Cellular and Molecular Biology

Golnarnik *et al. Cell. Mol. Biol.*2016, 62 (1): 34-37
ISSN: 1165-158X
doi: 10.14715/cmb/2016.62.1.7

Original Research

The *E-selectin* S149R polymorphisms in breast cancer in a northern Iran population

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Abstract: Breast cancer is a complex polygenic disorder that genetic factors play an important role in disease etiology. E-selectin mediates the adhesion of tumor cells to endothelial cells. This interaction is associated with metastatic dissemination. Expression of *E-selectin* on the endothelium is a hallmark of inflammation. This study was performed to evaluate the association of *E-selectin* S149R polymorphisms and the risk of breast cancer. A total of 100 patients with breast cancer and 120 healthy sample donors (controls) were recruited in this study. Genomic DNA was extracted from fresh blood samples and genotyping of the E-selectin genes was performed using PCR-restriction fragment length polymorphism (PCR-RFLP). The frequencies of the CC, AC and AA genotypes were 2%, 84%, and 14% in patients and 2%, 12%, and 86 % in controls, respectively, while the A and C allelic frequency was 56%, 44% in patients and 92%, 8% in controls, respectively. Statistical analysis showed that there is a significant difference between two groups (P<0.0001). It is concluded that the *E-selectin* S149R polymorphisms is associated with the oncogenesis of breast cancer in a population in northern Iran.

Key words: *E-selectin*, gene polymorphism, breast cancer.

Introduction

Breast cancer is the most common type of cancer among women worldwide, and the incidence of breast cancer is increasing in the developing world (1). It is widely recognized that breast cancer development is the consequence of complex interactions between the genome, lifestyle, and environment. Many factors including family history, high mammographic density, postmenopausal hormone use, early age of menarche, late first pregnancy, breastfeeding for short periods, lower parity, and a longer interval between births have been confirmed to be associated with breast cancer susceptibility (2).

This cancer is influenced by environmental and genetic factors. Cell adhesion molecules have been shown to have important roles in breast cancer invasion and metastasis (3). Selectins are calcium-dependent cell adhesion molecules present on a variety of cell types, first identified for their role in leukocyte trafficking (4). The ligands of selectins constitute a heterogenous group of heavily glycosylated proteins, all of which bear the tetrasaccharide sialyl-Lewisx (sLex) or its isomer, sialyl-Lewisa (sLea) (5). *E-selectin* is a surface glycoprotein molecule expressed on endothelial cells upon activation by cytokines (6). *E-selectin* supports the rolling of leukocytes on activated endothelial cells and efficiently mediates the adhesion of circulating monocytes and lymphocytes to endothelial cells (7). It has been demonstrated that expression of CD44 as a functional *E-selectin* ligand may be important in breast cancer metastasis (8). *E-selectin*, an inflammation inducible endothelial cell adhesion molecule, has been reported to play an important role in homing metastatic cancer cells (9).

Several findings regarding *E-selectin* polymorphisms have been described (10, 11). One polymorphism of *E-selectin* is the single base A to C transition polymorphism in exon 4, which results in an amino acid substitution serine to arginine at position 149 of EGF-domain of the *E-selectin* protein (Ser149Arg) (12).

The present study was aimed to study the potential association of S149R polymorphism of *E-selectin* with the risk of developing breast cancer in an Iranian population.

Materials and Methods

Clinical samples

In the present study 220 subjects including 100 women with breast cancer and 120 healthy women as the control group were assessed. Patients were selected considering breast cancer reports in tumor biopsy or clinical examination. Patients that have been received any type of chemotherapy or radiotherapy was excluded. The controls were selected of women came to the Razi Hospital, (-Rasht, -Iran) for health tests considering the absence of cancer in themselves and their first degree relatives and negative report of breast mass in mammography or clinical breast exam and diagnosed psychiatric diseases. Patients that have been received any type of chemotherapy or radiotherapy was excluded. All samples including controls and Cases were collected from the single Hospital. 5 ml of blood sample was collected into 10 ml K3-EDTA vacationer tube from both breast cancer cases and controls. All the samples were stored at -20°C. Written informed consent was obtained before sample collection. This form was provided considering the ethnicity rules of health ministry.

Received September 11, 2015; **Accepted** January 03, 2016; **Published** January 11, 2016

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Table 1. Distribution of genotypes and alleles in breast cancer patients and healthy controls.

	Patients (n=100) n(%)	Controls (n=120) n(%)	OR (95% CI)	p value
Allelic frequencies				
A (Ser) allele	112(56%)	221(92%)	Reference	-
C (Arg) allele	88(44%)	19(8%)	9.13 (5.29-15.76)	0.0001
Genotypic frequencies				
AA	14(14%)	103(86%)	Reference	-
AC	84(84%)	15(12%)	41.20 (18.82-90.17)	0.0001
CC	2(2%)	2(2%)	7.35 (0.95-56.46)	0.0549

Genomic DNA extraction

DNA extraction was done by GPP solution kit (Gen Pajoohan, Iran) as previously described (13). Extracted DNA was observed and confirmed by electrophoresis on 0.1% agarose gel containing etidium bromide. The concentration and purity of DNA were assessed with a Nanodrop (Thermo) with 260/280 measurement ratio and at the wavelength of 260 and 280 nm.

Genotype analysis

Genotype analysis was performed using the PCR–RFLP method. The following primers were employed for PCR amplification 5`-TCTATGGCACTCTGTAG-GAC-3` (forward) and 5`- AGAACCAGACTTACTT-TGCTC-3` (reverse) (BIONEER, Republic of Korea). The primers were designed by means of Oligo7 software (version 7.54, USA). The amplification procedure was carried in a total reaction volume of 25 µl, containing 2.5 µl 10X PCR buffer, 2 µl deoxy ribonucleotide triphosphates (1.25 µmol/L), 0.5 µl MgCl2 (25 mmol/L), 1.25 µl of each primer (25 mmol/L), 15.3 µl dH2O, 2 µl DNA (100 ng/µl) and 0.2 µl Taq DNA polymerase (5 U/µl) (Biflux, Japan). After an initial denaturation at 94°C for 5 min, the DNA was amplified by 35 cycles of 94°C for 45 Sec, 52°C for 45s and 72°C for 45s, with a final extraction at 72°C for 5 min on the mini PCR (Bio-Rad), then the products electrophoresed on a 2% agarose gel, to allow detection by ethidium bromide staining. The PstI restriction enzymes (Fermentase, USA) were used for RFLP. The restriction products were analyzed by electrophoresis in 2 % agarose gel and visualized using UV fluorescence after staining with ethidium bromide. PstI cannot digest the C allele (240 bp) but generates 98 bp and 142 bp fragments for the A allele. So heterozygote samples had a combination of both alleles (240 bp, 142 bp and 98 bp bands). (Figure 1)

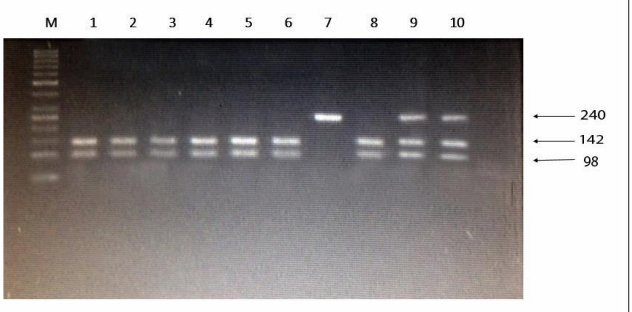

Figure 1. *E-selectin* SNP S149R PCR product stained by Ethidium Bromide on 2% Agarose Gel-Electrophoresis. Representative gel pictures of *E-selectin* S149R. The RFLP pattern of *E-selectin* S149R shows bands at 240 bp in wild-types (CC); 240bp, 142bp, and 98bp in heterozygotes (AC); and 142bp and 98bp in variant homozygotes (AA).

Statistical analysis

All statistical analyses were performed using MedCalc statistical software (Version 12.1, Mariakerke, Belgium). Differences in genetic distributions between patients and the controls were calculated by Pearson's chi-square (χ^2) test. Odds ratios (OR) and 95% confidence intervals (95%CI) were estimated using an unconditional logistic regression model. The results were considered statistically significant when $P<0.05$.

Results

Our study group consisted of 100 patients with pathologically confirmed breast cancer and 120 healthy control subjects (age range, 38-67 years). The length of PCR products for *E-selectin* was 240nt. The frequencies of *E-selectin* genotypes have been estimated using a PCR-RFLP. The observed genotype frequencies for polymorphism were in accordance with the Hardy–Weinberg equilibrium. The prevalence of genotype frequencies for AA, AC and CC were 86%, 12% and 2% in controls, and 14%, 84% and 2% in patients, respectively. Statistical analysis showed that there is significant difference between two groups ($P<0.0001$). The results indicated that the subgroups with AC genotypes were associated with increased risk of breast cancer (OR= 41.2, 95% CI= 18.82 – 90.17, $P< 0.0001$). Moreover, significant association was found in allele frequencies ($P< 0.0001$). All information about allele and genotype frequencies and associated ORs (95%CI) for patient cases and controls are summarized in table 1.

Discussion

Breast cancer is a multifactorial disease resulting from the interaction between genetic and environmental factors (14). Expression of *E-selectin* on the endothelium is a hallmark of inflammation (15). Consequently, elevated *E-selectin* expression on tumor-associated vasculature is observed in many types of cancers including breast cancer (16). *E-selectin* appears to play an equally important role in the metastatic process. Current evidence suggests that cancer cells expressing *E-selectin* ligands are able to bind to epithelial cells, which are activated by various stimuli, and initiate a process of trans-endothelial migration that has similarities with leukocyte rolling and diapedesis (17). Expression of sLex and sLea-the natural ligands of *E-selectin*- has been shown to be elevated in breast cancer cells (18).

In this study, we investigated the association between potentially functional polymorphism of *E-selectin* (S149R) and risk of breast cancer in a North Iranian population. In this case-controls study we evaluated the role of *E-selectin* S149R polymorphism in 100 patients and 120 controls. Our results suggest that there is a significant association in genotype distribution between cases and controls ($P< 0.0001$). The individuals with AC genotypes were associated with increased risk of breast cancer (OR= 41.2, 95% CI= 18.82 – 90.17, $P<0.0001$). The absolute number of carriers of the CC genotype in our sample were very small (2 patients and 2 controls), a fact which may be responsible for the unexpected CC frequencies. The study finding supports the important role of *E-selectin* in the progression of breast cancer and the potential prognostic role of the *E-selectin* S149R polymorphism.

It has been demonstrated that *E-selectin* genetic variations have a crucial role to increasing the risk in many kind of disease. Kontogianni and colleagues reported that the *E-selectin* S128R CC genotype may be related to poorer prognosis of breast cancer (16). It was also shown that the *E-selectin* S128R C allele may confer an increased susceptibility to gastric and pancreatic cancers development (19, 20). Alecssandro and colleagues have been suggested that the *E-selectin* S128R polymorphism can functionally affect tumor-endothelial interactions as well as motility and signaling properties of neoplastic cells that may modulate the metastatic phenotype (21). Also it has been shown that *E-selectin* S128R gene polymorphism is associated with the breast cancer in Malaysian population (22). It has been shown postoperative platelet activation is related to the S149R polymorphism, which enhances the risk of adverse events after coronary artery bypass grafting (12). It was shown that S128R polymorphism is a constitutional factor associated with a higher risk of relapse and death in patients treated for colorectal cancer (23). Wang et al also suggested that the *E-selectin* gene polymorphisms A561C, G98T were significantly associated with increased risk of coronary artery disease (24). One of the most common polymorphisms of E-selectin is the A to C transversion at position 561 of exon 4. This causes the substitution of serine 128 by an arginine residue (S128R) in the mature protein, which eventually results in a variant with higher affinity for E-selectin ligands (16).

This study showed that women who were AC heterozygotes and carriers of the Arg allele genotype had a significant increased risk of breast cancer. Patients who were carriers of the Ser allele genotype showed a significant association with poorly differentiated tumors.

In conclusion, the S149R polymorphism might confer an increased oncogenesis of breast cancer. Larger scaled studies are required to further investigate the prognostic utility of the polymorphisms for evaluation as predictive markers.

Acknowledgments

The authors express their appreciation to all the participants for their contribution to this study. We also would like to thank Biology Department- Faculty of Science-University of Guilan, Rasht, Iran.

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