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# Comparative Analyses of Villin and HER-2 Genes Expression in Breast Cancer

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ABSTRACT

**Background:** It has been previously demonstrated that HER-2 (human epidermal growth Factor receptor 2) positive breast cancers are associated with an aggressive nature. Villin is an actin bundling protein that plays a key role in actin reorganization and cell remodeling during stress. In this study, we aimed to investigate the correlation of Villin gene expression with HER-2 in breast cancer patients.

**Methods:** Samples of 42 patients with breast cancer, and 3 controls were collected. Expression of Villin and HER-2 genes were monitored with real-time PCR using pre-designed primers. Student T-test was used to compare the means between the groups.

**Results:** The mean age of the patients was 50±4.11years. Expression of the Villin gene was decreased in 28 samples (18 and 10 samples with negative and strongly HER-2 positive, respectively). Villin gene expression was increased in 14 samples (7, 2 and 5 samples with negative, weakly positive, and strongly HER-2 positive, respectively). The expression of Villin was significantly correlated with HER-2 positive status (P = 0.00057)

**Conclusions:** We found that Villin gene expression is associated with HER-2 positivity and may be a predicting factor in aggressive breast cancer.

## Introduction

Breast cancer is the most common cancer among Iranian women with a rate of 24 per 100,000.[1, 2] In 2008, almost 1.4 million women were diagnosed with breast cancer worldwide and approximately 459,000 deaths were recorded.[3] Lifetime risk of developing breast cancer for women is estimated to be 1 in 8.[4,5]

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Cancer is a progressive disease, made of tumor cells and multiple components which compete with the normal micro-environment. Therefore, cellular morphology and structure is the cornerstone of cancer diagnosis, staging, and therapy.[6]

With recent developments in genetics and molecular biology, breast cancer has been classified into different subtypes based on tumor markers of estrogen (ER), progesterone (PR) and human epidermal growth factor receptors (HER-2).[7-9] HER-2 is a member of receptor tyrosine kinase family and its overexpression is found in 20–30% of breast cancers, many of which are PR/ER negative.[10,11] HER-2 positive breast cancers are associated with aggressive course, malignant behaviors, higher recurrence, and reduced overall survival.[10,12,13]

Villin is an actin bundling protein that plays a key role in cell. It is usually found in microvilli of the brush border and is mainly expressed in normal gastrointestinal organs such as stomach, pancreas, bile duct, and intestines; as well as the corresponding adenocarcinomas.[14, 15] Also, Villin2 (ezrin) protein has been implicated in various human cancers and is closely linked with metastatic progression and aggressive cancer types.[16] Effects of Villin protein in aggressive breast cancer have not been thoroughly studied.

In this study, we aimed to investigate the correlation of Villin gene expression with HER-2 in breast cancer patients and its effects on pathophysiological and clinical findings.

## Methods

Samples of 42 patients with breast cancer, and 3 controls were collected from Iran National Tumor Bank (INTB; The Cancer Institute, Imam Khomeini Hospital, Tehran, Iran). RiboEx (Gene All biotechnology, Korea) was used for RNA extraction. Tissues were homogenized and vortex mixed for 10 seconds. After adding chloroform (200 µl), the samples were centrifuged at 1200 rpm for 15 minutes at 4°C. The supernatant was collected and centrifuged after adding isopropanol (500 µl). The RNA pellet was washed with ethanol and dried then eluted in DEPC treated water.

Extracted RNA was quantified with a NanoDrop ND-2000 spectrophotometer (NanoDrop Technologies, Wilmington, DE). The quality of RNA was validated with gel electrophoresis. The complementary DNA (cDNA) was generated using cDNA reverse transcription kit (Takara Bio Inc., Japan). Synthesized cDNA was tested with housekeeping control. To monitor gene expression, real-time PCR (Takara Bio Inc., Japan) was used with pre-designed primers (Table 1).

**Table 1.** Sequences of pre-designed primer used to assess genes expression

| Gene | | Sequence | |
|---|---|---|---|
| Villin | Fwd | 5'-GGCCAGCCAAGATGAAATTA-3' | 60.0 |
| | Rev | 5'-CTCAAAGGCCTTGGTGTTGT-3' | 60.1 |
| Gapdh | Fwd | 5'-GAAGGTGAAGGTCGGAGTCA-3' | 60.2 |
| | Rev | 5'-AATGAAGGGGTCATTGATGG-3' | 59.6 |

The data of HER-2 expression was extracted from Iran National Tumor Bank database. HER-2 score of 0 and 1 was considered as negative, while +2 was considered as weakly positive and scores of +3 and +4 were considered as HER-2 positive (Strongly positive).[17]

We used SPSS (version 19.0; IBM Corp., Armonk, NY) for statistical analysis. T-test was used to compare the means and standard deviations between the two groups. Values of $P < 0.05$ were considered statistically significant.

## Results

The mean age of the patients was 50±4.11years (Ranging from 33 to 74 years). Of these patients, 3 (7.1%) were in stage I, 21 (50%) were in stage II, 17(40.5%) were in stage III, 1 (2.3 %) was in stage IV. There were 5 (11.9%) patients with M1 and 37(88.09%) patients with M0. Seventeen (40.5%) patients were HER-2 positive (15 were strongly positive; 2 were weakly positive) and 25 (59.5%) patients were HER-2 negative.

Comparison of cancerous and normal tissues revealed that expression of the Villin gene was decreased in 28 samples (18 and 10 samples with negative and strongly HER-2 positive, respectively). Villin gene expression was increased in 14 samples (7, 2 and 5 samples with negative, weakly positive, and strongly HER-2 positive, respectively) (Figures 1 and 2, Table 2). The expression of Villin was significantly correlated with HER-2 positive status ($P = 0.00057$).


**Figure 1.** Villin gene expression and HER-2 protein expression in breast cancer patients the cut of line shows standardization based on normal breast tissue

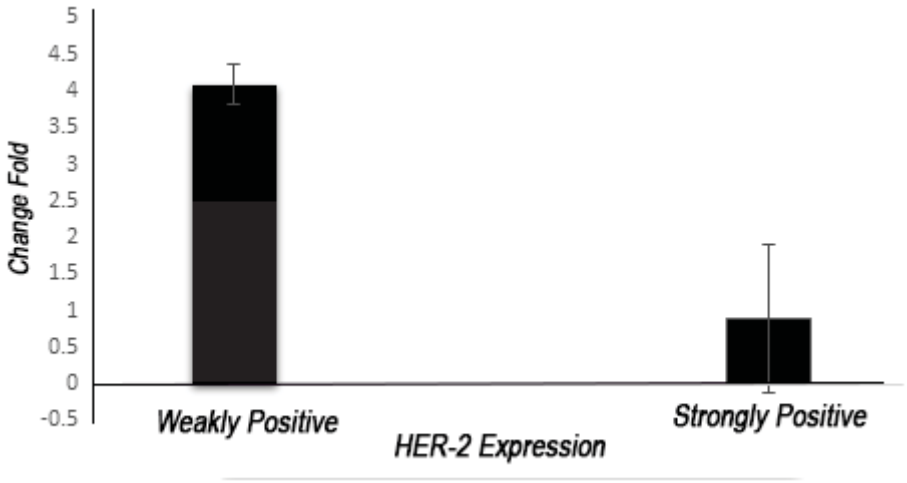


**Figure 2.** The average of Villin gene expression amongst different HER-2 expressing tumor tissues (P = 0.00057)

**Table 2.** the expression of Villin based on HER-2 receptor expression among breast cancer patients

| Patient number | HER-2 Expression | Villin Gene expression | Average±SD | P-value |
|---|---|---|---|---|
| 1 | Weakly positive | 4.29 | 4.01±0.28 | 0.00012 |
| 2 | Weakly positive | 3.90 | 1.03±1.71 | |
| 3 | negative | 0.46 | | |
| 4 | negative | 0.31 | | |
| 5 | negative | 0.09 | | |
| 6 | negative | 1.15 | | |
| 7 | negative | 0.49 | | |
| 8 | negative | 0.095 | | |
| 9 | negative | 6.59 | | |
| 10 | negative | 2.80 | | |
| 11 | negative | 0.40 | | |
| 12 | negative | 0.01 | | |
| 13 | negative | 0.06 | | |
| 14 | negative | 0.07 | | |
| 15 | negative | 0.59 | | |
| 16 | negative | 0.07 | | |
| 17 | negative | 0.74 | | |
| 18 | negative | 0.50 | | |
| 19 | negative | 0.09 | | |
| 20 | negative | 1.43 | | |
| 21 | negative | 0.03 | | |
| 22 | negative | 0.01 | | |
| 23 | negative | 0.06 | | |
| 24 | negative | 1.06 | | |
| 25 | negative | 0.06 | | |
| 26 | negative | 5.49 | | |
| 27 | negative | 3.0 | | |
| 28 | Strongly positive | 0.4 | 0.89±1.01 | 0.00019 |
| 29 | Strongly positive | 0.3 | | |
| 30 | Strongly positive | 0.02 | | |
| 31 | Strongly positive | 1.20 | | |
| 32 | Strongly positive | 0.07 | | |
| 33 | Strongly positive | 0.20 | | |
| 34 | Strongly positive | 0.10 | | |
| 35 | Strongly positive | 0.10 | | |
| 36 | Strongly positive | 0.01 | | |
| 37 | Strongly positive | 0.90 | | |
| 38 | Strongly positive | 3.0 | | |
| 39 | Strongly positive | 0.95 | | |
| 40 | Strongly positive | 1.30 | | |
| 41 | Strongly positive | 1.90 | | |
| 42 | Strongly positive | 2.90 | | |

## Discussion

In this study, we demonstrated that the expression of the cytoskeletal protein Villin is decreased in breast cancer patients and it is significantly associated with HER-2 positive status.

Increased motility is a critical step in migration and invasion of the malignant cells, which is regulated by the reorganization of the actin cytoskeleton.[18,19] Villin is an important protein for actin reorganization and cell remodeling during stress.[20, 21] While Villin is an actin binding protein in the structure of microvilli, strong body of evidence suggests that this protein can also induce actin severing at physiologically relevant calcium concentrations; a function that is associated with cell migration.[19,22]

Villin is mainly expressed in normal gastrointestinal organs, and corresponding adenocarcinomas.[15,23] Villin expression was also inconsistently reported in cancers of kidney, lung, endometrium, and ovary.[24-26] In all breast adenocarcinoma cases tested in previous reports, Villin expression was negative.[15, 24, 26] In the studies by Moll *et al.*[24], and Bacchi *et al.*[26], ER status was not mentioned. Yang and colleagues evaluated the Villin protein expression with ER monitoring.[15] They used immunohistochemical staining for Villin detection, which was negative in all breast cases, regardless of normal breast tissue, in situ, invasive, or metastatic carcinoma.[15] This finding is consistent with ours. We assessed Villin gene expression with RT-PCR and found that Villin expression is generally decreased in breast cancer.

Moreover, we assessed the correlation between Villin expression and HER-2 positive status. HER-2 (ERBB2) is a tyrosine kinase transmembrane receptor that plays a key role in intracellular signaling pathways of tumor cells.[27] This family of tyrosine kinase receptors also includes HER1 (epidermal growth factor receptor, EGFR), HER3, and HER4. HER-2-positivity is associated with invasiveness and poor prognosis; however, it provides information to identify patients who may benefit from targeted therapies.[28]

We found that Villin gene expression is significantly correlated with HER-2 positive status and may be a predicting factor in aggressive breast cancer. To the best of our knowledge, we are the first to report such correlation in breast cancer and further studies are warranted to elucidate these findings.

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