RESEARCH ARTICLE

Editorial Process: Submission:09/30/2024 Acceptance:03/12/2025

PTEN and *HES1* Gene Expression Alteration in Breast Cancer: Any Association with Tumor Histomorphological Features or Invasive Behavior?

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Abstract

Background: Genomic techniques and the evaluation of epigenetic and proteomic alterations are increasingly used to predict breast tumors' behavior and histopathological patterns. While some studies have linked *PTEN* and *HES1* gene expression to tumor aggressiveness and metastatic behavior, others have not confirmed these associations. This study aimed to assess *PTEN* and *HES1* gene expression in breast cancer lesions and explore their relationship with tumor morphological features and behavior. **Methods:** This cross-sectional study involved 50 women with breast cancer. Tumor and adjacent normal breast tissue (50 each) were collected to assess *PTEN* and *HES1* gene expression using Real-Time PCR. RNA extraction was performed using the Trizol method, followed by cDNA synthesis. Gene expression changes were quantified using the ΔΔCT method. Statistical analysis was conducted using SPSS version 23.0, with significance set at $p \leq 0.05$. **Results:** *PTEN* down-regulation was observed in 70.0%, and *HES1* up-regulation in 98.0% of cases. No significant association was found between *PTEN* down-regulation and tumor-related characteristics, except for a significantly higher mean age in patients with down-regulated *PTEN* ($p = 0.003$). *HES1* expression intensity, with a mean fold change of 6.193 (median: 4.560, SD: 5.116), showed no significant relationship with any histopathological features or invasive behavior. **Conclusion:** Older patients showed reduced *PTEN* expression. Additionally, elevated *HES1* was identified in 98% of patients and lower *PTEN* in 70%. However, these gene alterations do not seem to reliably predict aggressive tumor behavior in our population, possibly due to the limited sample size. Further studies with long-term follow-up are needed to fully evaluate the prognostic significance of these markers.

Keywords: *PTEN*- *HES1*- Breast cancer- Prognostic marker- Tumor suppressor gene

Asian Pac J Cancer Prev, **26 (3)**, 935-941

Introduction

Breast cancer is the most commonly diagnosed cancer and the second leading cause of cancer-related deaths among women worldwide [1]. In the United States, approximately 1 in 8 women will develop breast cancer in their lifetime, making it the leading cause of cancer mortality in women by 2024 [2]. Even when combined with men, female breast cancer remains the fourth leading cause of cancer-related deaths worldwide, behind lung, colorectal, and liver cancers, highlighting its significant global burden [3, 4].

While early-stage breast cancer often responds well to treatments like surgery, radiation, and drug therapies, about one-third of patients progress to metastatic disease. These metastases, often resistant to conventional therapies, pose significant therapeutic challenges, with aggressive forms frequently leading to fatal outcomes [2, 5].

To address these challenges, recent research focuses on genomic techniques and the evaluation of epigenetic and proteomic defects [6–8]. In breast cancer, efforts are directed toward understanding tumor biology and identifying biomarkers related to therapeutic response. While therapies such as monoclonal antibodies have been developed based on gene expression and genetic pathways, the major challenge remains a deeper understanding of metastatic and genetic mechanisms to develop more effective treatments [9–11].

One key factor in these mechanisms is the regulation of the cell cycle, which determines how a cell responds to external stimuli. Disruptions in this regulation often lead to resistance against anti-cancer therapies. *PTEN*

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(phosphatase and tensin homolog deleted on chromosome 10), a critical tumor suppressor and "guardian of the genome," plays a central role in regulating cell death, invasion, proliferation, and survival [12]. *PTEN* inhibits the phosphatidylinositol 3 kinase (PI3K)/Akt pathway, which is involved in these processes [12–17]. Loss of *PTEN* function through mutations, promoter methylation, or protein degradation is frequently observed in tumors, including breast cancer, and contributes to altered tumor cell behavior and survival [18, 19].

In addition to *PTEN*, the Hairy and Enhancer of Split Homolog-1 (*HES1*) gene also plays a crucial role in cell differentiation and cycle arrest [20, 21]. Elevated *HES1* expression has been associated with various cancers, including lung, ovarian, and colon cancer, as well as embryonic brain tumors [22–24]. In addition, *HES1* regulates cell proliferation, survival, and metastasis through multiple signaling pathways [25], though its role in breast cancer remains underexplored [26, 27]. Notably, research indicates a significant interaction between *HES1* and *PTEN* as reduced *HES1* expression correlates with decreased AKT activity and increased *PTEN* levels, while elevated *HES1* expression suppresses *PTEN* and increases AKT pathway activity [28, 29]. These interactions, particularly involving the AKT and Epithelial-mesenchymal transition (EMT) pathways, are associated with breast cancer cell migration and invasiveness [30]. Ultimately, it seems that both *PTEN* and *HES1* expression, individually or interactively, can impact tumor cell behavior. In this regard, reduced *PTEN* and increased *HES1* expression have been associated with increased tumor survival and poorer patient outcomes [31]. In this study, we aim to investigate whether these gene alterations are present in breast cancer lesions within our population and examine their connection to tumor morphological characteristics.

Materials and Methods

Study design, setting, and participants

This cross-sectional study included 50 women diagnosed with breast cancer who were referred to the Cancer Institute at Imam Khomeini Hospital Complex in Tehran between January 1 and December 31, 2020. According to the Declaration of Helsinki, the Institutional Review Board of the Tehran Medical University approved this study with the ethics code IR.TUMS.IKHC.REC.1399.253. Malignancies were confirmed through histopathologic and immunohistochemistry (IHC) studies as indicated. Tumor samples were obtained from the tumor bank of the Cancer Institute for research purposes. The sample size was determined through power analysis [32, 33], with a desired 80% power and 0.05 significance level and effect size of 0.56, indicating that 50 tumor tissue and 50 adjacent normal breast tissue samples would be sufficient to detect statistically significant differences in gene expression. Consequently, 100 tissue samples were collected, comprising 50 tumor tissues and 50 adjacent normal breast tissues, under the supervision of a pathologist.

Sample Collection and Preservation

All tissue samples were received in fresh state, separated during surgery and immediately placed in liquid nitrogen to preserve RNA integrity. They were stored at -80°C (frozen) until further analysis.

RNA Extraction

Total RNA was extracted from the tissue samples using the Trizol RNA extraction kit (Sigma-Aldrich). During the first stage, 50 mg of target tissue was carefully crushed using a sterile razor blade and then transferred to a 1.5 ml tube, and the remaining steps were performed according to the kit instructions. The quantity and purity of RNA were measured using a Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific). The RNA purity was confirmed by measuring the absorbance ratios at 260/280 nm and 260/230 nm, which were in the acceptable ranges of 1.8–2.2 and 1.7–1.9, respectively.

cDNA Synthesis and Real-Time PCR

cDNA was synthesized from the extracted RNA using a reverse transcription kit (Biofact, Korea). Gene expression levels of *HES1* and *PTEN* were analyzed using the Real-Time PCR method with SYBR GREEN (Takara Co., Japan). Data obtained from the Real-Time PCR were analyzed using melting curves, and the CT number was calculated to quantify gene expression levels.

Gene Expression Measurement Method

To assess gene expression levels, the ΔΔCT method was employed to ensure the accuracy of synthesized cDNA. First, the CT value of the target gene was obtained and compared to the CT value of a housekeeping gene, which served as an internal control due to its stable expression. The difference between the CT values of the target gene and the housekeeping gene was calculated as ΔCT. Subsequently, the ΔΔCT value was derived by subtracting the ΔCT of normal breast tissue from the ΔCT of tumor tissue. The fold change in gene expression was calculated using the formula:

$$\text{Fold change} = 2^{(-\Delta\Delta CT)}$$

A fold change greater than 1 indicated an increase in gene expression, while a fold change less than 1 signified a decrease.

Statistical analysis

Quantitative variables were presented as mean ± standard deviation (SD), while categorical variables were reported as frequencies and percentages. The Chi-square test was used to analyze qualitative variables. The normality of data distribution was assessed using the Kolmogorov-Smirnov test. For non-normally distributed data, the Mann-Whitney test was applied. Nominal variables were evaluated, and score variables were analyzed using the T-test when normal distribution was confirmed. The ROC curve was utilized to assess the predictive value of the CT number for tumor prognosis, determining the optimal cutoff point along with its sensitivity and specificity. A p-value ≤ 0.05 was considered statistically significant.

Statistical analyses were performed using SPSS version 23.0 (IBM, Armonk, NY).

Results

Clinical and Pathological Features of Individuals and Lesions

A total of 50 patients with invasive breast cancer were studied. The mean age of patients was 51.22±12.95 years in the range of 20 to 81 years. The mean tumor size was also 4.00±2.00 cm. (Figure S1). Histopathological features of the tumor are summarized in Table 1. Most of the tumors had invasive ductal patterns (74.0%). Regarding tumor grade (Nottingham scoring), 18%, 50.0%, and 26.0% of tumors were grade 1, 2, and 3, respectively, and remained unknown in 6.0%. Lymphatic invasion was observed in 36 cases (72%), vascular invasion in 33 cases (66%), and perineural invasion in 15 cases (30%). Additionally, axillary lymph node involvement was found in 30 cases (60%), and lymph node extracapsular extension was present in 10 cases (20%). Distant metastases were detected in 11 cases (22%).

Hormonal Receptors Status

In terms of hormonal receptor status, 17 cases (34%) were estrogen receptors (ER)-positive, while 33 cases (66%) were ER-negative. Progesterone receptor (PR) positivity was observed in 15 cases (30%) and PR negativity in 35 cases (70%). Furthermore, 25 cases (50%) were Her2-positive, and 25 cases (50%) were Her2-negative. Six cases (12%) were classified as triple-negative breast cancer (Table 1).

PTEN Gene Alterations

In our study, *PTEN* gene down-regulation was observed in 70% of breast cancer patients (Figure S2). Furthermore, as shown in Table 2, none of the baseline tumor characteristics or histopathological features reached a statistically significant association with *PTEN* down-regulation, except for patient age, which was significantly higher in those with reduced *PTEN* expression (p-value = 0.003). (Figure S4) Notably, patients with *PTEN* down-regulation exhibited a smaller average tumor size (3.75±1.54 cm) than those without *PTEN* down-regulation (4.57±2.75). However, this difference did not achieve statistical significance (p-value = 0.191) (Figure S3).

HES1 Gene Alterations

Up-regulation of the *HES1* gene occurred in 98% of cases (Figure S2), with only one patient not exhibiting up-regulation. This patient, a 77-year-old woman, had a 2.0 cm invasive ductal carcinoma (grade 3) with lymphatic, vascular, and perineural invasion but no axillary lymph node involvement or distant metastasis. The mean *HES1* expression (fold change) was 6.193, with a median of 4.560 and a standard deviation of 5.116. No significant association was observed between *HES1* expression intensity and any tumor characteristics, histopathological features, hormonal profiles, or *HER2* status (Table 3, Figures S5 and S6).

Table 1. Baseline Characteristics of the Included Patients

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Age (years), mean ± SD	51.22 ± 12.95
Tumor size (cm), mean ± SD	4.00 ± 2.00
Histological features, n (%)	
Infiltrating ductal (NOS)	47 (94.0)
Infiltrating lobular (NOS)	1 (2.0)
Other patterns	2 (4.0)
Tumor grade, n (%)	
Grade 1	9 (18.0)
Grade 2	25 (50.0)
Grade 3	13 (26.0)
Unknown	3 (6.0)
Invasive behavior, n (%)	
Axillary lymph node involvement	30 (60.0)
Lymph node extracapsular extension	10 (20.0)
Vascular invasion	33 (66.0)
Lymphatic invasion	36 (72.0)
Perineural invasion	15 (30.0)
Hormonal receptor, n (%)	
ER Positive	17 (34)
ER Negative	33 (66)
PR Positive	15 (30)
PR Negative	35 (70)
HER2 Positive	25 (50.0)
HER2 Negative	25 (50.0)
Triple Negative	6 (12.0)

HR, Hormone Receptor; ER, Estrogen Receptor; PR, Progesterone Receptor; *HER2*, Human Epidermal Growth Factor Receptor 2; NOS, Not Otherwise Specified

Discussion

In recent years, genomic techniques, along with the evaluation of epigenetic and proteomic alterations, have been utilized to predict the behavior and histopathological patterns of breast tumors [34]. Notably, changes in the expression of certain genes have been linked to more aggressive tumor behavior and, consequently, to disease prognosis. Studies have highlighted the relevance of *PTEN* and *HES1* gene expression alterations in relation to the pathological patterns, aggressiveness, and metastatic behavior of breast tumors [26]. However, some studies have reported a lack of significant associations [35]. In this context, the role of *PTEN* and *HES1* expression in breast cancer patients from Iran remains unclear.

Our findings revealed a 70% decrease in *PTEN* expression and a 98% increase in *HES1* expression among the study cohort. These results suggest that more than two-thirds of breast cancer patients experience down-regulation of *PTEN*, while nearly all exhibit up-regulation of *HES1*. The increase in *HES1* expression appears to be a consistent finding across all patients, whereas *PTEN* down-regulation is observed in the majority. These findings align with the literature but demonstrate variability across studies. For instance, Sajjadi et al. [36]

Table 2. Correlation between Tumor Characteristics and *PTEN* Expression

	With *PTEN* down-regulation, n (%)	Without *PTEN* down-regulation, n (%)	P-value
Age (years), mean ± SD	54.85±11.81	42.73±11.76	0.003
Tumor size (cm), mean ± SD	3.75±1.54	4.57±2.75	0.191
Histological feature			
Infiltrating ductal (NOS)	33 (94.3)	14 (93.3)	0.350
Infiltrating lobular (NOS)	1 (2.9)	0 (0.0)	
Other patterns	1 (2.9)	1 (6.7)	
Tumor grade			
Grade 1	5 (14.3)	4 (26.7)	0.740
Grade 2	18 (51.4)	7 (46.7)	
Grade 3	10 (28.6)	3 (20.0)	
Unknown	2 (5.7)	1 (6.7)	
Invasive behavior			
Lymphatic invasion	25 (71.4)	11 (73.3)	0.239
Lymph node extracapsular extension	7 (20.0)	3 (20.0)	0.974
Axillary lymph node involvement	21 (60.0)	9 (60.0)	0.239
Vascular invasion	22 (62.9)	11 (73.3)	0.474
Perineural invasion	10 (28.6)	5 (33.3)	0.255
Distant metastasis	7 (20.0)	7 (46.7)	0.09
Hormonal receptor Status			
ER Positive	13 (37.1)	3 (20.0)	
ER Negative	22 (62.9)	12 (80.0)	0.224
PR Positive	15 (42.9)	4 (26.7)	
PR Negative	20 (57.1)	11(73.3)	0.401
HER2 Positive	17 (48.6)	8 (53.3)	
HER2 Negative	18 (51.4)	7 (46.7)	0.384
Triple Negative	5 (14.3)	1 (6.7)	0.447

HR, Hormone Receptor; ER, Estrogen Receptor; PR, Progesterone Receptor; *HER2*, Human Epidermal Growth Factor Receptor 2; NOS, Not Otherwise Specified

Table 3. The Relationship between *HES1* Expression Intensity and Characteristics of Tumors

	HES1 Expression Intensity*	P-value
Histology		
Invasive ductal carcinoma	6.20 ± 5.222	
Other	6.01 ± 4.30	0.935
Vascular invasion		
Yes	6.53 ± 5.03	
No	5.48 ± 2.30	0.503
Perineural invasion		
Yes	6.59 ± 5.85	
No	6.01 ± 3.43	0.717
Extracapsular extension (axillary lymph nodes)		
Yes	9.66 ± 6.84	
No	5.70 ± 3.83	0.075
Histological grade		
Grade 1	7.21 ± 5.82	
Grade 2	6.59 ± 4.92	
Grade 3	4.92 ± 3.31	
Unknown	5.37 ± 2.87	0.81
Lymph node involvement		
Yes	6.94 ± 5.88	
No	4.98 ± 3.19	0.212
M stage		
M0	4.26 ± 2.57	
M1	9.79 ± 8.80	0.087
Hormone receptor status		
Triple-negative	9.25 ± 7.06	
Other	5.96 ± 4.63	0.262
ER status		
Positive	6.10 ± 5.26	
Negative	6.83 ± 4.72	0.258
PR status		
Positive	5.80 ± 4.64	
Negative	6.97 ± 4.55	0.083
Her2 status		
Positive	5.89 ± 2.73	
Negative	7.41 ± 5.43	0.633

* Data are presented as mean ± standard deviation (SD); HR, Hormone Receptor; ER, Estrogen Receptor; PR, Progesterone Receptor; *HER2,* Human Epidermal Growth Factor Receptor 2

reported reduced *PTEN* protein expression in 46.1% of patients, noting a correlation between decreased *PTEN* expression and increased hormone receptor and *HER2* expression. Fan et al. [37]similarly reported that 24.7% of their breast cancer patients exhibited low *PTEN* expression levels, while 55.2% had moderate expression levels. Furthermore, a study by Li et al. in 2018 found increased *HES1* expression in only 35.3% of patients with invasive ductal carcinoma [26]. These variations suggest that the expression patterns of *PTEN* and *HES1* may be influenced by the demographic and histopathological characteristics of the tumors.

While the expression levels of *PTEN* and *HES1* vary across populations and studies, our findings underscore the high specificity of *HES1* overexpression in breast cancer patients within our cohort, suggesting its potential prognostic significance. However, when examining the relationship between *PTEN* and *HES1* expression and the histopathological features of tumors, we found no significant association between decreased *PTEN* expression and key tumor characteristics such as histological subtype, tumor grade, lymphovascular or perineural invasion. This suggests that, in our patient population, *PTEN* expression alone may not serve as a reliable prognostic marker for predicting tumor behavior.

In contrast to our findings, some studies have confirmed a relationship between decreased *PTEN* expression, increased *HES1*, and adverse histological and prognostic tumor behavior. For instance, Li et al. reported a significant association between increased *HES1* expression and higher TNM stage, lymph node metastasis, negative estrogen receptor status, and triple-negative breast cancer. In their study, elevated *HES1* expression was linked to a poorer prognosis in cancer patients [26]. Similarly, Fan et al. [37] demonstrated that low, medium, and high *PTEN* expression levels correlated with 10-year disease-free survival rates of 42.3%, 55%, and 81%, respectively, and 10-year overall survival rates of 65.0%, 84.2%, and 90.5%. Increased gene methylation was also associated with an increased risk of recurrence and mortality.

Interestingly, the study by Prvanović et al. (2021) did not find a relationship between *PTEN* expression and adverse outcomes in triple-negative breast cancer patients, aligning with our results [35]. Meanwhile, Sajjadi et al. [36] observed that decreased *PTEN* expression was linked to higher expression of hormone receptors and *HER2*. However, they found that it was reduced *PTEN* protein expression—not *PTEN* gene expression—that was associated with worse clinical outcomes, including disease-free survival.

It is important to acknowledge that our findings may be limited by the relatively small sample size. Notably, the comparison of various variables, such as the association of tumor size and tumor grades with *PTEN* expression, as well as the connection link between *HES1* expression and PR status, axillary lymph node metastasis, and other tumor profiles, with notable observed differences could reveal a more meaningful association with a larger sample size.

Furthermore, the absence of long-term survival data, a crucial indicator of prognosis in breast cancer, represents another limitation of our research. Further studies with larger cohorts, comprehensive survival analyses, and exploration of related signaling pathways such as AKT could enhance our understanding of the prognostic significance of *PTEN* and *HES1* expression in breast cancer.

In conclusion, our study found decreased *PTEN* expression in 70% of breast cancer patients and increased *HES1* expression in 98%. However, reduced *PTEN* expression and/or *HES1* intensity do not seem to reliably predict aggressive tumor behavior in this population. Given the observed differences lacked statistical significance, larger studies with long-term follow-up are needed to thoroughly evaluate the prognostic value of these markers.

Author Contribution Statement

M.Z. and S.K. wrote the first draft of the manuscript. M.Z. collected the data, and Z.N. and A.A. conceived the idea. S.K. revised the manuscript, aided by input from N.Z. and A.A., who provided valuable feedback and contributed to the manuscript's refinement.

Acknowledgements

Ethics approval

The Institutional Review Board of the Tehran Medical University approved this study with the ethics code IR.TUMS.IKHC.REC.1399.253

Data availability statement

The data used to support the findings of this study are available from the corresponding author upon request.

Conflict of interest disclosure

The authors declare that they have no competing interests.

Funding statement

The research did not receive financial support from any institution.

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