS, Law PT, Chen Z, Thomas M, Banks L, et al. Oncogenicitiy comparison of human papillomavirus type 52 E6 variants. J Gen Virol 2019 Mar;100(3):484–96.

[3] Louie KS, de Sanjose S, Mayaud P. Epidemiology and prevention of human papillomavirus and cervical cancer in sub-Saharan Africa: a comprehensive review. Trop Med Int Health 2009 Oct;14(10):1287–302.

[4] Scheurer ME, Danysh HE, Follen M, Lupo PJ. Association of traffic-related hazardous air pollutants and cervical dysplasia in an urban multiethnic population: a cross-sectional study. Environ Health 2014 Jun 13;13(1):52.

[5] Faghihloo E, Sadeghizadeh M, Shahmahmoodi S, Mokhtari-Azad T. Cdc6 expression is induced by HPV16 E6 and E7 oncogenes and re-presses E-cadherin expression. Cancer Gene Ther 2016 Nov 11.

[6] Faghihloo E, Saremi MR, Mahabadi M, Akbari H, Saberfar E. Prevalence and characteristics of Epstein-Barr virus-associated gastric cancer in Iran. Arch Iran Med 2014 Nov;17(11):767–70.

[7] Weiderpass E, Ye W, Tamimi R, Trichopolous D, Nyren O, Vainio H, et al. Alcoholism and risk for cancer of the cervix uteri, vagina, and vulva. Cancer Epidemiol Biomarkers Prev 2001 Aug;10(8):899–901.

[8] Gonzalez-Cao M, Iduma P, Karachaliou N, Santarpia M, Blanco J, Rosell R. Human endogenous retroviruses and cancer. Cancer Biol Med 2016;13(4):483–8.

[9] Boeke JD, Stoye JP. Retrotransposons, endogenous retroviruses, and the evolution of retroelements. In: Coffin JM, Hughes SH, Varmus HE, editors. Retroviruses. Cold spring harbor. (NY): Cold Spring Harbor Laboratory Press; 1997.

[10] Vargiu L, Rodriguez-Tomé P, Sperber GO, Cadeddu M, Grandi N, Blikstad V, et al. Classification and characterization of human endogenous retroviruses; mosaic forms are common. Retrovirology 2016 Jan 22;13:7.

[11] Riahi Rad Z, Riahi Rad Z, Goudarzi H, Goudarzi M, Mahmoudi M, Yasbolaghi Sharahi J, et al. MicroRNAs in the interaction between host-bacterial pathogens: a new perspective. J Cell Physiol 2021 Sep;236(9): 6249–70. https://doi.org/10.1002/jcp.30333. Epub 2021 Feb 18. PMID: 33599300.

[12] Jern P, Sperber GO, Blomberg J. Use of endogenous retroviral sequences (ERVs) and structural markers for retroviral phylogenetic inference and taxonomy. Retrovirology 2005 Aug 10;2:50.

[13] Dadashi M, Khaleghnejad S, Abedi Elkhichi P, Goudarzi M, Goudarzi H, Taghavi A, et al. COVID-19 and influenza Co-infection: a systematic review and meta-analysis. Front Med 2021;8:681469.

[14] Dewannieux M, Blaise S, Heidmann T. Identification of a functional envelope protein from the HERV-K family of human endogenous retroviruses. J Virol 2005 Dec;79(24):15573–7.

[15] Tugnet N, Rylance P, Roden D, Trela M, Nelson P. Human endogenous retroviruses (HERVs) and autoimmune rheumatic disease: is there a link? Open Rheumatol J 2013 Mar 22;7:13–21.

[16] Singh M, Cai H, Bunse M, Feschotte C, Izsvák Z. Human endogenous retrovirus K rec forms a regulatory loop with MITF that opposes the progression of melanoma to an invasive stage. Viruses 2020 Nov 13;12(11):1303.

[17] Gonzalez-Hernandez MJ, Swanson MD, Contreras-Galindo R, Cookinham S, King SR, Noel Jr RJ, et al. Expression of human endogenous retrovirus type K (HML-2) is activated by the Tat protein of HIV-1. J Virol 2012 Aug;86(15):7790–805.

[18] Hajikhani B, Goudarzi M, Kakavandi S, Amini S, Zamani S, van Belkum A, et al. The global prevalence of fusidic acid resistance in clinical isolates of Staphylococcus aureus: a systematic review and meta-analysis. Antimicrob Resist Infect Control 2021 May 1;10(1):75.

[19] Saei Ghare Naz M, Kariman N, Ebadi A, Ozgoli G, Ghasemi V, Rashidi Fakari F. Educational interventions for cervical cancer screening behavior of women: a systematic review. Asian Pac Cancer Prev 2018;19(4):875–84.

[20] Robatjazi S, Nikkhahi F, Niazadeh M, Amin Marashi SM, Peymani A, Javadi A, et al. Phenotypic identification and genotypic characterization of plasmid-mediated AmpC β-lactamase-producing Escherichia coli and Klebsiella pneumoniae isolates in Iran. Curr Microbiol 2021 Jun;78(6):2317–23.

[21] Baghbanbashi S, Mousavi SMJ, Dabiri H, Hakemi-Vala M, Goudarzi H, Hamzehloo G, et al. Rifampin resistance among individuals with extrapulmonary tuberculosis: 4 years of experience from a reference laboratory. New Microbe. New Infect 2021 Jan 20;40:100841.

[22] Rycaj K, Plummer JB, Yin B, Li M, Garza J, Radvanyi L, et al. Cytotoxicity of human endogenous retrovirus K-specific T cells toward autologous ovarian cancer cells. Clin Cancer Res 2015 Jan 15;21(2): 471–83.

[23] Wang-Johanning F, Liu J, Rycaj K, Huang M, Tsai K, Rosen DG, et al. Expression of multiple human endogenous retrovirus surface envelope proteins in ovarian cancer. Int J Cancer 2007 Jan 1;120(1):81–90.

[24] Jo JO, Kang YJ, Ock MS, et al. Expression profiles of HERV-K Env protein in normal and cancerous tissues. Genes Genom 2016;38: 91–107.

[25] Jeon KY, Ko EJ, Oh YL, Kim H, Eo WK, Kim A, et al. Analysis of KAP1 expression patterns and human endogenous retrovirus Env proteins in ovarian cancer. Genes Genomics 2020 Oct;42(10):1145–50.

[26] Iramaneerat K, Rattanatunyong P, Khemapech N, Triratanachat S, Mutirangura A. HERV-K hypomethylation in ovarian clear cell carcinoma is associated with a poor prognosis and platinum resistance. Int J Gynecol Cancer 2011 Jan;21(1):51–7.

[27] Wang-Johanning F, Rycaj K, Plummer JB, Li M, Yin B, Frerich K, et al. Immunotherapeutic potential of anti-human endogenous retrovirus-K envelope protein antibodies in targeting breast tumors. J Natl Cancer Inst 2012 Feb 8;104(3):189–210.

[28] Chen T, Meng Z, Gan Y, Wang X, Xu F, Gu Y, et al. The viral oncogene Np9 acts as a critical molecular switch for co-activating β-catenin, ERK, Akt and Notch1 and promoting the growth of human leukemia stem/progenitor cells. Leukemia 2013 Jul;27(7):1469–78.

[29] Armbruester V, Sauter M, Roemer K, Best B, Hahn S, Nty A, et al. Np9 protein of human endogenous retrovirus K interacts with ligand of numb protein X. J Virol 2004 Oct;78(19):10310–9.

[30] Ma W, Hong Z, Liu H, Chen X, Ding L, Liu Z, et al. Human endogenous retroviruses-K (HML-2) expression is correlated with prognosis and progress of hepatocellular carcinoma. Biomed Res Int 2016;2016: 8201642.

[31] Hu L, Hornung D, Kurek R, Ostman H, Blomberg J, Bergqvist A. Expression of human endogenous gammaretroviral sequences in endometriosis and ovarian cancer. AIDS Res Hum Retroviruses 2006 Jun;22(6):551–7.

[32] Menendez L, Benigno BB, McDonald JF. L1 and HERV-W retrotransposons are hypomethylated in human ovarian carcinomas. Mol Cancer 2004 Apr 26;3:12.