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ORIGINAL ARTICLE

PTEN loss is not associated with trastuzumab resistance in metastatic breast cancer

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Summary

Purpose: *Although the clinical benefits of trastuzumab are well known, intrinsic or acquired resistance is a commonly encountered clinical condition. A potential resistance mechanism is aberrant downstream signal transmission due to loss of phosphatase and tensine homologue (PTEN). This study investigated the relationship between trastuzumab response and loss of PTEN in metastatic breast cancer patients.*

Methods: *Patients with histologically confirmed human epidermal growth factor receptor 2 (HER2) positive metastatic breast cancer, who were treated with trastuzumab were enrolled into the study. PTEN expression was immunohistochemically evaluated.*

Results: *The patient median age was 50 years. Of 38 patients, 6 (15.8%) showed PTEN loss. No statistically significant difference was found between trastuzumab response, overall survival (OS) and progression-free survival (PFS) and PTEN loss (p=0.538).*

Conclusion: *The activation of phosphatidylinositol 3-kinase (PI3K) pathway resulting from PTEN loss was not found to be correlated with trastuzumab response and survival. PTEN loss should not lead to exclusion of patients from the potential to benefit from trastuzumab administration.*

Key words: *breast cancer, HER2, PI3K pathway, PTEN loss, trastuzumab*

Introduction

With the progressive identification of the genetic and biological characteristics of breast cancer, important advancements in diagnosis, prevention and treatment of this disease were achieved. Among these advancements, the most important ones were hormone receptors and human HER2. HER2 is amplified or overexpressed in approximately 20% of the patients with breast cancer [1]. Breast cancer patients in whom HER2 is overexpressed should receive trastuzumab as a part of their treatment. Although a significant part of the patients respond to trastuzumab-based therapy at the beginning, progression is generally observed during the 1st year of therapy. Recently, some resistance mechanisms leading to this condition were tried to be identified [2,3].

PTEN gene is a tumor suppressor, homologue to tyrosine phosphatase and tensine, localized on 10q23.3 chromosomal band, which is a genomic domain characterized by frequent loss of heterozygosity in many types of human tumors. As a tumor suppressor gene, PTEN controls many cellular functions, such as growth, proliferation, cell survival and migration [4-6]. Germline PTEN mutations leads to Cowden syndrome, which is a hereditary cancer predisposition syndrome characterized by high incidence of breast, uterus, thyroid and skin neoplasms [7]. PTEN is inactivated as a result of epigenetic silence due to mutation or, more commonly, to methylation [8]. PTEN dephosphorylates and thereby inactivates the p110 catalytic unit of PI3K. Approximately 25% of

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breast cancer cases harbor oncogenic-activating mutations in the p110 catalytic unit of their PI3K [9]. Activating mutations occurring in the gene that encodes the p110-alpha catalytic subunit of PI3K may be an important factor leading to tumor progression. PTEN loss is associated with genetic instability and aneuploidy [10] and was found to be associated with decreased response to trastuzumab [11]. Activation of PI3K pathway results in activation of some kinases, including Akt1, Akt2 and Akt3. Activated Akt1 is known to be antiapoptotic. Downstream proliferative effectors of PI3K pathway also include mammalian target of rapamycin (mTOR) complex. Ras/raf/MEK/ERK pathway is a critical signal transduction pathway for many growth factor receptors.

Methods

In this study, we retrospectively evaluated 38 patients with histologically diagnosed HER2-positive breast cancer who were treated with first-line trastuzumab for metastatic disease. Patients with positive hormone receptors, soft tissue or bone metastases received antihormonal therapy in addition to trastuzumab. Hormone receptor negative patients with soft tissue or bone metastases received capecitabine. All patients with visceral metastases, regardless of hormone receptor status received taxane-containing regimens. The relationship between PTEN expression, and disease characteristics, therapeutic response and prognosis was assessed.

Immunohistochemistry (IHC)

In the tissue fixed with formalin, streptavidine-biotin-peroxidase immmunohistochemical staining was used to demonstrate PTEN immunoexpression. In this method, 3 μm sections were transferred to positively charged microscope slides from paraffin-embedded tissues and were deparaffinized at 37°C overnight. Following this, deparaffinization was completed by soaking them for 5 min in three separate xylenes. The sections were immersed in two different 96% ethanol solutions and endogenous tissue peroxidase activity was inhibited by using 3% hydrogen peroxide (in methanol). The sections which were washed with distilled water underwent antigen recovery using citrate buffer (pH 6.0) in microwave oven in order to reveal the antigens that have been masked. The slides were cooled at room temperature for 20 min, they were washed using two different phosphate buffer solutions (PBS) and, in order to prevent non-specific staining, the tissues were subjected to protein blockade for 10 min (Histostain Bulk Kit, Invitrogen LAB-SA Detection System, UK). Following the blockade, 1:100 diluted PTEN (Klon 28H6, Novocastra, Leica Microsystems, UK) was dripped on separate cross sections and incubated for 60 min at room temperature. At the end of this time, the sections were washed using two different PBSs and kept in biotinylated secondary antibody (Histostain Bulk Kit, Invitrogen LAB-SA Detection System, UK) for 10 min. Thereafter, they were re-washed with PBS, streptavidine peroxidase (Histostain Bulk Kit, Invitrogen LAB-SA Detection System, UK) was instilled and were incubated for 10 min. 3,3'-diaminobenzidin (DAB) chromogene was instilled to the sections washed with PBS, and they were incubated for 5 min. The sections washed with distilled water were counterstained using Mayer Hematoxylene and were dehydrated by treating with ethanol. The tissue sections that were taken to xylene and mounted using mounting medium were evaluated under light microscope (BX51 Olympos, Japan).

All stained sections were examined by two pathologists who were blinded to histological grade, hormone status and HER2 results. In all subjects, invasive area, in situ area and surrounding breast tissue were separately evaluated for presence of PTEN antibody. Nuclear staining was obtained in the presence of PTEN. Due to the variations in the intensity of staining, a scoring was used [11]. The percentage of cells that were semi-quantitatively positively stained for PTEN and the intensities of staining were multiplied and the result was recorded as immunoreactive score (IRS). Intensities of staining were graded as 0 (negative), 1 (weakly positive), 2 (moderately positive) and 3 (strongly positive). Staining was graded as 0 (<5% cells), 1 (5-25% cells), 2 (26-50% cells), 3 (51-75% cells), and 4 (>75% cells). Based on the results, IRS 0-3 was recorded as 0, IRS 4-6 as 1 positive, IRS 7-9 as 2 positive and IRS 10-12 as 3 positive. PTEN negativity was defined as IRS 0 or 1 and PTEN positivity was defined as IRS 2 or 3 (Figure 1A and B).

Statistics

OS was evaluated by two different approaches: OS was either calculated from the date of the initial diagnosis of breast cancer (OS-1) or from the starting date of the trastuzumab-containing therapy during the metastatic period (OS-2) to death or the last follow-up date. PFS was defined as the time from the starting date of the trastuzumab-containing therapy to the date on which disease progression was detected. Response to trastuzumab-based therapy was evaluated every 8-12 weeks using the modified RECIST (Response Evaluation Criteria in Solid Tumors) [12]. For statistical analyses, NCSS (Number Cruncher Statistical System) 2007 & PASS (Power Analysis and Sample Size) 2008 statistical software (Utah, USA) were used. Study data were evaluated using descriptive statistics (mean, standard deviation, median, frequency, percentage) and quantitative data were compared using Mann-Whitney U test. Qualitative data were compared using chi-square test, Yates chi-square test, Yates corrected chi-square test and Fisher's Exact test. Survival analyses were performed using Kaplan-Meier method analysis and log

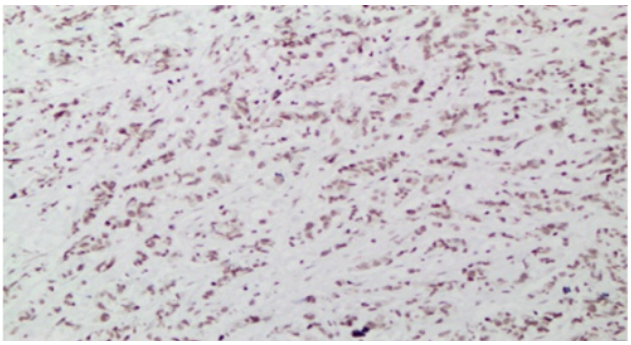

Figure 1A. Strongly positive PTEN expression (PTEN immunohistochemical stain x20)..

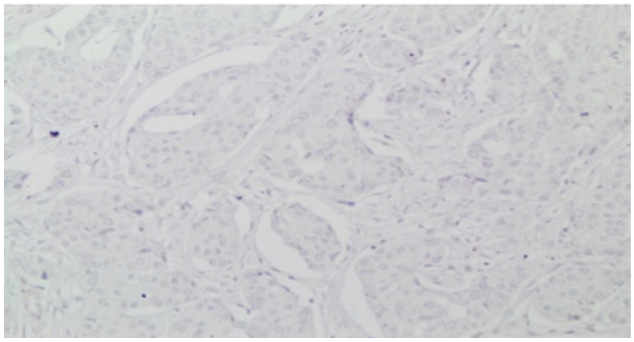

Figure 1B. Negative PTEN expression (PTEN immunohistochemical stain x20).

rank analysis. A p value <0.05 was considered as significant.

Results

Thirty eight patients were incuded in the study. Their median age was 50 years (range 30-84). Descriptive characteristics of the patients are shown in Table 1. Median follow-up time was 38.6 months and median PFS was 12.37 months. When evaluated for trastuzumab response, 9 patients (23.7%) had progressive disease, 19 (50%) had partial response, 7 (18.4%) had complete response and 3 (7.9%) had stable disease.

Immunoreactive scoring showed PTEN loss in 6 patients (15.8%) and absence of PTEN loss in 32 patients (84.2%). No significant difference was found between PTEN loss and grade, stage at the time of diagnosis, hormone receptor status and metastatic sites (p>0.05). However, all patients with PTEN loss were postmenopausal. The response rates with trastuzumab in the patients without PTEN loss were higher, not reaching however statistical significance (Table 2). Median PFS was 12.63 and 9.63 months in patients with and without PTEN loss (p>0.05). Again, in the presence of PTEN loss, OS-1, OS-2 and PFS did not show statistically significant difference (Table 3).

Of the patients with PTEN loss, 5 (83.3%) survived and 1 died. Of the patients without PTEN loss, 26 (81.3%) survived and 6 died. As seen in Figure 2, when survival rates were evaluated by PTEN status, no statistically significant difference was found between 5-year survival rates (p>0.05)

Discussion

Although trastuzumab-based adjuvant therapy yields a decrease of 50% in the risk of recurrence in patients with HER2-positive early breast cancer, approximately 15% of the patients develop metastatic disease despite adjuvant therapy [13]. In some patients with HER2-positive breast cancer, trastuzumab has no effect or resistance to therapy develops in the short-term. Understanding the mechanisms that underlie trastuzumab resistance is dramatically important to determine new anti-HER2 therapeutic strategies. Researchers tried to describe the genetic mechanisms by which this resistance occurs but no important evidence that could be implemented in daily practice has been found until now. In our study, approxi-

Table 1. Patient characteristics

Characteristics	*N (%)*
Menopausal status	
Postmenopausal	24 (63.2)
Premenopausal	14 (36.8)
Breast operation	
Yes	25 (65.8)
No	13 (34.2)
Histological grade	
1	1 (2.6)
2	23 (60.5)
3	14 (36.8)
Stage at diagnosis	
I	1 (2.6)
II	5 (13.1)
III	11 (29)
IV	21 (55.3)
Hormone receptors	
Positive	14 (36.8)
Negative	24 (63.2)
Adjuvant chemotherapy	
Yes	13 (34.2)
No	25 (65.8)
Adjuvant radiotheraphy	
Yes	10 (26.3)
No	28 (73.7)
Metastatic site	
Bone/soft tissue	19 (50)
Visceral organ	19 (50)
Additional therapy with trastuzumab	
Anti-hormonal therapy	6 (15.8)
Capecitabine	3 (7.9)
Taxane-based therapy	29 (76.3)

Table 2. Trastuzumab response according to PTEN status

Trastuzumab response	*No PTEN loss N (%)*	*PTEN loss N (%)*	*p-value**
Progression	7 (21.9)	2 (33.3)	0.569
Partial response	17 (53.1)	2 (33.3)	0.613
Complete response	7 (21.9)	0 (0)	0.660
Stable disease	1 (3.1)	2 (33.3)	0.059

*Fisher's exact test

Table 3. Survival according to PTEN

	No PTEN loss	*PTEN loss*	*p-value**
Median OS-1 (mo)	34.42	38.6	0.841
Median PFS (mo)	9.63	12.63	0.749
Median OS–2 (mo)	15.76	26.65	0.128

*Mann-Whitney U test
OS: overall survival, PFS: progression-free survival, mo: months

mately 25-30% of the patients showed resistance to trastuzumab-based therapy.

PTEN loss is one of the genetic alterations that is thought to have the potential to lead to trastuzumab resistance. It is functional in the PI3K/Akt pathway that transmits signals coming from extracellular molecules that stimulate cell growth and proliferation by receptor tyrosine kinases, such as growth factors and cytokines, to intracellular environment. Increased intracellular PTEN levels and the related Akt inactivation cause G1 arrest of the cell cycle and apoptosis. In cells without PTEN function, cell proliferation is increased [14]. The importance of PTEN expression in HER2-positive breast cancer has been recently understood.

Previous studies reported an incidence of 15-48% for PTEN loss in sporadic breast cancer cases [15-18]. In our patient group, PTEN loss was found to be 15.8%. When the clinical response rates were examined, our study did not show a statistically significant difference between the groups with and without PTEN loss. Progression rate was 33.3% in the group with PTEN loss and 21.9% in the group without PTEN loss with trastuzumab therapy, but this difference did not reach statistical significance (p>0.569).

Although PTEN mutations that cause PTEN loss are rarely seen in sporadic breast cancer, loss of heterozygosity, PTEN haploinsufficiency and epigenetic PTEN downregulation are commonly seen mechanisms [19]. It is unknown whether genetic and epigenetic oncogenic changes in PTEN occur in early or late stages. In the present study, all patients with PTEN loss were postmenopausal with median age 59.6 years, when in the general patient population the median age was 50 years. Although no statistical significance was detected in the general patient population (most likely due to the small number of patients with PTEN loss), interestingly, all patients with PTEN loss were within postmenopausal period.

Activating mutations of the PI3K pathway were reported to be correlated with advanced age [20]. Although the literature contains clear information stating that this may also be the case for PTEN, it is reasonable that PTEN loss is more commonly seen at advanced age.

There are some publications suggesting that PTEN loss is a negative predictive factor for trastuzumab response in HER2-positive metastatic breast cancer or, in other words, it is associated with trastuzumab resistance [21-24]. There are also some publications that assert the contrary of this hypothesis. In a study performed by Barbareschi et al., 48 patients who used trastuzumab

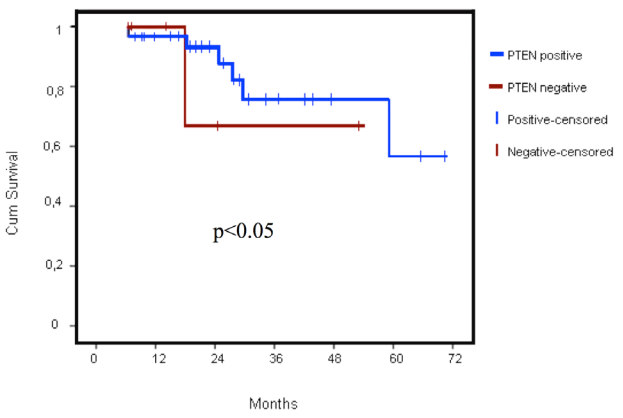

Figure 2. Overall survival-2 according to PTEN status

for the treatment of metastatic breast cancer were assessed and, as a result, the activation of PI3K pathway due to PTEN loss or PI3KCA mutations were not correlated with trastuzumab response and clinical course [25]. However, similar to other studies presented in the literature, this study underlined the limited number of cases and, thereby, the need for further studies.

In a study performed by Jinno et al., patients with HER2-positive breast cancer were given paclitaxel and trastuzumab as neoadjuvant therapy. In this study, 8 of 24 patients (33%) showed PTEN loss while no significant correlation was detected between PTEN loss and/or PIK3CA mutations [26].

In our study no statistically significant differences were found between the groups with and without PTEN loss in terms of clinical response, PFS and OS rates. The scarcity of the cases and the variations of the results in the literature underline the need for a meta-analysis. Based on the results obtained from existing studies and our study, no result strong enough to suggest a change in the daily practice considering PTEN status was found.

Consequently, no adequate evidence was reached to demonstrate that immunohistochemically determined status of the PTEN gene was prognostic or predictive. Larger and more homogeneous case series are needed to analyze this condition.

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