RESEARCH ARTICLE

Prognostic Role of PTEN Gene Expression in Breast Cancer Patients from North-East Iran

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Abstract

Background: PTEN protein is one of the most important tumour suppressor factors which is detectable by immunohistochemistry. The goal of the present study was to investigate the prognostic role of PTEN gene expression in breast cancer patients. Materials and Methods: This descriptive-analytical study was conducted on 100 breast cancer patients referred to Sabzevar hospitals in the north-east of Iran between 2010 and 2011, who were followed up to 2015. PTEN gene expression in tissue samples was determined using specific monoclonal antibodies and data were analyzed using Chi-square test and Fisher's exact test. Patient survival was analyzed after 4 years of follow-up using the Cox regression model. Results: PTEN gene expression was evident in 70 of 100 cnacer samples but was found at high levels in all non-cancer samples. There was an inverse significant relationship between PTEN gene expression and tumour stage or tumour grade ($p<0.001$). The expression of PTEN in invasive ductal tumours was lower than in non-invasive tumours. There was also an inverse significant relationship between the hazard of death and PTEN gene expression ($p<0.001$). In addition, there was an inverse significant relationship between tumour stage and hazard of death ($p<0.001$). Conclusion: These findings indicate that lack of PTEN gene expression can be a sign of a worse prognosis and poor survival in breast cancer cases.

Keywords: PTEN - breast carcinoma - immunohistochemistry - survival - prognosis

Asian Pac J Cancer Prev, **17 (9)**, 4527-4531

Introduction

Breast cancer is considered as one of the most important malignancies in women in the U.S. (Cianfrocca and Goldstein, 2004). In western countries, one in 8-12 individuals is diagnosed with breast cancer. Breast cancer is the second leading cause of mortality in women (Dunning et al., 1999). In Asia, the prevalence of breast cancer has been increasing during the recent years (Lam et al., 2005). Moreover, the mean age of onset of breast cancer is reduced in Iran (Golmohammadi and Pejhan, 2012). Breast carcinoma has mainly two forms: non-invasive and invasive. Non-invasive carcinomas account for about 15-30% of all breast cancer cases and include ductal carcinoma in situ (DCIS) and lobular carcinoma in situ (LCIS). Invasive carcinomas comprise 70-85% of breast cancer cases and include invasive ductal carcinoma (IDC) as well as invasive lobular carcinoma (ILC) (Kumar et al., 2007).

A series of genetic and environmental factors are involved in the development of breast cancer. Genes activated in breast cancer fall into two categories: those inhibiting tumour progression which are called tumour suppressors such as P53 and PTEN genes, and the ones whose expression result in tumour progression or acceleration (de Assis and Isoldi, 2013; Golmohammadi et al., 2013). Phosphatase and tensin homolog deleted on chromosome ten (PTEN) is a tumour suppressor gene on chromosome 10q23 consisting of 11 exons. New studies are concentrated on this gene. The PTEN gene is deactivated in some malignant tumours including breast cancer (Bogdanova et al., 2013; Yu et al., 2015; Zhang et al., 2015). The functional role of this gene is the induction of apoptosis in cancer cells. The PTEN gene product plays a role in the generation of chemotaxis signals and inhibition of cell proliferation. PTEN deletion can increase progression of prostate carcinoma in mice and human (Wang et al., 2003; Dean et al., 2014; Troyer et al., 2015). In the research conducted by Wan et al., it was indicated that PTEN gene regulates numerous cellular responses on tumour cell chemotaxis. Moreover, in vitro studies have indicated that the injection of PTEN to mice lacking the gene can reduce metastasis of cancer cells to the lung (Wan et al., 2007). Not only is the dysfunction of PTEN gene associated with several cancers, but also it can lead to cardiovascular diseases, diabetes, Parkinson's disease and schizophrenia (Gori et al., 2009).

PTEN gene plays the role of a tumour suppressor, and

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its mutation is associated with the rapid metastasis and increased tumour invasive power in some tumours (Sakr et al., 2010; Yang et al., 2010; Okutur et al., 2015; Troyer et al., 2015). However, there is a controversy about PTEN role in different cancers. It has been reported that PTEN does not play any significant role in the development of endometrial carcinoma in Iranian cases (Kafshdooz et al., 2015), but there is a little information about its role in breast cancer in Iran. Considering different functional roles of PTEN gene in breast cancer, further complementary studies are needed to better determine its mechanism (Boosani and Agrawal, 2013).Therefore, the present study aimed to investigate the relationship between PTEN gene expression and the length of survival in breast cancer patients along with pathological and histological parameters in Iranian patients.

Materials and Methods

Collection and Preparation of Samples

The present descriptive-analytical study was conducted on 100 breast cancer tissue samples of patients referring to Sabzevar hospitals in the north east of Iran between 2010 and 2011, and they were followed up to 2015. This study was performed according to the guidelines of the committee of ethics in Sabzevar University of Medical Sciences in Sabzevar, Iran. Samples were collected before the patients receive any radiotherapy and/or chemotherapy. The healthy adjacent tissue was used as the control sample. The order of procedures in summary includes: fixing samples in 10% formalin, tissue processing, fixing samples in paraffin and then moulding, preparing 4-micron sections of all samples using Litzerotary microtome and finally staining with hematoxylin and eosin. Tumours were divided into grades 1-3 according to the diagnosis of malignancy by two pathologists (double-blinded) and determining tissue grades was based on mitosis, polymorphism and presence or absence of a gland in samples.

Evaluation of PTEN Gene Expression by Immunohistochemistry

PTEN gene expression in samples was investigated by immunohistochemistry. The order of immunochemistry procedures may be summarized as follows: Temperature and antibody concentrations were used according to the kit's instructions; microwave and citrate buffer with the temperature of 100°C was used for 10 minutes to demask the location of the antigenic indices of the samples; in order to inhibit the activity of endogenous peroxidise, the slides were kept in 3% hydrogen peroxide solution for 30 minutes and then rinsed 5 times with phosphate buffer saline (PBS). Afterwards, primary specific rabbit monoclonal PTEN antibody manufactured by Novocastra Co was applied on slides and rinsed three times by PBS after being incubated, and then the second antibody was added. After each stage, slides were rinsed three times with PBS. HRP-conjugated streptavidin which can oxide diaminobenzidine (DAB) was used for staining the cells. Finally, the slides were investigated using a light microscope equipped with the advanced motic plus 2 camera, and images were taken from them. It should be noted that the slides were separately investigated by two pathologists. After the investigation, cells with less than 10% colouring were considered negative, and those with more than 10% of brown stain were considered positive. Samples were divided into positive and negative degrees considering PTEN gene expression or its lack (Sakr et al., 2010; Wikman et al., 2012).

Follow up

All patients were followed up after their diagnosis for 48 months; calls were made to their family or themselves to follow up their current status.

Statistical analysis

Data analysis was performed using SPSS 11.5, and chi-square and Fisher's exact tests were performed when required. Using Cox's proportional hazard Regression Model, the relationship between the hazard of death of those patients with PTEN expression as well as tumour stage and tumour grade were also evaluated. Significant differences were considered when p-value was less than or equal to 0.05 in all.

Results

Demographic, Pathological and Histological Findings

The average age of breast cancer patients was 47.11±13.86; and minimum and maximum ages were 25 and 82 years, respectively. Among 100 cases of malignancy, 82 cases were IDC, 13 cases were non-invasive lobular carcinoma, and 5 cases were ILC. Moreover, 32 cases were in the CIS stage; in other words, cancerous cells were limited to a single lobule or duct and surrounding fat tissues were not infected. The classification of patients according to tumour stage and tumour grade with PTEN gene expression is shown in Table 1.

PTEN Gene Expression in Samples

PTEN gene was expressed at a high level in 70 malignancy samples (70%) (Table 1). All noncancerous samples expressed PTEN gene at a high level. No significant relationship was observed between tumour grade and tumour stage ($p>0.05$). However, there was an inverse significant relationship between PTEN gene expression and tumour stage in malignant mammary gland tissues ($p<0.001$).

There was also an inverse significant relationship between PTEN gene expression and tumour grade ($p<0.001$). Moreover, PTEN gene expression was less in tumours that had metastasized. PTEN gene was not expressed in 28 cases (28%) of malignant invasive ductal tumours, but in non-invasive tumours PTEN gene was not expressed only in 2 cases (%2). In other words, a significant relationship was observed between tumour type and PTEN gene expression (Fisher's exact test) ($P<0.04$).

Immunohistochemical Studies of PTEN Gene Expression

During the specific immunohistochemical staining, cancerous cells expressing PTEN gene became brown (Figure 1). As it can be observed in Figure 1, PTEN

Table 1. Expression Levels of PTEN Gene in Different Tumour Stages and Grades

PTEN gene expression	Positive		Negative		Total	
	N	Percent	N	Percent	N	Percent
Stage						
Zero	24	24%	8	8%	32	32%
One	36	36%	8	8%	44	44%
Two and three	9	9%	5	5%	14	14%
Four	1	1%	9	9%	10	10%
Total	70	70%	30	30%	100	100%
Grade						
Grade 1	14	14%	3	3%	17	17%
Grade 2	45	45%	7	7%	52	52%
Grade 3	11	11%	20	20%	31	31%
Total	70	70%	30	30%	100	100%

Table 2. Estimation of Cox's Proportional Hazard Regression in Breast Cancer Patients

	Standard Error	Coefficient	P - value
Grade			0.63
Grade 1	0.53	0.47	0.34
Grade 2	0.43	0.1	0.81
Stage			
Stage 0	0.99	-5.01	0
Stage 1	0.95	-4.61	0
Stage 2	0.88	-2.61	0
Stage 3	0.81	-1.36	0.09
PTEN Expression	0.4	-1.1	0
Age	0.01	0.01	0.22

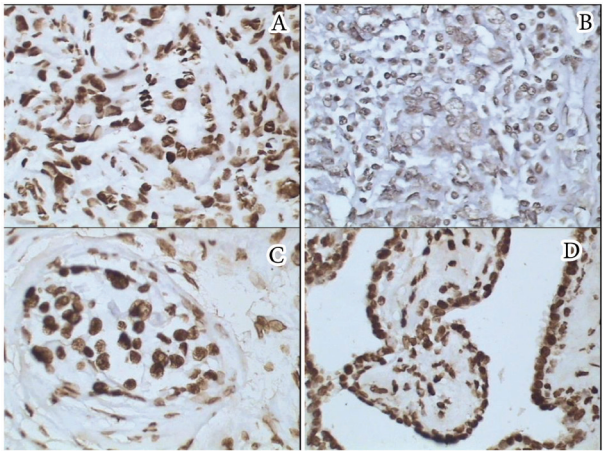

Figure 1. Immunohistochemical Expression of PTEN in Breast Cancer and Normal Tissues (×400). A: Grade 1; B: Grade 2; C: Grade 3; D: Normal mammary gland tissue. This image shows malignant cells, in more than 50% of which PTEN gene has been expressed (brown dots).

gene expression decreased in malignancy samples with increased grade. In healthy breast tissues, stromal cells and mammary gland ducts cells showed a high level of PTEN gene expression.

Survival determination

The present study showed that 49 patients died during 4 years follow up. After investigations of the baseline hazard functions by Log-minus-log plot, the Cox Regression Model was tested. As the exp(coefficient) shows hazards, the results in Table 2 show that the hazard of death of patients in stage 0 is very low in comparison to patients in stage 4 (0.007 times). Patients in stage 4 have 100 times more hazard of death in comparison to patients in stage 1 and 13 times more in comparison to patients in stage 2. But there is no significant difference in comparison to patients in stage 3. This table also shows that hazard of death in patients without PTEN gene expression is 3 times more than patients with PTEN gene expression. Also, this table shows that there are no significant differences between different tumor grades or different ages.

Discussion

The present study indicated that there is a significant relationship between decreased PTEN gene expression and tumour stage. Thus, developed breast cancer tumours at higher stages expressed PTEN gene less. Previous studies showed that the risk of metastasis of cancerous cells to other tissues is greater in breast cancer patients without PTEN gene expression (Tsutsui et al., 2005; Wikman et al., 2012). This study is consistent with the aforementioned studies. In the present study, PTEN gene was only expressed in 70 (70%) of 100 samples. Negative PTEN gene expression samples were more frequently observed in patients with higher stages of the disease. The report by Yang et al. in China indicated that PTEN gene was not expressed in 24 of 50 breast cancer patients (Yang et al., 2010). The present study is different from the aforementioned research in two respects: PTEN gene expression and sample size. The present research investigated PTEN gene expression in 100 breast cancer patients while the one by Yang investigated 50 malignancy samples. On the other hand, it is also likely that difference in PTEN gene expression is likely to be related to geographic factors. The study by Koo et al. (2015) indicated that patients with low PTEN gene expression showed poor response to chemotherapy in breast cancer patients (Koo et al., 2015); also, the study by Zhou et al. showed that the lack of PTEN gene expression was associated with drug resistance in leukemia cells. It should be noted that via the inhibition of MDM2 and the activity of a few other genes such as p53, the PTEN gene product causes physiological death in cancerous cells. Resistance is induced in the apoptosis of malignant cells with the loss of PTEN gene expression (Zhou et al., 2003). PTEN gene inhibits MDM2 activity

via an antagonistic signal role of phosphatidyl inositol. As a result, the protein of this gene remains in cytoplasm and is degraded by enzymes. MDM2 gene is an oncoprotein nullifying the activity of P53 while PTEN gene maintains P53 function. Therefore, PTEN gene expression makes the tumour sensitive to chemotherapy (Mayo et al., 2002; van den Broek et al., 2011). A study by Zhang et al. on breast cancer indicated that 84 of 146 (57.5%) breast cancer patients were associated with PTEN gene expression while all of the healthy samples were associated with it (Zhang et al., 2013). The healthy samples of the present research were also associated with PTEN gene expression. This result is consistent with that of the aforementioned study in this regard. However, there is a difference in PTEN gene expression in malignant samples in the two geographic regions. New studies have indicated that different genetic and non-genetic mechanisms including epigenetic factors are involved in the regulation and performance of PTEN gene, and that some of these factors have not been identified to date. Hence, PTEN gene expression in breast cancer is recommended to be investigated in several geographic regions.

Comprehensive analysis of the PTEN gene has indicated that not only it is a tumour suppressor, but also it plays a role in breast cancer prognosis (Jones et al., 2013; Mandal et al., 2015). In the present study, PTEN gene was not expressed in patients in the progress stage of the disease (Stage IV). It is probable that due to the removal of inhibitory factors from the cycle of cell division in malignant cells without PTEN gene expression, the rate of division and invasiveness in them increases significantly. In the present study, PTEN gene expression in invasive ductal tumours was less than non-invasive tumours, and the difference was statistically significant. Therefore, it is likely that one of the reasons for increased invasiveness in invasive ductal tumours is the lack of PTEN gene expression in them. Studies by Jones et al. indicated that complete loss of the function of PTEN protein in tumours increases invasive power and significantly reduces the oestrogen receptors (Jones et al., 2013). In the present study, PTEN gene was expressed less in malignant breast tumours at higher grades. This study is consistent with the aforementioned research.

The PTEN gene product is active in the regulation of cellular oxidative stresses and apoptosis (Kitagishi and Matsuda. 2013). New studies have indicated that PTEN gene can be used as a marker for the evaluation of survival (Neto et al., 2012). On the other hand, the PTEN gene product affects the expression of other genes which are important in breast cancer for response to treatment. However, some of the actions of this gene remain unknown to date (Song et al., 2012). In another study, Baig et al. showed that genetic alterations such as PTEN gene mutation are associated with breast cancer (Baig et al., 2011). In the present research, hazard of death in patients without PTEN gene expression was more than those associated with it. Therefore, this specific marker can be used for prognosis. However, pathological parameters must be considered along with PTEN gene expression because in the present study, there was an inverse significant relationship between high stage and PTEN expression. Also, our study showed that the hazard of death is high in patients with a higher stage. This indicates that besides the investigation of new markers, pathological parameters which are usually used in the prognosis are important. Other studies confirm this fact (Sharif et al., 2009). It should be noted that for a timely diagnosis and treatment, it is necessary to use new markers such as the examination of PTEN gene expression along with the investigation of pathological parameters in breast cancer, the second most lethal cancer in women.

In conclusion, the present study showed that, considering the fact that the average age of the onset of breast cancer is decreasing in Iran, new prognosis markers such as PTEN are suggested to be used along with pathological and histological parameters in order to determine the situation of patients. The length of survival of patients without PTEN gene expression is less than those with it. This finding can be helpful in the prognosis and treatment process of these patients.

Acknowledgements

Authors would like to express their gratitude to Sabzevar University of Medical Sciences Research Council for approving this project and funding it (Grant number 90005). We also thank pathologists Dr. Mohammad Reza Mohajeri and Dr. Farshad Marouzi, as well as laboratory experts Ms. Landarani and Ms. Mahmoodi for conducting part of the histological tasks and Dr. N. Shomoossi for editorial assistance. It is necessary to announce that, there is no conflict of interest regarding the authors of this article.

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