ORIGINAL ARTICLE

The evaluation of human endogenous retroviral env expression in normal and cancerous tissues of the breast

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Background: Both internal and external risk factors can accelerate the progression of breast cancer which is the reason why clinicians have tried to find new biomarkers for this health problem. Human endogenous retrovirus-W (HERV-W) can be one of these biomarkers, as it has been mentioned that some genes of this virus are able to have either higher or lower expression in numerous cancerous cells. In this study, we aimed to compare HERV-W envelope expression in breast cancer tissues and normal ones since its effects on this malignancy have not been clear. **Materials and Methods:** We collected 46 breast cancer tissues and their normal adjacent ones. After extracting the RNA of breast samples, we evaluated the expression of HERV-W envelope syncytin-1 and 2 using quantitative real-time polymerase chain reaction (PCR) in different kinds of breast cancer stages. **Results:** Data showed that more than 13% of patients had a family history of breast cancer; moreover, approximately half of the tissues were estrogen receptor or progesterone receptor positive. Lymph node metastasis was seen in 52% of the patients, and about 40% of tumors were larger than 2 cm. Real-time PCR showed that syncytin-1 and 2 had upward regulation with ($^{*}P < 0.05$) and ($^{**}P < 0.01$), respectively. **Conclusion:** As the expression of HERV-W Env (syncytin-1, syncytin-2) was higher in breast cancerous tissues in comparison with normal ones, we believe that these genes may have a role to play in monitoring patients suffering from this type of cancer. However, further studies are needed to confirm this hypothesis.

Key words: Breast, cancer, syncytin- 2, syncytin-1

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INTRODUCTION

Scientists are constantly making discoveries increasing our knowledge about carcinogenesis; however, early diagnosis of breast cancer has still been a challenge for the health-care system since this type of malignancy is the second leading cause of cancer death.[1,2] Indeed, breast cancer has been identified as the most common type of cancer, and it is the fifth cause of mortality among Iranian women. Moreover, it is responsible for the second and third causes of death in developed and developing countries, respectively, since its standardized incidence rate (ASR) is about 28/100,000 people, and the incidence continues to grow.[3-8] It has been reported that both internal and external risk factors, ranging from obesity, smoking, consumption of alcohol, and the level of melatonin hormone to genetic and epigenetic ones can affect the progression of breast cancer.[9-12] This is the reason why clinicians have tried to find new biomarkers for this health problem. It seems that one of these risk factors is infectious diseases which are likely to mutate the genome of human cells by stimulating different kinds of signaling pathways. Recently, it has been published that either a number of bacteria or viruses can trigger cells to divide uncontrollably,[10,11] but the effects of human endogenous retrovirus (HERV) in patients

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suffering from breast cancer have not been confirmed. This virus entered the germ cell line since 30–40 million years approximately, which has been transmitted vertically into the host's genome.[13] Nowadays, it is estimated that about 8% of human genes are compromised by HERVs, and numerous functional proteins can be produced by several HERV-W genes which can result in the progression of tumor cells.[14] Exogenous retroviruses are separated into three distinct classes, including I (gammaretrovirus-and *epsilonretrovirus*-like), class II (*betaretrovirus*-like), and class III (spumaretrovirus-like). HERV-W belongs to the class 1 retrovirus group employing tryptophan tRNA for its primer site binding.[15] It should be mentioned that HERV elements are flanked on both the 5′ and 3′ends of the provirus by long terminal repeats which regulate their expression, and this infectious agent is likely to change the expression of cellular genes.[16-18] In fact, it has been identified that HERV-W can have some roles in the maintenance of human pregnancy.[19] This virus is able to progress a number of diseases, such as multiple sclerosis.[20] However, scientists are at the crossroads whether, or not the envelope (syncytin-1 and 2) of this virus can affect breast cancer detrimentally as it has been reported that some genes of this virus are able to have either higher, or lower expression in numerous cancerous cells.[20-25] In this study, we aimed to compare the HERV-W envelope in breast cancer tissues and normal ones to evaluate its effect on breast cancer progression.

MATERIALS AND METHODS

Samples

This study is financially supported by the Research Department of the School of Medicine, Shahid Beheshti University of Medical Sciences: (Grant no 20789) (IR.SBMU.MSP.REC.1399.24). HERV-W env expression in 46 cancerous tissues (mean age: 68.9 ± 3.45) and their adjacent normal tissues, which were at least 5 cm away from tumors, were evaluated from breast cancer patients in Imam Hussain and Taleghani Hospital between 2018 and 2019 in Tehran, Iran. All tissues were stored in RNA later (QIAGEN GmbH, Hilden, Germany) in −20°C, and expert pathologists confirmed the stage of malignant tissues. The inclusion criteria of this study were set as the patients who provided informed consent, and the samples which confirmed the stage of breast cancer tissues by histological experts were included in the study. However, the samples of those patients who were treated either by chemotherapy or radiation were excluded from this study.

RNA extraction

In the current study, after suspending samples in 1 mL RNX-Plus solution (CinnaGen, Iran) reagent, we homogenized them and added chloroform. We added RNX-plus solution and chloroform three times to remove all the proteins by centrifuge. Afterward, isopropanol precipitated the RNA of the supernatant during 15 min of centrifuging at 12,000 rpm, and finally the RNA was diluted with 50 µL of DEPC-treated water. To confirm the purity of RNA, all RNA samples were run in agarose gel electrophoresis in order to observe 5 S, 18 S, and 28 S bands.

cDNA synthesis

To convert RNA into DNA, we provided a 20 µL reaction containing 1 µL random hexamer, 9 µL master mixes of cDNA synthesis kit (Biofact, Daejeon, South Korea) and 10 µL of RNA samples. The incubation program was 40 min at 50°C and 10 min at 95°C in Bio Intellectica polymerase chain reaction (PCR). The synthesized cDNA was then diluted twice in sterile water.

Quantitative real-time polymerase chain reaction

We assessed syncytin-1 and 2 expressions using real-time RCR analysis. 10 µL BIOFACT™ 2X real-time PCR master mix (for SYBR Green I; BIOFACT, South Korea), 6 µL sterile water, 1 µL forward 10 pmol, 1 µL reverse primer 10 pmol, and 2 µL cDNA in a final 20 µL volume were both combined and incubated in one cycle at 95°C for 10 min, 40 cycles at 95°C for 30 s; 55°C for 30 s, 72°C for 30 s in Rotor-Gene 6000 real-time rotary analyzer (Corbett Life Sciences, Sydney, Australia) in 36-well Gene Discs. The melt curve was between 60°C and 95°C. Internal control was the GAPDH housekeeping gene, and the values for the relative quantification were calculated based on 2 – ΔΔct expression formula. The list of primers used in this study is summarized in Table 1.[21]

Statistical analysis

To analyze the results of syncytin-1 and 2 expressions, we used GraphPad Software, San Diego, CA, USA. Experimental data are expressed by mean ± standard deviation of three independent assays. Statistical significance was calculated using ANOVA tests and Student's *t*-test. ($P < 0.05$) was used for the differences.

RESULTS

In this study, 46 cancerous tissues (68.9: mean age) and their adjacent normal ones were collected. Data showed that more than 13% of patients had a family history of breast cancer;

Table 1: Nucleotide sequences of primers used for real-time polymerase chain reaction

Gene	Forward primer (5'–3')	Reverse primer (5'–3')
GAPDH	ATGTTCGTCATGGGTGTGAA	GGTGCTAAGCAGTTGGTGGT
Syncytin 1	TTCACTGCCCACACCCAT	CCCCATCAGACATACCAGTT
Syncytin 2	GCTGCTGTACAACCAGTAGCTC	TTCTCTTGCCTGACCTTGAAT

moreover, approximately half of the tissues were estrogen receptor, or progesterone receptor positive. Lymph node metastasis was seen in 52% of the patients, and about 40% of tumors were larger than 2 cm.

Slight up-regulation of syncytin-1 in breast cancerous tissues

Given is a figure illustrating syncytin-1 in breast cancerous tissues increased slightly, experiencing an up-ward regulation with (*$P < 0.05$) [Figure 1]. When we divided our results according to the pathological data, we witnessed that tumors with stage I and II had lower expression compared to stages III and IV [Figure 1b].

Syncytin-2 expression in breast cancerous tissues

Figure 2, which had a similar pattern (with a higher intensive trend), showed a surge in an expression of syncytin-2 in breast cancerous tissues with (**$P < 0.01$) [Figure 2]. It should be mentioned that stages III and IV had (***$P < 0.001$) and stages I and II revealed (*$P < 0.05$) in comparison with normal samples.

DISCUSSION

Given the fact that different kinds of studies have discussed the role of HERV families on numerous cancer tissues,[20-25] it is assumed that HERV can cause breast cancer progression. Among HERV members, it has been reported that HERV-W has the possible ability to mutate the genome of cells detrimentally.[26-29] Take, for example, a study conducted by Larsen and colleagues in Denmark, which introduced syncytin as a potential prognostic factor in colorectal cancer. They reported that the more syncytin expression can be observed, the less rectal cells can be alive while it was not true in colon cancer tissues.[26] However, it has been reported that some researchers did not find any changes in cancerous cells in comparison with normal ones. To illustrate, neither the transcripts nor the translation of syncytin in leukemia and lymphoma cells was found.[30]

Although there are some reports of the expression of HERV-W in various cancers, it is still unclear which genes of this virus can contribute to breast cancer pathogenesis. In this study, we attempted to compare HERV-W env expressions in breast cancerous tissues and normal ones as they can be employed as a biomarker to diagnose this health problem. Our data showed that there was a significant increase in Syn1 and Syn2 expression in breast tumors when we compared them with the matched normal tissues, experiencing $P < 0.05$ and $P < 0.01$, respectively. Numerous studies have assessed these genes in breast cancer,[31-37] for instance, Bjerregaard and colleagues said that breast cancer cells can express high levels of HERV-env. In fact, they illustrated that the more these genes are expressed in cancerous tissues, the more breast cancer-endothelial cells can fuse with each other since these cells produce ASCT-2 (a receptor for syncytin).[31] The HERV-W Env can stimulate T47D human breast carcinoma cells to be resistant to radiotherapy as syncytin-1 homologous protein moves to the external surface of the plasma membrane of these cells.[32] Among 165 premenopausal lymph node–negative women in 2007, immunocytochemical showed that syncytin-1 was expressed in 38% of the patients, and it was described as a prognostic biomarker to diagnose this type of cancer.[33]

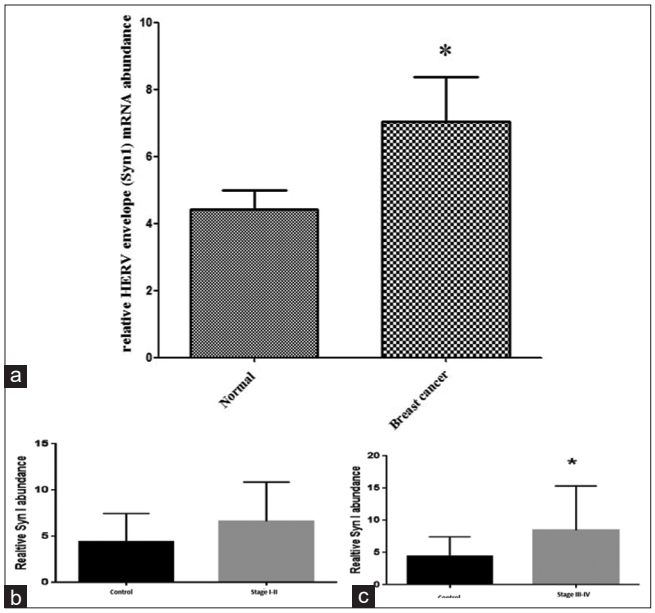

Figure 1: (a-c) Given is a figure illustrating syncytin-1 in breast cancerous tissues increased slightly, experiencing an up-ward regulation with (*$P < 0.05$). HERV = Human endogenous retrovirus

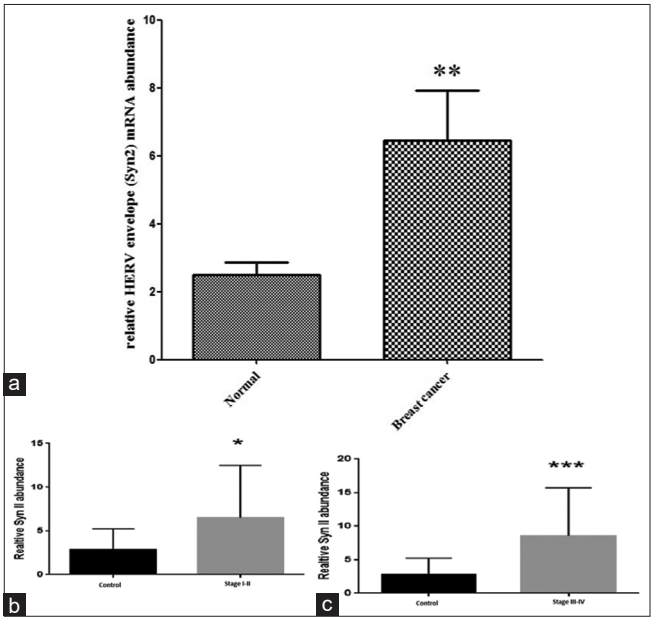

Figure 2: (a-c) Syncytin-2 had a similar pattern (with a higher intensive trend), and showed a surge in expression of syncytin-2 in breast cancerous tissues with (**$P < 0.01$). HERV = Human endogenous retrovirus

Experiments on another breast cancer cell line called MCF-7 illustrated that steroid hormones and selected endocrine disruptive chemicals in these cells can up-regulate syncytin expression.[34] Trophoblast cells, cancer-cancer cells, and cancer-host cell fusions can be related to syncytin-1 while in healthy individuals cell fusions never occurred.[35] Moreover, Durnaoglu *et al*. mentioned the disastrous effects of HERV-W env on breast cancer and some other types of malignancies, including endometrial cancer, leukemia, urothelial cells, and human hepatocellular carcinoma.[36] It was reported that preeclampsia (a common pregnancy disorder with poor trophoblast differentiation) and vascular dysfunction in the placenta have a down-regulated expression of syncytin while the opposite trend is true in multiple sclerosis tissues, endometrial carcinoma, breast cancer cell lines, and specimens.[37]

CONCLUSIONS

As the expression of HERV-W Env (syncytin-1, syncytin-2) was higher in breast cancerous tissues in comparison with normal ones, we believe that these genes may have a role to play in monitoring patients suffering from this type of cancer. However, further studies are needed to confirm this hypothesis.

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Conflicts of interest

There are no conflicts of interest.

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