

Tissue Expression of Prohibition-I and Its Relationship With Prognostic Factors in Breast Cancer

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KEYWORDS

Breast Neoplasm;
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ABSTRACT

Background & objective: Globally, breast cancer is the most common malignancy among females. Prohibition (PHB)-I, a homologous protein, was initially introduced as a suppressor gene for amplification process. Further, the protein has a key role in the cell cycle and is capable of inhibiting DNA transcription in many cell types. Therefore, its possible role in different types of human malignancies is of interest.

The current study aimed at examining the relationship between the tissue distribution of PHB-I and prognostic factors of breast cancer.

Methods: Paraffin-embedded tissue specimens of 33 patients diagnosed with breast cancer at Omid teaching Hospital, Mashhad, Iran were studied and a commercial monoclonal antibody was used to perform immunohistochemistry (IHC). The relationship between PHB-I tissue expression with age, disease stage, tumor grade and size, as well as hormone receptor status including estrogen (ER) and progesterone (PR) receptors, and Her-2 receptor were evaluated.

Results: The Immunohistochemical analysis showed a relative increase in PHB-I tissue expression along with higher tumor grade ($P=0.057$). In addition, higher expression of ER and PR were observed ($P=0.027$ and 0.009 , respectively). The age of patients and other prognostic factors including Her-2 receptor status and disease stage did not statistically correlate with PHB-I expression.

Conclusion: An increased expression of PHB-I was observed in the breast cancer tumors of the current study patients compared with the anatomically healthy margin. Its coloration with some prognostic factors such as disease grade and expression of ER and PR might indicate the PHB-I potential application for diagnostic and patient management purposes.

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Introduction

Breast cancer is the most common cancer among females. According to the World Health Organization (WHO) statistics, one out of 8-10 females is affected by breast cancer (1). Likewise in Iran, one out of 10-15 females is probably affected by breast cancer; however, the age of incidence of this cancer in Iranian populations is almost one decade earlier than that of

the developed countries. It is noteworthy that breast cancer is the second cause of death resulting from cancer in Iran (2). Prognostic evaluations are essential in order to set disease management. Biomarkers are different proteins released from the cancer tissue (3). By examining these factors in serum or tissue samples, a model for prognosis assessment in many malignancies including breast cancer can be provid-

ed. The factors including biomarkers in the prognosis of the patients are continuously updated and revised.

Prohibition (PHB)-I is a homologous protein introduced for the first time as the suppressor gene of amplification during highly conserved evolution (4-6). Further studies revealed that this gene is located on the long arm of Chromosome 17. This area is genetically attached to the site predisposing the early onset of breast cancer (7). Moreover, it is observed that PHB-I has a major role in the cell cycle and can inhibit DNA transcription in different cell types (8-10).

It is observed that PHB-I in breast cancer cells binds to the p53 protein and increases p53 transcriptional activity (11). Therefore, PHB-I has a pivotal role in the function of "check point control" of p53 protein during the cell cycle. Additionally, studies demonstrate that PHB-I interacts with Rb protein, which inhibits the growth via binding and inhibiting E2F (12, 13). In some studies, a relationship between basal like and luminal type B tumors and PHB-I expression is reported (14). These findings indicate that PHB-I might have a tumor suppressor activity through regulation of the translation. Moreover, there are numerous studies indicating the definitive anti-tumorigenic role of PHB-I in some other cancers such as prostate cancer (15), gastric cancer (16), and hepatocellular malignancies (17).

In addition, PHB-I may act as an anchor in the cell membrane for C-Raf. Ras protein controls the cell signaling and collaborates with Raf protein kinase and finally activates MAPK (mitogen-activated protein kinase); thereby, biological order of growth is transferred and the cell growth is performed. In the absence of PHB-I, C-Raf activity stops; therefore, PHB-I may also play a key role in the progression of malignant transformation (18).

Some studies reported the increased expression of PHB-I gene in breast cancer (19), prostate cancer (20), and bladder cancer, and its significant relationship with prognostic factors such as disease stage and disease grade. On the other hand, some studies on gastric adenocarcinoma (16) and human glioma cell carcinoma (21) reported a significant

reduction in expression of PHB gene. The increase or decrease of PHB-I expression is probably related to its subcellular status, which there are limited studies in this field (22). According to these controversial results, the role of PHB-I as a tumorigenic or tumor suppressor is still unclear; therefore, the current study aimed at investigating the distribution of PHB-I both in tumoral tissue and anatomically normal margin in Iranian patients with breast cancer.

Method and Material

The current cross sectional study was conducted on formalin fixed paraffin embedded (FFPE) tissue samples from 33 patients with breast cancer. The samples were obtained from the Department of Pathology, Omid teaching Hospital, Mashhad, Iran.

Patients' data including age, hormone receptor status (ER, PR, and Her2 receptors), involved lymph nodes and metastasis condition, tumor grade (based on the pathologist's report), and disease stage (according to American Joint Committee on Cancer Protocol) (23) were extracted from patients' clinical files and recorded.

Immunohistochemistry (IHC) on FFPE samples was conducted based on LSAB (the labeled streptavidin-biotin) technique (24). Briefly, 4-micrometer tissue sections were prepared from the paraffin blocks. To ensure the fixation quality, hematoxylin and eosin (H & E) staining was performed. Next, special staining for PHB-I was conducted using the primary specific antibody. Thereafter, tissue pieces were floated on the slides coated with poly-alanine and immunohistochemical study of PHB-I was performed in the membrane of tumor cells using mouse PHB-I polyclonal antibody from Antibody Online Company (Cat. No. Orb80752), according to the manufacturer's guidelines. For deparaffinization, the slides were dipped in xylene (for six minutes, twice), followed by immersion into 100%, 95%, 85% and 75% alcohol, respectively for two to three minutes. The slides were then treated with hydrogen peroxide in order to block the endogenous peroxidase activity. Afterwards, the tissue pieces were incubated with antimouse-PHB-I polyclonal antibody (Enzo life sciences, USA, dilu-

tion of 1:400, overnight at 4°C) and after the washing steps, the streptavidin-peroxidase-conjugated secondary antibody was added. Finally, the stained slides were washed with DAB (diaminobenzidine) in order to stop the color reaction.

Finally, slides were examined under a microscope with 40X and 100X magnifications. Gastric tumor tissue and anatomically normal marginal tissue samples, confirmed by an expert pathologist, served as positive and negative controls, respectively. The intensity of staining and abundance of positive cells were scored semi-quantitatively as provided in Tables 1 and 2, respectively. The intensity of observed stained cells

scored 1 to 3 (weak to strong), and frequency of positive stained cells was determined as 0-25, 26-50, 51-75, and 76-100 (score 1 to 4) by an expert pathologist. The final score of PHB-I tissue expression was calculated by multiplying the recorded abundance and intensity scores. Data analysis was performed based on the calculated overall scores.

Data analysis

Categorical variables such as age, stage of the disease, and different hormone receptors were analyzed with SPSS version 16.0. A P-value of <0.05 was considered statistically significant in all calculations.

Table 1. Examining the Intensity of Staining in Immunohistochemistry Studies of Breast Cancer Tissue With 100X Magnification

Score	Intensity of staining
1	Weak (light brown)
2	Moderate (chestnut brown)
3	Strong (dark brown)

Table 2. Examining the Severity of Staining in Immunohistochemistry Studies of Breast Cancer Tissue With 100X Magnification

Score	Abundance of Positive Cells
1	Staining \leq 25% of cells
2	Staining 26%-50% of cells
3	Staining 51%-75% of cells
4	Staining of \geq 76% of cells

Results

The mean age of the patients was 45 ± 1.5 years. Figure 1 represents the IHC staining of control samples and tumor tissues at different stages, respectively. Average tissue distribution of PHB-I in the studied patients scored 3.97 ± 3.50 . PHB-I tissue distribution score ranged 0 to 12. The frequency of the status of prognostic receptors among the patients is shown in Table 3.

Also, tissue expression of PHB-I did not significantly correlate with the stage of cancer ($P=0.499$) (Table 5), similarly, it had no significant relationship with tumor size ($P=0.394$). Likewise, the incidence of

metastasis and the number of involved lymph nodes were independent of PHB-I expression ($P=0.491$ and 0.258 ; respectively).

As shown in Table 4, the average PHB-I tissue distribution in ER-positive patients was higher than that of the ER-negative group ($P=0.027$). Also, PHB-I tissue distribution in the PR-positive group was significantly higher than those of the PR-negative patients ($P=0.009$). No statistically significant relationship was observed between tissue expression of PHB-I and Her2 receptors ($P=0.911$). Tissue expression of PHB-I in patients with grade 1 was rather lower than that of grade 2 (2.27 ± 2.14 vs. 4.80 ± 3.58) ($P=0.057$).

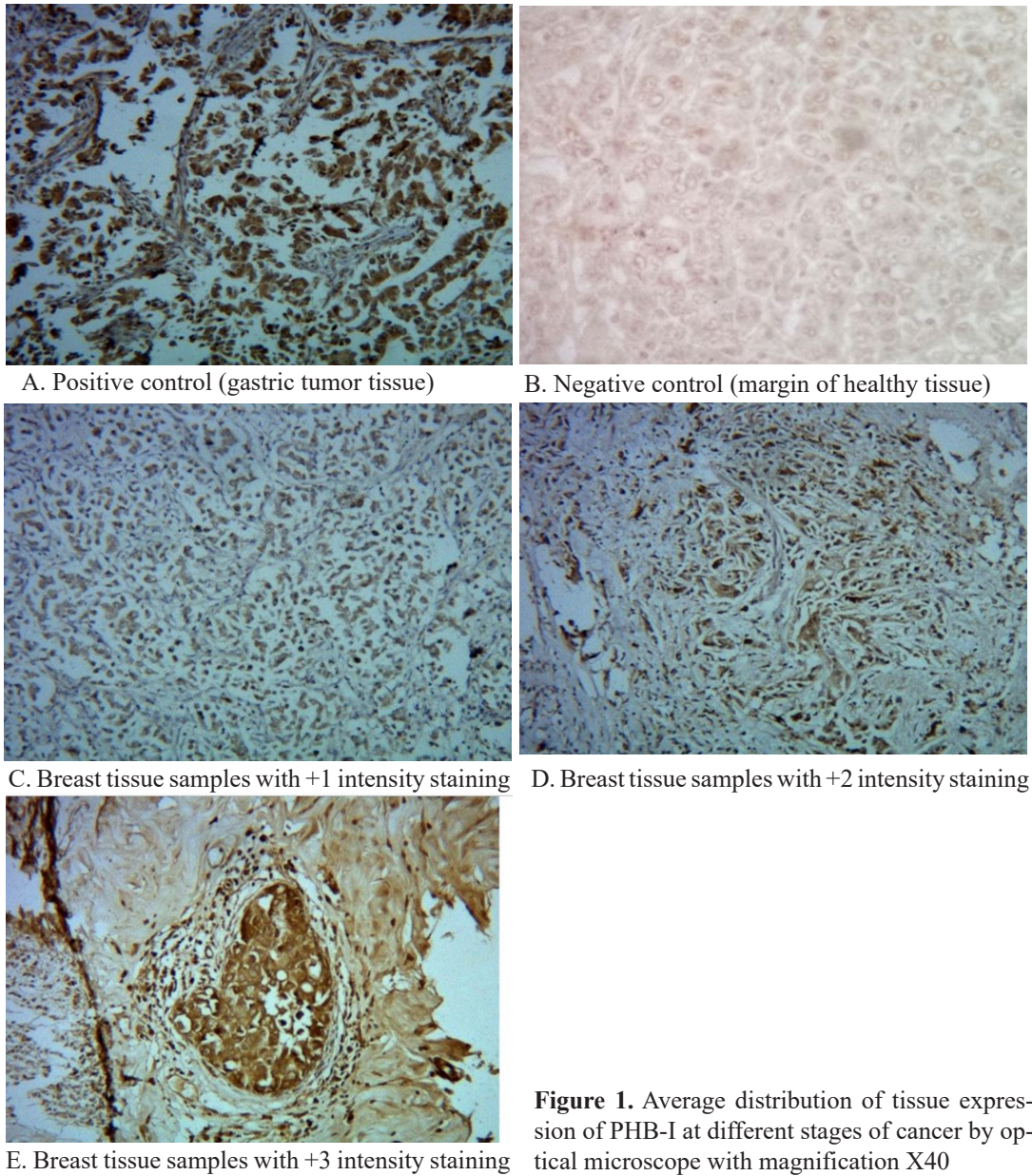


Figure 1. Average distribution of tissue expression of PHB-I at different stages of cancer by optical microscope with magnification X40

Table 3. Frequency of the Status of Intended Different Receptors Among the Studied Patients

Type of Receptors	ER (N) %	PR (N) %	HER-2 (N) %
Positive	(22) 66.7	(20) 60.6	(17) 51.5
Negative	(11) 33.3	(13) 39.4	(16) 48.5
Total	(33) 100	(33) 100	(33) 100

Table 4. PHB Expression Score in Positive/Negative Patients for ER, PR, and Her2

Type of Receptors	Positive	Negative	P value*
ER	4.95±3.59	2.18±2.27	0.027
PR	5.25±3.47	2.15±2.47	0.009
HER2**	3.75±3.50	3.95±3.24	0.911

*Student t test

**Negative and 1+ for Her2 were grouped as positive and 2+ and 3+ were grouped as negative.

Table 5. The Average Tissue Distribution of PHB-I and Correlation With Staging of Cancer

Group	Average Tissue Distribution of PHB-I
Stage 1	4.95
Stage 2	6.75
Stage 3	3.28
Stage 4	9.24

Discussion

The concentration of biomarkers provides helpful data for a physician to determine disease severity and prognosis. Until now, multiple prognostic factors and predictive factors such as CA125, CA19-9, and CA 15-3 are identified for breast cancer, and the value of some of them is completely proved; however, still no consensus is reached regarding other proposed factors (25-27).

It is essential to determine the prognosis of patients with cancer in order to set proper management of disease and also predict the disease outcome. Today, overall risk evaluation can be performed through clinical risk scoring systems (28). The status of tumor markers at the time of diagnosis is one of the tools used to determine the prognosis of breast cancer and subsequently the effect of adjuvant treatments such as chemotherapy. Improved prognostic models with newly included factors may provide developed clinical management schedules and treatment protocols. One of such new biomarkers is PHB-I, which might be promising for diagnostic purposes.

It is noted that one of the best methods to measure PHB-I is immunohistochemistry in which the rate of tissue expression can be determined based on the intensity and percentage of staining of the tumor tissue samples (29). In one study, immunohistochemical evaluations of breast cancer showed an increase in PHB-I expression in breast cancer cells and revealed a significant relationship between the increased expression and the breast tumor grade. Also, a direct relationship was observed between the increased PHB-I expression and clinico-pathological features such as ER and PR receptors (19). However, still summative studies are needed to further explore such observed relationships.

Results of the current study showed that the level of PHB-I expression increases along with an increase in the disease grade and also other prognostic factors such as ER- and PR-positivity. There are few studies linking between PHB-I and ER/PR, Her2 and prognosis of the patients and the results are controversial (30-32). Some factors including statistical issues and the included patients' characteristics such as grade at the time of diagnosis might cause such contradictory observations.

No statistically significant relationship was observed between PHB-I and Her2 and disease stage. However, larger sample sizes might reveal a significant relationship. Due to contradictory results of the studies still additional studies preferably in a multicenter model are needed to confirm the results.

Conclusion

The current study showed an increase in PHB-I expression in breast cancer specimens in a sample of Iranian patients. A statistically significant relationship between tissue distribution and some prognostic factors such as ER and PR was observed. With the observed correlations, PHB-I might be a promising factor in breast cancer.

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Ethical approval

The current study was reviewed and approved by the Ethical Committee of the Research Council of Mashhad University of Medical Sciences (Ethical

code: 930825). All procedures in the current study were performed in accordance with the ethical standards of the institutional/national research committee and with the 1964 Helsinki declaration and its additional amendments.

Conflict of Interest

Authors declared no conflict of interest.

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