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Up Regulation of Ezrin and Radixin with respect to Grade of Tumors in Breast Cancer Patients

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ABSTRACT

Background: Breast cancer (BC) is one of the main causes of death among women in Iran. Biomarkers involved in promotion and progression of disease is very important in management and control of BC outcomes. In this research, we aim to estimate the expression levels of Ezrin and Radixin, as two important factors in morphogenesis, endocytosis, exocytosis, adherence, and migration of cells, in BC patients and their relationship with pathological factors.
Methods: One hundred and thirteen BC patients were involved in this research. Relative expression of Ezrin and Radixin genes were estimated with quantitative real-time PCR. Pathological data include the histology, tumor size, grade, lymphovascular invasion and clinical TNM (Tumor, Node, and Metastasis) staging of patients were recorded based on the pathology report and their relationship with relative expression of Ezrin and Radixin were estimated.
Result: According to result Ezrin were over expressed in tumor samples in comparison to adjacent normal tissue. There is a significant relationship between over expression of Ezrin and Radixin and grade of tumor and necrosis. Also there is a direct relationship between relative expression of Ezrin and Radixin expression.
Conclusions: These data support the role of Ezrin and Radixin in the biology of breast cancer and additional studies needed that determine the Ezrin and Radixin associated with phenotype and may validate them as markers of cancer progression and as a potential target for cancer therapy.

Keywords: Ezrin, Radixin, Breast cancer, Grade

INTRODUCTION:

Breast cancer (BC) is one of the main causes of death among women in Iran. Although recent improvement of screening methods increased the survival rate of BC patients, but there is a long way to manage and control the outcomes of disease (1-6). Common features of all cancers are inappropriate cell survival, uncontrolled cell proliferation and abnormality of cellular morphogenesis. Actin and microtubule cytoskeletons play a key role in these cellular processes and stabilizing cell membrane (7, 8). Abnormal organization of cell skeleton is one of the most basic features of modified tumor cells. Ezrin and Radixin as a member of Ezrin- Radixin-Moesin (ERM) group play a main role in linkage of actin filaments to the apical cell membrane (9). ERM proteins provide a regulated connection between cell membrane and cytoskeleton. It also gives the cells ability to organize complex proteins in special parts. Therefore, Ezrin and Radixin proteins play a critical role in morphogenesis, endocytosis, exocytosis, adherence, and migration of cells.

Ezrin in intestinal and renal proximal tubular epithelium is expressed. The name of Ezrin is derived from the microvillus which is first found in the microvilli of epithelial layer of intestinal tissue. Ezrin is highly specialized and detected protein in colorectal carcinoma (CRC). The loss of Ezrin protein expression has shown to have a correlation with poor prognosis and survival in CRC (10). High levels of Ezrin may regulate c-Src activation in tamoxifen resistance BC cells (11).

Both epithelial and mesenchymal cancers were expressed Ezrin and Radixin. According to the investigations, most of the normal epithelial tissues have high Ezrin expression in comparison to low Ezrin expression in normal mesenchymal tissues, such as smooth muscle, skeletal muscle, and endothelium (12). This research aim is to estimate the relative expression of Ezrin and Radixin in tumor samples in comparison with corresponding normal tissue and their relationship with pathological factors in BC patients.

Materials and Methods:

Patients and specimens: biological materials were provided by Iran National Tumor Bank which is founded by Cancer Institute of Tehran University of Medical Sciences, for Cancer Research. One hundred and thirties patients with BC who underwent surgery in Cancer Institute of Iran for this study were selected. Patients have received any chemotherapy or radiotherapy prior to surgery. Patients were diagnosed with BC based on histopathological examination. The pathological data of patients were recorded according to the pathology report include the histology, tumor size, grade, lymphovascular invasion and clinical TNM (Tumor, Node, and Metastasis) staging. Subjects with chronic or acute inflammatory diseases or any other synchronize primary tumor were also excluded from the study. All samples with full observation of preparation and preservation processes of standard protocols were prepared base on ethical permission and obtaining written informed consent from all donors.

RNA extraction and real-time quantitative RT-PCR:

Primer sequences for Ezrin and Radixin were designed and synthesized by TakapouZist Company, Iran. Forward and reverse primers for Ezrin were 5'-GGCCAGCCAAGATGAAATTA-3' and 5'-CTCAAAGGCCTTGGTGTTGT-3' respectively and for radixin were 5'-AAA GCC AAT CGA CAA AAA GG-3' and 5'-GTTTCTCCTCCCTAGCCTGA-3'. Forward and reverse primers for GAPDH were 5'-GAAGGTGAAGGTCGGAGTCA-3' and 5'-AATGAAGGGGTCATTGATGG-3' respectively.

RNA using GeneAll® Hybrid-RTM kit (GeneAll Biotechnology, Seoul, Korea) according to the manufacturer's instructions was extracted. After normalization of all extracted RNA to 1μg, RNA was reverse transcribed into single strand cDNA using Takara kit (Takara, Japan) fallowing the protocols. The quantity and purity of extracted RNA by using Nano-Drop (Technologies, ND-2000) was analyzed. The product was used for quantitative RT-PCR using syber green /ROX (Takara, Japan) real time PCR master mix based on the protocol

of rotor gene quantitative RT-PCR thermal cycler (Qiagen, USA). The amplification protocol comprised of 1 cycle at 95 °C for 4 min followed by 40 cycles at 95 °C for 15 s, 59 °C for 30 s, and then 72 °C for 30 s. The cycle threshold (CT) by ROTOR GENE software was determined. The relative expression of the studied genes to the housekeeping gene was calculated by $2-\Delta\Delta CT$ formula (13).

Statistical analysis:

Data were presented as mean ± SE, and number (percentage). Statistical differences of Ezrin and Radixin expression level between tumor samples and normal adjacent tissue were determined by two tailed Mann–Whitney U test. Comparison of gene expression between two groups in variables like lympho-vascular invasion also by two tailed Mann–Whitney U test was determined. Comparison of gene expression between more than two groups in variables like histological grade and TNM staging by K independent sample and kruskal-wallis test were determined. Pearson's correlation coefficient analysis was used to calculate correlation between genes expression and tumor size. A P value less than 0.05 was considered statistically significant.

Results:

The expression of Ezrin and Radixin in one hundred and thirteen BC patients was detected by qRT-PCR. The results showed that the expression level of both genes was strongly expressed in breast cancer tissues, particularly in Ezrin. The expression levels of Ezrin and Radixin (normal) and their matched adjacent normal tissues (control) were also detected by qRT-PCR. Clinico-pathological characteristics of study population are showed in Table 1. The patient's age range was between 31 and 77 years old with average of 47.49±10.73. There were 21(16.15%) patient with gradeI, 59(45.3%) were in gradeII , 44(33.8%) in gradeIII and 6(4.04) patients data not applicable.

Expression levels of Ezrin and Radixin in tumor samples and normal adjacent tissue of BC patients is shown in Figure 1. Although it is not significant for Radixin P value>0.05, the mean value of Ezrin and Radixin expression in tumor sample is increased in comparison to the related normal adjacent tissue.

Association between relative expression of Ezrin and Radixin and pathological factors of tumor such as Grade, Stage, Lymphovascular and perineural invasion is evaluated in this research. As indicated in Table 2, there is significant relationship between relative ex-

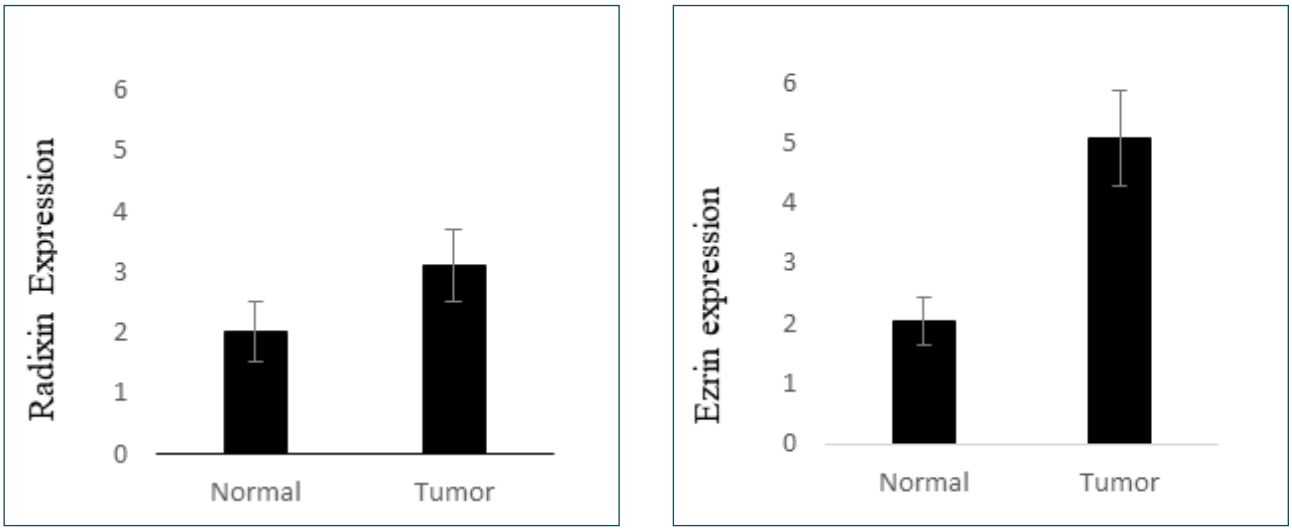

Figure 1. Ezrin and Radixin expression levels in tumor samples in comparison to the related normal adjacent tissue. (Ezrin p value =0.005) , (Radxin p value =0.09) Radixin expression in breast patient (left) and Ezrin expression levels in breast cancer (right) when comparing paired normal and tumor gene array data. The bars represent the proportions of tumor samples (One hundred and thirteen BC patients) that show a higher expression of the selected gene compared to normal samples .

Table 1. Pathological characteristics of study population.

Variable	Patients number (%)
Stage	
I	10 (7.6)
II	63 (48.4)
III	52 (40)
Data not applicable	5(3.8)
Grade	
I	21 (16.15)
II	59 (45.3)
III	44 (33.8)
Data not applicable	6 (4.06)
Tumor size	
>5	94(7.3)
≤5	31(23.8)
Data not applicable	5(3.8)
Necrosis	
Yes	62(47.6)
No	60(46.1)
Data not applicable	8(6.1)
Lymphatic invasion	
Yes	72(55.38)
NO	45(34.6)
Data not applicable	3(2.3)
Vascular invasion	
Yes	88(67.7)
NO	37(28.4)
Data not applicable	5(3.8)
Perineural invasion	
Yes	37(28.4)
NO	85(65.4)
Data not applicable	8(6.1)
Age(year)	
<50 years	71 (54.6)
≥50 years	59 (45.4)

Table 2. Association between Ezrin and Radixin relative expression and pathological characteristics in BC tumors

pathological Parameters		Radixin	P value	Ezrin	P value
Stage	I-II	3.25±0.25	0.63	1.73±0.16	0.26
	III-IV	3.05±0.32		1.45±0.19	
Tumor size		3.1±0.19	0.98	1.6±0.12	0.24
Necrosis	Yes	3.28±0.29	0.46	1.9±0.19	0.02
	No	2.97±0.27		1.34±0.15	
Lymphatic invasion	Yes	1.51±0.15	0.39	2.66±0.36	0.15
	No	1.77±0.28		3.31±0.23	
Vascular invasion	Yes	3.47±3.6	0.302	1.49±0.13	0.058
	No	2.47±4.2		2.07±1.97	
Perineural Invasion	Yes	3.34±0.38	0.42	0.25±0.057	0.196
	No	2.97±0.24		0.28±0.043	
Grade	I	1.51±0.12	0.014	0.54±0.07	0.011
	II	3.11±0.28	0.000	2.31±0.20	0.000
	III	3.9±0.35	0.20	1.53±0.17	0.07

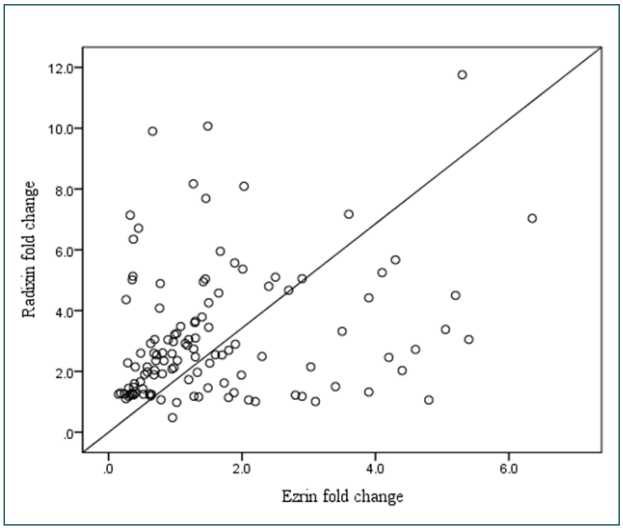

Figure 2. Correlation between Ezrin and Radixin relative expression in BC patients. (r=0.257 and p value =0.005

pression levels of Ezrin and Radixin and grade of tumors ($p< 0.05$).

Bivariate correlation between Ezrin and Radixin is shown in a single scatter plot (Figure 2). As indicated, there is a positive relationship between Ezrin and Radixin relative expression in BC patients ($r= 0.257$, $P< 0.01$).

Discussion:

In this study relative expression change of Ezrin and Radixin in BC patients were evaluated. We investigate the relationship between Ezrin and Radixin expression fold change and clinico-pathological factors including tumor size, grade, stage, necrosis presence, lympho-vascular and perineural invasion.

Ezrin and Radixin are belonging to ERM family, which act as linkers between the actin cytoskeleton and a variety of membrane-anchoring proteins. This role of ERM proteins is essential for many processes that basically are vital for the micro invasion and metastasis. Other processes of tumor progression such as apoptosis, cell adhesion, motility, surface structures and morphogenesis of cells are also mediated by ERM proteins. Previous studies show that cell survival is promoted by Erzin and Radixin through restricting Rho1 activation, which lead to activation of Jun N-Terminal (JNK) pathway signaling and apoptosis (14). Poor prognosis of Ezrin is conferring by many investigations that indicate the relation of Ezrin to malignant tumor progression, invasion and metastasis (14), Although studies reporting that Erzin and Radixin have a direct relationship with tumor pathogenesis that

is few in number. Evidence supporting the role of Erzin and Radixin in cancer behavior, especially in invasion and metastasis has begun to accumulate.

Expression of Ezrin, was analyzed in over 5,000 patients with breast, lung, prostate cancers and sarcomas using tissue microarray immunohistochemistry by Benjamin et al. In general, Ezrin was expressed at higher levels in sarcomas than in carcinomas. A significant association were found between Ezrin over expressions and histological grade in sarcomas and poor outcome in breast cancer (12).

The results show there is a significant relationship between Ezrin and Radixin relative expression and grade of tumors in BC patients. This finding is consistent with Valdman et al investigation. They report the over expression of Ezrin in prostate cancer and high-grade prostatic intraepithelial neoplasia (HGPIN) in comparison to the adjacent normal tissue with greater expression level of Ezrin in HGPIN compared with prostate cancer (15). Weng et al, found that over expression of Ezrin in soft tissue sarcoma patients is strongly associated with the development of metastases, and consequently with poor survival (16). A similar association was found in pediatric osteosarcoma patients by Khanna et al, (17).

The results show there is also a positive correlation between expression levels of Ezrin and Radixin in BC patients. The ERM family has consistently been found as co-expressed in the majority of cultured cells (18). The role of ERM proteins in tumor progression and metastatic human carcinoma is identified (19-21). In this study, we did not find any correlation between the size of the tumor and expression levels of the Ezrin and Radixin.

Investigations indicate that the phosphorylation status of ERM proteins beside their sub cellular localization is related to tumor progression. The results show there is a relationship between lymph node metastasis and moesin and radixin over expression in pancreatic cancer. Ezrin phosphorylation, also found to be involved in lymph node metastasis in primary pancreatic cancer (20). Change in Ezrin localization is associated with a poor prognostic in invasive breast carcinomas (22). Delocalization of ERM proteins in cells like translocation from the membrane to the cytoplasm is found to be related with several dysfunctions such as impair adhesion of cell-cell or cell-matrix which can lead to the acquisition of an invasive phenotype in tumor cells (23).

Conclusion:

These data support a role for Ezrin and Radixin in the biology of BC and the need for additional studies that determine the Ezrin and Radixin associated phenotype and may validate them as markers of cancer progression and as a potential target for cancer therapy.

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Conflict of interest:

There is no conflict of interest

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