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 comparison 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.

Keywords: Cervical cancer, gag, HERV-K env, np9, rec

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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 synthesise 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 intraepithelial 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 synthesised 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, 1 ul 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.

GAPDH housekeeping gene was used as an internal control, and based on 2^{−ΔΔct} 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 GraphPadPrism 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 1. The clinical characteristics of 12 cervical cancer patients

| Age | FIGO stage | Histology | Tumour size | Lymph node metastasis |
|-----|------------|-----------|-------------|----------------------|
| >50 | IA2        | Squamous cell carcinoma | <2 | 10 % |
| <50 | IB1        | Adenocarcinoma | >2 | 33.4 % |

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

| Gene | Forward primer (5’-3’) | Reverse primer (5’-3’) |
|------|------------------------|------------------------|
| GAPDH rec | ATGGTTCGTCATGGGTGTGAA | GGTGCTAAGCAGTTGGTGGT |
| 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.

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
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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FIG. 4. No changes of np expression in cervical tissues.
Availability of data and materials

Please contact author for data requests.

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Not applicable.

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