PTEN and p53 Gene Expressions in Breast Cancer Specimens and their Clinicopathological Significance

Hamzeh Salmani*, Arshad Hosseini**♦, Asaad Azarnezhad***,****, Hussain Ahmad*****

*Department of Medical Biotechnology, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran
**Department of Medical Biotechnology, School of Allied Medicine, Iran University of Medical Sciences, Tehran, Iran
***Department of Molecular Medicine and Genetics, Faculty of Medicine, Kurdistan University of Medical Sciences, Sanandaj, Iran
****Department of Medical Genetics, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
*****Department of Medical Biotechnology, School of Advanced Technologies in Medicine, International Campus, Tehran University of Medical Sciences, Tehran, Iran

Abstract

Background: Breast cancer is the second leading cause of cancer death after lung cancer. Discovering molecular biomarkers is necessary for disease management that includes prognosis prediction and preventive treatment. The aim of this study is to evaluate the expression value of p53 and PTEN as molecular biomarkers of breast cancer and their relation with clinicopathological characteristics.

Methods: In this study, 100 breast cancer and 20 normal samples were subjected to investigation. Total RNA was isolated and we measured RNA expression by real-time RT-PCR. Data were analyzed by REST 2009 and SPSS.

Results: Gene expression results showed up-regulation of P53 in 53 breast cancer subjects and PTEN in 52 breast cancer subjects compared with normal controls. However, there was lower P53 expression in 25 breast cancer samples compared to normal tissues. PTEN expression was lower in 26 breast cancer samples than normal tissues. p53 showed a significant relationship to HER2 receptor (P=0.024) and menopausal status (P=0.013); no significant relationships existed with other clinico-pathological parameters (P>0.05). PTEN had the only significant correlation with lymphatic invasion (P=0.046) without any relation with other clinicopathological features (P>0.05). PTEN expression had no significant association with p53 expression in the studied population (P=0.074).

Conclusion: Combined detection of PTEN and p53 may have the potential to estimate the pathobiological behavior and prognosis of breast cancer. Due to the heterogeneous nature of cancer and the presence of different factors involved in the clinical situation of breast cancer, we suggest a study of a larger population and more biomarkers.

Keywords: Breast cancer, P53, PTEN

♦Corresponding Author:
Arshad Hosseini, PhD
Department of Medical Biotechnology, School of Allied Medicine, Iran University of Medical Sciences, Hemmat Highway, Postal code: 14155-5983, Tehran, Iran
Tel: 098-021-86704604
Fax: 0098-021-86552633
Email: hoseini.a@iums.ac.ir
ash-hosseini@Sina.tums.ac.ir

Middle East Journal of Cancer

Introduction

Although there are numerous advances in diagnosis and treatment of cancers, they remain a major problem. Following lung cancer, breast cancer (BC) is the second leading cause of cancer death and remains a major health problem.[1,2] More than half of BC incidences currently occur in developed countries, with a rapidly increasing incidence rate in these geographical areas.[3] The importance of biomarker investigations has been highlighted in recent years. Disease management, prognosis, predictive utility, and personalized medicine are advantages of discovering BC biomarkers.[4] Exploring the correlation of molecular biomarkers with clinicopathological parameters can assist with cancer prevention and management.

Phosphatase and tensin homolog (PTEN) and TP53 are tumor suppressor genes located on chromosomes 10q23 and 17p13, respectively. These proteins are involved in DNA repair, apoptosis, proliferation, and cell cycle progression. Therefore, a defect in their functions and changes in their expressions may result in uncontrollable tumor cell growth, escape from cell cycle arrest, and apoptosis signals.[5,6]

PTEN and p53 are the most frequently mutated genes in human cancers, particularly primary BC.[6-8] It is reported that mutation followed by disruption in the regular function of these proteins leads to angiogenesis, drug resistance, and defects in apoptosis.[7,8] Different studies that discuss deregulation of p53 in BC have controversial findings. However, most supported the overexpression of a mutant p53 and some reported reduced expression of p53 in BC.[10-12] Some studies reported up-regulated expression of the biomarker PTEN. In some breast tumors, other studies reported down-regulation of this biomarker.[14]

Correlation of PTEN and p53 expression with clinicopathological features of BC patients have been investigated in different areas and different ethnicities with contradictory results.[9-12] In this study, we aimed to evaluate the mRNA expressions of p53 and PTEN genes and investigate the correlation of their expression signature with clinicopathological parameters in patients affected with BC.

Materials and Methods

Samples and patients

Breast carcinoma tissues were obtained from the Cancer Institute of Imam Khomeini Hospital (Tumor Bank section, Tehran, Iran). Fresh tissues were divided into two parts: the first slice was frozen in liquid nitrogen and then preserved at -80°C. The second was sent to the Pathology Laboratory of Imam Hospital for supplementary investigation. Pathological parameters that included age, stage, grade, tumor size, lymph node metastasis, vascular and lymph invasion, and immunohistochemical situation of ER, PR, and HER-2 receptors were collected. We considered 20 normal specimens and 100 tumor samples diagnosed between 2011 and 2014 for this study. The Ethics Committee of Tehran University of Medical Sciences approved this study. All patients signed an informed consent form prior to the use of their tissue samples according to the Declaration of Helsinki (DoH).

RNA extraction

We extracted the RNA according to previously reported instructions.[13] Briefly, the total RNA was extracted from 40 mg frozen tissue using Qiazol reagent according to the supplier's instructions (Qiagen, Hilden, Germany). The tissues were homogenized with a mortar and pestle. Spectrophotometry was used to determine the quantity and purity of all extracted RNA for downstream applications. Next, a 1% agarose gel electrophoresis was carried out to confirm the quality and integrity of the RNA samples. RNase inhibitor (Thermo Fisher Scientific, USA) was used to prevent RNA degradation. The isolated RNA were maintained at -80°C until further use to avoid degradation.

DNase treatment and cDNA synthesis

DNase reagent was used according to the manufacturer's instructions (Thermo Fisher

Table 1. Correlation of p53 and phosphatase and tensin homolog (PTEN) expression with clinicopathological characteristics.

	PTEN expression (N=78)			p53 expression (N=78)		
	Low PTEN	High PTEN	*P*-value	Low p53	High p53	*P*-value
Number, N (%)	26 (33.3)	52 (66.7)		25 (32.1)	53 (67.9)	
Age,						
≤45 (%)	11 (14.5)	14 (18.4)	0.303	6 (7.9)	19 (25)	0.305
>45 (%)	15 (19.7)	36 (47.4)		19 (25)	32 (42.1)	
Tumor size,						
≤5 cm (%)	23 (30.7)	39 (52)	0.523	21 (28)	41 (54.7)	0.531
>5 cm (%)	3 (4)	10 (13.3)		3 (4)	10 (13.3)	
Tumor grade, N (%)			0.203			0.316
I	2 (2.60)	10 (12.80)		4 (5.10)	8 (10.30)	
II	15 (19.20)	20 (25.60)		14 (17.90)	35 (44.90)	
III	9 (11.50)	22 (28.20)		7 (9)	31 (39.70)	
Tumor stage, N (%)			0.372			0.474
I	0 (0)	2 (2.90)		1 (1.40)	1 (1.40)	
II	12 (17.10)	29 (41.4)		14 (20.0)	27 (38.60)	
III	11 (15.70)	16 (22.90)		6 (8.60)	21 (30.0)	
Histology, N (%)			0.559			0.761
Invasive ductal carcinoma	22 (28.9)	39 (51.3)		21 (27.6)	40 (52.6)	
Other	4 (5.3)	11 (14.5)		4 (5.3)	11 (14.5)	
Metastasis, N (%)	6 (7.7)	13 (16.7)	0.449	3 (3.8)	16 (20.5)	1.000
Lymphatic invasion, N (%)	12 (23.5)	12 (23.5)	0.046	8 (15.7)	16 (31.4)	1.000
Vascular invasion, N (%)	17 (25.8)	21 (31.8)	0.125	10 (15.2)	28 (42.4)	0.431
Necrosis, N (%)	11 (18.6)	17 (28.8)	0.598	10 (16.9)	18 (30.5)	0.572
Lobular carcinoma *in situ*, N (%)	0 (0)	6 (7.7)	0.845	1(1.3)	5 (6.4)	0.877
Ductal carcinoma *in situ*, N (%)	16 (21.9)	30 (41)		33 (45.2)	13 (17.8)	
Premenopausal, N (%)	15 (20.5)	25 (34.2)	0.623	8 (11)	32 (43.8)	0.013
Postmenopausal, N (%)	10 (13.7)	23 (31.5)		16 (21.9)	17 (23.3)	
IHC ER receptor positive, N (%)	18 (23.1)	30 (38.5)	0.459	18 (23.1)	30 (38.5)	0.221
IHC PR receptor positive, N (%)	16 (20.5)	30 (38.5)	0.810	18 (23.1)	28 (35.9)	0.141
IHC Her2 receptor positive, N (%)	6 (7.7)	13 (16.7)	1.000	2 (2.6)	17 (21.8)	0.024

Scientific, USA) to prevent genomic DNA contamination and nonspecific binding. Treated RNA was then reverse transcribed using random hexamer primers and a cDNA synthesis kit according to the manufacturer's instructions (Thermo Corporation, Germany).

Primer design and quantitative expression examination (qPCR)

The correct reference sequences of each gene were retrieved from the NCBI nucleotide database (NCBI GenBank). The appropriate primers were designed with Primer 3 and Oligo 7 software. The specificities of all primers were subsequently checked by performing BLAST. Primers sequences for PTEN, p53, and GAPDH (reference internal control) genes were: F: CCAGGACCA-GAGGAAACCT, R: GCTAGCCTCTGGATT TGA; F: GTTCCGAGAGCTGAATGAGG, R: TTATGGCGGGAGGTAGACTG; and F: AAG-GTCGGAGTCAACGGATTTG, R: GCCAT GGGTGGAATCATATTGG, respectively. The lengths of the amplified products were: PTEN (241 bp), p53 (122 bp), and GAPDH (150 bp).

Real-time quantitative analysis of gene expression was performed in a Rotor Gene 2000 Real-Time PCR machine (Qiagen, USA) using the SYBR Green method (AccuPower Green Star qPCR Master Mix; Bioneer, Korea) according to the manufacturer's instructions. Each of the gene expression assays were performed in duplicate 10 µl reactions. PCR cycling was performed as follows: one cycle at 95ºC for 10 min, 40 cycles at 95ºC for 20 sec, and 60ºC for 45 sec. In each qPCR reaction, an extra melting curve analysis from 60ºC to 95ºC was run to confirm specific amplification.

Pathology

Pathologists from the Pathology Laboratory (Imam Hospital, Tehran, Iran) assessed all specimens. ER, PR, and HER-2 status were scored by the pathologists by imunohistochemical examination based on the Hercep test standards, American Society of Clinical Oncology, and the College of American Pathologists HER-2 testing guidelines. Correlation of expression

Table 2. Correlation between expressions of p53 and PTEN in breast cancer (BC).

Correlation between p53 and PTEN gene expression			
	Up-regulation -p53	Down-regulation-p53	*P*-value
Up-regulation- PTEN	39	13	0.074
Down-regulation- PTEN	14	12	

PTEN: Phosphatase and tensin homolog

changes in patients with clinicopathological features was analyzed.

Statistical analysis

The cycle threshold (CT) values provided by RT-qPCR were used to calculate the relative fold expression change according to the 2-ΔΔCT method. Statistical analysis was carried out via IBM SPSS Statistics software version 16. The unpaired t-test and chi-square test were performed for statistical examination. *P*-values less than 0.05 were considered statistically significant.

Results
Patients and clinicopathological features

Patients had a mean age of 49.5 years (range: 27-77 years). Based on our examination, more than half of specimens (51 samples) were lymph node positive. Table 1 lists a comprehensive overview of the clinicopathological features in the studied population.

Expressions of p53 and phosphatase and tensin homolog (PTEN) in breast cancer (BC) specimens

In this study, we performed quantitative analyses of the PTEN and p53 gene expressions by real-time PCR. GAPDH, as the appropriate housekeeping gene, was used to normalize expression in the different samples. We excluded 22 out of 100 tumor samples from the study due to low quality tumor tissue and mRNA. A total of 20 normal tissue samples from the breast margin were used for relative expression assessment. We observed that PTEN up-regulated in 52 (66.7%) and down-regulated in 26 (33.3%) out of a total of 78 samples. In the TP53 gene, 53 (67.9%) samples overexpressed, while the remaining 25 (32.1%) had decreased expression. Totally, we observed significantly different expressions of PTEN (*P*=0.002) and p53 (*P*=0.003) in the BC

specimens compared with normal breast margin tissues.

Relationship between p53 and phosphatase and tensin homolog (PTEN) gene expressions and clinicopathological features of breast cancer (BC)

We compared the gene expression levels of p53 and PTEN with clinicopathological criteria according to the chi-square test to determine significant relations of each parameter with gene expression. According to table 1, there was only a significant relation of PTEN gene expression with lymphatic invasion (*P*=0.046). Other clinicopathological characteristics did not show any significant association with PTEN gene expression (*P*>0.05). p53 gene expression did not significantly correlate with lymph node metastasis, ER receptor status, PR receptor status, Her2 receptor status, vascular invasion, age, stage, and tumor grade (*P*>0.05). However, p53 expression had a significant relation to HER-2 receptor status (*P*=0.024) and menopausal status (*P*=0.013).

Relationship between p53 and phosphatase and tensin homolog (PTEN) expressions in breast cancer (BC)

PTEN expression in BC did not show a significantly positive correlation with p53 expression (*P*=0.074). Table 2 shows the relation between the two genes.

Discussion

Breast cancer is the second most common cancer worldwide and, by far, the most frequent cancer among women. Currently, it is the second cause of cancer death in more developed regions after lung cancer.[1] Discovering molecular biomarkers with prognostic, predictive, and diagnostic value is necessary for disease management.[4] p53 and PTEN genes are two

biomarkers that have an important role in BC.[14] This study has investigated the expression and clinicopathological significance of PTEN and p53 genes in 100 tumor samples (22 out of 100 samples excluded) and 20 normal samples (normal margin of breast tissue). To our knowledge, most researches have used immunohistochemistry tests in pathology laboratories that are based on antibodies to detect protein molecules.[11,15] However, this study used real-time qPCR to detect the RNA expression status of target genes as a quantitative method. This method has an advantage over the IHC (ImmunoHistoChemistry) test which is a semi-quantitative method. Real-time PCR is a highly sensitive, accurate, reproducible and quantitative method. Hence, it can be an alternative/complementary method to determine biomolecular status.[16,17] This work had other advantages that outweigh other studies of the same subject, which included the simultaneous assessment of two tumor suppressors and selection of an appropriate population from Tehran province, which was representative of different ethnicities in Iran. According to a number of studies, different populations and ethnicities show various expressions of these biomarkers.[18]

In the current study, we measured the expressions of PTEN and p53 and compared them with clinicopathological parameters. Expression of p53 in BC has been reported to vary from 9% to 69%. According to our results, the TP53 gene overexpressed in 67.9% of tumor tissues and 32.1% of tumors had decreased expression. Therefore, this finding approximately supported a number of previous studies and slightly contrasted other studies.[9,19,20] RNA expression of PTEN down-regulated in 33.3% and up-regulated in 66.7% of tumor tissue samples, which was similar to other studies.[21] It has previously been reported that loss of PTEN expression occurs in 30% of sporadic BC and 72% of cases with a familial history of BC.[22] Loss and/or down-regulation of PTEN and p53 might be related to loss of heterozygosity or promoter methylation events.

As shown in table 1, p53 had a significant relation to HER-2 receptor ($P=0.024$) and menopausal status ($P=0.013$). This gene did not have any relation to stage, tumor grade, ER and PR receptor status, ethnicity, age, tumor size, the number of positive lymph nodes, lymph node metastasis, lymphatic invasion, and vascular invasion ($P>0.05$). In BC, various tumor suppressors, including p53, are mutated. Mutations in this gene are associated with tumor growth, increased angiogenesis, dysfunction of apoptosis, and chemotherapy treatment resistance.[23,24] Studies in other countries have shown that the expression of the p53 gene in BC can also be considered an important factor in disease prognosis.[25] Information about p53 status in certain cancers may be helpful in disease management of the diagnosis, therapeutic decisions, and prognosis prediction.[26] Previous literatures have demonstrated that p53 expression has a significant positive correlation with high proliferation index (MIB1), increased grade values, a negative correlation with steroid receptor status, and a significant association with lower age, larger tumor size, ductal morphology, and high tumor grade.[27] Patients' survival and response to therapy have been reportedly predicted based on knowledge of the p53 mutation status.[28] In one study, expressions of PTEN and PIK3CA in tumor tissue significantly increased compared to normal tissue. Expressions of PTEN and PIK3CA in tumor tissue showed no correlations with metastasis in the lymph nodes.[29]

According to table 1, a significant relation existed between lymphatic invasion ($P=0.046$) and the PTEN gene. We did not observe any correlations with other clinicopathological factors ($P>0.05$). The PI3K/PTEN pathway plays a vital role in carcinogenesis. This pathway controls multiple important cellular functions such as metabolism, cell proliferation, apoptosis, migration, and survival. Inappropriate regulation of this route often occurs in BC and the lack of PTEN expression has been suggested as a major mechanism for resistance against HER-2 treatment.[30,31] Rapid metastasis of some tumors has been found to be associated with mutations in

p53 and PTEN.[32] Although some studies indicated no correlations between PTEN and clinicopathological parameters, other reports showed an association between loss of PTEN expression and high tumor grade, presence of lymphatic invasion, and a high proliferative index (22, 33).[27,36] Furthermore, it has been reported that ER+ status, small tumor size, and low HER-2 expression correlated with loss of PTEN.[34] This discrepancy among different literatures probably resulted from the small sample size, different ethnicities, and genetic heterogeneity.

PTEN and p53 are two tumor suppressor genes most commonly related to various cancers. It has been indicated that PTEN and p53 are functionally related through the PI3K signaling pathway.[5] PTEN also affects cellular functions independently of the PI3K pathway via direct binding to p53 and prevention of its degradation.[35] Although PTEN and p53 are not the only mechanisms to suppress tumor progression in cancer, their co-expression status could have significance in disease management. Our results have revealed that expression status of PTEN and p53 did not significantly correlate (P=0.074). We did not investigate gene mutations in PTEN and p53 in the present study and false-negative or false-positive findings might be other limitations.

In conclusion, the results of this study had some consistencies and inconsistencies with previous literatures due to genetic heterogeneity of BC between different geographical regions, differences in ethnicities, and the variety of sample size. The expression status of PTEN showed a significant correlation with lymphatic invasion, while p53 had a significant association with menopausal and HER-2 receptor status. Assessments of PTEN and p53 expressions might provide information helpful to the management of BC, regardless of the underlying mechanisms that drive gene expression alterations in cancer cells.

Conflict of interests

No conflict of interest is declared.

References

1. Jiemin, Ma; Ahmedin, Jemal. Breast cancer statistics. In: Ahmad, Aamir, editor. Breast cancer metastasis and drug resistance. New York: Springer; 2013.p.1-18.
2. Moloudi K, Neshasteriz A, Hosseini A, Eyvazzadeh N, Shomali M, Eynali S, et al. Synergistic effects of arsenic trioxide and radiation: Triggering of intrinsic pathway of apoptosis. *Iran Biomed J*. 2017; [Epub ahead of print].
3. Ferlay, J; Héry, C; Autie, P; Sankaranarayanan, R. Global burden of breast cancer. In: Christophe, Li, editor. Breast cancer epidemiology. Lyon: Springer; 2010.p.1-19.
4. Patani N, Martin LA, Dowsett M. Biomarkers for the clinical management of breast cancer: international perspective. *Int J Cancer*. 2013;133(1):1-13.
5. Song MS, Salmena L, Pandolfi PP. The functions and regulation of the PTEN tumour suppressor. *Nat Rev Mol Cell Biol*. 2012;13(5):283-96.
6. Yin Y, Shen WH. PTEN: a new guardian of the genome. *Oncogene*. 2008;27(41):5443-53.
7. Teodoro JG, Evans SK, Green MR. Inhibition of tumor angiogenesis by p53: a new role for the guardian of the genome. *J Mol Med* (*Berl*). 2007;85(11):1175-86.
8. McCubrey JA, Steelman LS, Kempf CR, Chappell WH, Abrams SL, Stivala F, et al. Therapeutic resistance resulting from mutations in Raf/MEK/ERK and PI3K/PTEN/Akt/mTOR signaling pathways. *J Cell Physiol*. 2011;226(11):2762-81.
9. Depowski PL, Rosenthal SI, Ross JS. Loss of expression of the PTEN gene protein product is associated with poor outcome in breast cancer. *Mod Pathol*. 2001;14(7):672-6.
10. Shoman N, Klassen S, McFadden A, Bickis MG, Torlakovic E, Chibbar R. Reduced PTEN expression predicts relapse in patients with breast carcinoma treated by tamoxifen. *Mod Pathol*. 2005;18(2):250-9.
11. Tsutsui S, Inoue H, Yasuda K, Suzuki K, Higashi H, Era S, et al. Reduced expression of PTEN protein and its prognostic implications in invasive ductal carcinoma

of the breast. *Oncology*. 2005;68(4-6):398-404.

12. Rosanelli GP, Steindorfer P, Wirnsberger GH, Klimpfinger M, Ratschek M, Puerstner P, et al. Mutant p53 expression and DNA analysis in human breast cancer comparison with conventional clinicopathological parameters. *Anticancer Res.* 1995;15(2):581-6.

13. Azarnezhad A, Sharifi Z, Seyedabadi R, Hosseini A, Johari B, Sobhani Fard M. Cloning and expression of soluble recombinant HIV-1 CRF35 Protease-HP Thioredoxin fusion protein. *Avicenna J Med Biotechnol.* 2016; 8(4): 175-181.

14. Kurose K, Gilley K, Matsumoto S, Watson PH, Zhou XP, Eng C. Frequent somatic mutations in PTEN and TP53 are mutually exclusive in the stroma of breast carcinomas. *Nat Genet*. 2002;32(3):355-7.

15. Yamashita H, Nishio M, Toyama T, Sugiura H, Zhang Z, Kobayashi S, et al. Coexistence of HER2 over-expression and p53 protein accumulation is a strong prognostic molecular marker in breast cancer. *Breast Cancer Res*. 2004;6(1):R24-30.

16. Vanden Bempt I, Vanhentenrijk V, Drijkoningen M, Wlodarska I, Vandenberghe P, De Wolf-Peeters C. Real-time reverse transcription-PCR and fluorescence in-situ hybridization are complementary to understand the mechanisms involved in HER-2/neu overexpression in human breast carcinomas. *Histopathology*. 2005;46(4):431-41.

17. Collins R, Xiang D, Christie A, Leitch M, Euhus D, Rao R, et al. Abstract P1-07-04: Comparison of HER2 expression by immunohistochemistry (IHC) using automated imaging system and fluorescence in situ hybridization (FISH). A retrospective analysis of 2853 cases. *Cancer Res*. 2012;72(24 Supplement): Abstract nr P1-07-04.

18. Chlebowski RT, Chen Z, Anderson GL, Rohan T, Aragaki A, Lane D, et al. Ethnicity and breast cancer: factors influencing differences in incidence and outcome. *J Natl Cancer Inst*. 2005;97(6):439-48.

19. Lee DS, Kim SH, Suh YJ, Kim S, Kim HK, Shim BY. Clinical implication of p53 overexpression in breast cancer patients younger than 50 years with a triple-negative subtype who undergo a modified radical mastectomy. *Jpn J Clin Oncol.* 2011;41(7):854-66.

20. Di Cristofano A, Pandolfi PP. The multiple roles of PTEN in tumor suppression. *Cell.* 2000;100(4):387-90.

21. Bose S, Crane A, Hibshoosh H, Mansukhani M, Sandweis L, Parsons R. Reduced expression of PTEN correlates with breast cancer progression. *Hum Pathol.* 2002;33(4):405-9.

22. Bakarakos P, Theohari I, Nomikos A, Mylona E, Papadimitriou C, Dimopoulos AM, et al. Immunohistochemical study of PTEN and phosphorylated mTOR proteins in familial and sporadic invasive breast carcinomas. *Histopathology.* 2010;56(7):876-82.

23. Liang Y, Besch-Williford C, Benakanakere I, Thorpe PE, Hyder SM. Targeting mutant p53 protein and the tumor vasculature: an effective combination therapy for advanced breast tumors. *Breast Cancer Res Treat.* 2011;125(2):407-20.

24. Hofseth LJ, Hussain SP, Harris CC. p53: 25 years after its discovery. *Trends Pharmacol Sci.* 2004;25(4):177-81.

25. Porter PL. Global trends in breast cancer incidence and mortality. *Salud Publica Mex.* 2009;51 Suppl 2:s141-6.

26. Malamou-Mitsi V, Gogas H, Dafni U, Bourli A, Fillipidis T, Sotiropoulou M, et al. Evaluation of the prognostic and predictive value of p53 and Bcl-2 in breast cancer patients participating in a randomized study with dose-dense sequential adjuvant chemotherapy. *Ann Oncol.* 2006;17(10):1504-11.

27. Al-Joudi FS, Iskandar ZA, Rusli J. The expression of p53 in invasive ductal carcinoma of the breast: a study in the North-East States of Malaysia. *Med J Malaysia.* 2008;63(2):96-9.

28. Miller LD, Smeds J, George J, Vega VB, Vergara L, Ploner A, et al. An expression signature for p53 status in human breast cancer predicts mutation status, transcriptional effects, and patient survival. *Proc Natl Acad Sci U S A*. 2005;102(38):13550-5.

29. Palimaru I, Brügmann A, Wium-Andersen MK, Nexo E, Sorensen BS. Expression of PIK3CA, PTEN mRNA and PIK3CA mutations in primary breast cancer: association with lymph node metastases. *Springerplus*. 2013;2:464.

30. Parsons R. Human cancer, PTEN and the PI-3 kinase pathway. *Semin Cell Dev Biol.* 2004;15(2):171-6.

31. García JM, Silva J, Peña C, Garcia V, Rodríguez R, Cruz MA, et al. Promoter methylation of the PTEN gene is a common molecular change in breast cancer. *Genes Chromosomes Cancer*. 2004;41(2):117-24.

32. Yang J, Ren Y, Wang L, Li B, Chen Y, Zhao W, et al. PTEN mutation spectrum in breast cancers and breast hyperplasia. *J Cancer Res Clin Oncol*. 2010;136(9): 1303-11.

33. Bose S, Chandran S, Mirocha JM, Bose N. The Akt pathway in human breast cancer: a tissue-array-based analysis. *Mod Pathol.* 2006;19(2):238-45.

34. Pérez-Tenorio G, Alkhori L, Olsson B, Waltersson MA, Nordenskjöld B, Rutqvist LE, et al. PIK3CA mutations and PTEN loss correlate with similar prognostic factors and are not mutually exclusive in breast cancer. *Clin Cancer Res*. 2007;13(12):3577-84.

35. Lee YK, Park NH. Prognostic value and clinicopathological significance of p53 and PTEN in epithelial ovarian cancers. *Gynecol Oncol.* 2009;112(3):475-80.