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ORIGINAL ARTICLE

Overexpression of PIK3CA impacts global survival of patients with HER2 subtype breast carcinoma

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Summary

Purpose: *To investigate the expression of proteins fosfatidil-inositol-4,5-bifosfate 3-quinase (PIK3CA) and phosphatase and tensin homolog (PTEN) in HER2-positive breast cancer and verify their associations with clinical and pathological variables.*

Methods: *We assessed PTEN and PIK3CA status using immunohistochemistry (IHC), which was performed in formalin-fixed paraffin-embedded biopsies from 50 patients with HER2-positive breast cancer. Medical records were studied for collection of clinical-pathological information, including overall survival (OS). The HIC markers PTEN and PIK3CA were analyzed semi-quantitatively by two blinded independent researchers. The relationship between the variables were evaluated using the chi-square test and Kaplan-Meier curves plus log-rank test for survival.*

Results: *In IHC, the expression level of PIK3CA was 86%, and loss of PTEN expression was observed in 46% of the cases. The expression of the markers showed no significant correlation with each other or with the clinical and pathological parameters studied: tumor grade, staging, ER, PR, Ki67 and recurrence. The highest expression of PIK3CA was associated with lower number of deaths (p=0.016) and longer OS of patients (p=0.001). The PTEN marker showed no significant effect on OS.*

Conclusions: *The PIK3CA expression showed a protective effect in relation to the OS of patients with HER2-positive breast cancer.*

Key words: *breast cancer, immunohistochemistry, HER2, PIK3CA, PTEN, survival*

Introduction

Breast cancer is the most frequent cancer among women, impacting 2.1 million women each year, and also causing the greatest number of cancer-related deaths among females [1].

Tumors of *HER2*-positive subtype, generally negative for estrogen and progesterone hormone receptors (ER and PR) occur in 15 to 20% of breast cancers, conferring poor prognosis to patients [2,3]. These tumors are designated for target therapy with Trastuzumab. However, the therapeutic response is not satisfactory due to mechanisms involving resistance. There are some hypotheses about the mechanisms of therapeutic failure and studies about new strategies for the treatment of this subtype. However, there are still no definitive biomarkers and no substitute treatment for Trastuzumab [4-6].

Some studies have shown increased *PIK3CA* gene expression and loss of *PTEN* associated with *HER2* overexpression and possibly *PI3K/AKT/mTOR*

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pathway overactivation promoting tumor proliferation. Nevertheless, little is known about the expression of these biomarkers in relation to the clinical and pathological statuses of patients with *HER2*-positive breast cancer, making these genes possible research targets for these cases [7-10].

This study aimed to evaluate the expression of *PIK3CA* and *PTEN* by IHC in *HER2*-positive breast cancer and its relationship with clinical and pathological data, including global survival.

Methods

Study subjects

Tissue samples were obtained from 50 female patients with *HER2*-positive breast cancer. Biopsies were selected between 2013 to 2015, belonging to the archive of the Pathology Sector of Hospital de Clínicas de Porto Alegre. This study was approved by the Ethics Committe (CAAE number: 57627916.8.0000.5327). Clinical and pathological data from the patients, including the follow up time were assessed from the medical records.

Immunohistochemistry staining and analysis

The samples were submitted to immunohistochemistry for *PTEN* (Abcam, 1:100) and *PIK3CA* (Abcam, 1:100), at the Benchmark ULTRA Ventana Medical System automation platform (Tucson, Arizona USA). The analysis of the results was performed by two independent blinded pathologists, in a semi-quantitative way. The percentage of positive cells (0-100%) and reaction intensity (0-3) were evaluated, with subsequent creation of a score (0-300 points) [11]. Low and high levels of the marker's expression were defined by the mean score of expression (score 20).

Statistics

The data were presented as absolute frequencies and percentages, mean ± standard deviation or mean and 95% confidence interval. Normality was assessed by the Shapiro-Wilk test and categorical variables were compared by the chi-square and Kaplan-Meier plus log-rank tests for cumulative survival analysis using the SPSS 22.0 (SPSS Inc., Chicago, IL, USA). Only p values below 0.05 were considered statistically significant.

Results

Clinicopathological features

The clinical and pathological data of the sample studied are presented in Table 1. The mean age of the patients at the time of diagnosis was 54 years (32-89). The predominant histological type was the non-special ductal invasive carcinoma. Most patients had histological grade 3 and stage II (Table 1). The total follow-up time was 80 months (4-90 months). Eleven patients had recurrence of the disease and 6 patients died during the study period.

Expression of PTEN and PI3K protein

As demonstrated in Figure 1, the expressions of *PTEN* and *PI3K* were defined as brown stain in the cytoplasm and nucleus and on the cell membrane and in the cytoplasm, respectively. The level of immunohistochemical expression detected in *PIK3CA* was 86%. Loss of *PTEN* expression was observed in 46% of the cases.

Considering the expression of the markers *PIK3CA* and *PTEN* among themselves and in relation to

Table 1. Clinicopathological characteristics of patients

Characteristics	***n (%)***
Age years-old (min-max)	
Mean	54 (32-89)
Histological type	
Ductal invasive	44 (88)
Lobular invasive	6 (12)
Tumor grade	
1	2 (4)
2	23 (46)
3	25 (50)
Stage	
I	12 (24)
II	27 (54)
III	11 (22)
IV	0 (0)
ER	
Positive	33 (66)
Negative	17 (34)
PR	
Positive	32 (64)
Negative	18 (36)
Ki67	
<13%	2 (4)
>14%>	48 (96)
PIK3CA	
>20	43 (86)
<19	7 (14)
PTEN	
>20	27 (54)
<19	23 (46)
Disease relapse	
Yes	11 (22)
No	39 (78)
Death	
Yes	6 (12)
No	44 (88)

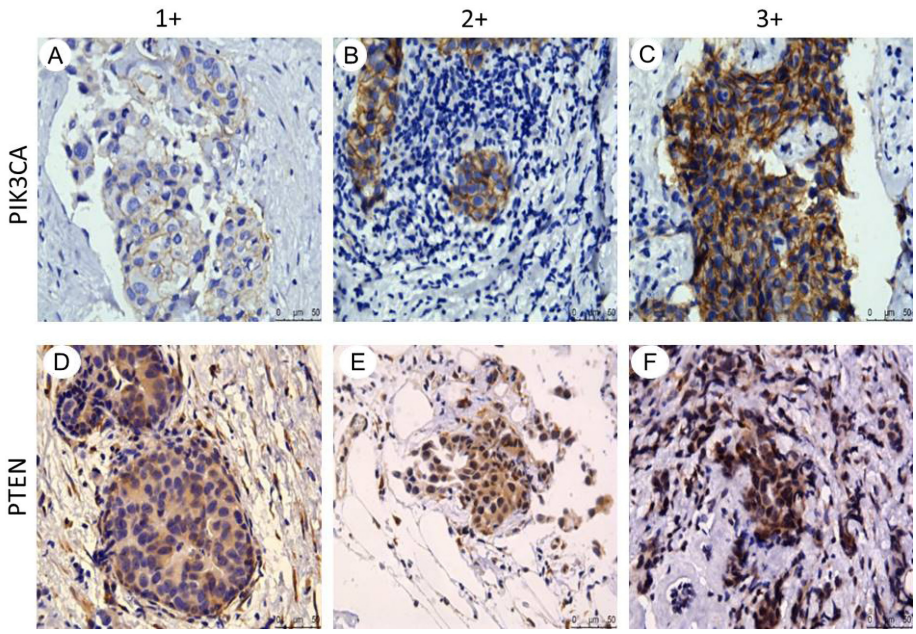

Figure 1. Representative images of PIK3CA and PTEN immunohistochemistry staining in breast cancer tissue. Weak 1+ **(A)**, moderate 2+ **(B)**, and strong 3+ **(C)** PIK3CA expression, and weak **(C)** PTEN expression. Weak 1+ **(D)**, moderate 2+ **(E)**, and strong 3+ **(F)** PTEN expression. Original magnification ×400.

Table 2. Analyses of PIK3CA and PTEN expression and clinicopathological features

Clinicopathological features	*PIK3CA*			*PTEN*		
	<19 n (%)	*>20 n (%)*	*p*	*<19 n (%)*	*>20 n (%)*	*p*
Tumor grade						
I	0 (0)	2 (4.7)		2 (8.7)	0 (0)	0.079
II	4 (57.1)	19 (44.2)	0.877	12 (52.2)	11 (40.7)	
III	3 (42.9)	22 (51.2)		9 (39.1)	16 (59.3)	
Stage						
I	0 (0)	11 (25.6)	0.52	6 (26.1)	5 (19.2)	0.672
II	5 (83.3)	22 (51.2)		10 (43.5)	17 (65.4)	
III	1 (16.7)	10 (23.3)		7 (30.4)	4 (15.4)	
IV	0 (0)	0 (0)		0 (0)	0 (0)	
ER						
Positive	3 (42.9)	30 (69.8)	0.692	20 (87.0)	13 (48.1)	0.006*
Negative	4 (57.1)	13 (30.2)		3 (13.0)	14 (51.9)	
PR						
Positive	4 (57.1)	28 (65.1)	0.692	18 (78.3)	14 (51.9)	0.077
Negative	3 (42.9)	15 (34.9)		5 (21.7)	13 (48.1)	
Ki67						
<13%	0 (0)	2 (4.7)	1	0 (0)	2 (7.4)	0.493
>14%	7 (100)	41 (95.3)		23 (100.0)	25 (92.6)	
Disease relapse						
Yes	2 (28.6)	9 (20.9)	0.641	3 (13.0)	8 (29.6)	0.189
No	5 (71.4)	34 (79.1)		20 (87.0)	19 (70.4)	
Death						
Yes	3 (42.9)	2 (4.7)	0.016*	2 (8.7)	3 (11.1)	1
No	4 (57.1)	41 (95.3)		21 (91.3)	24 (88.9)	

*Statistically significant finding (p>0.05).

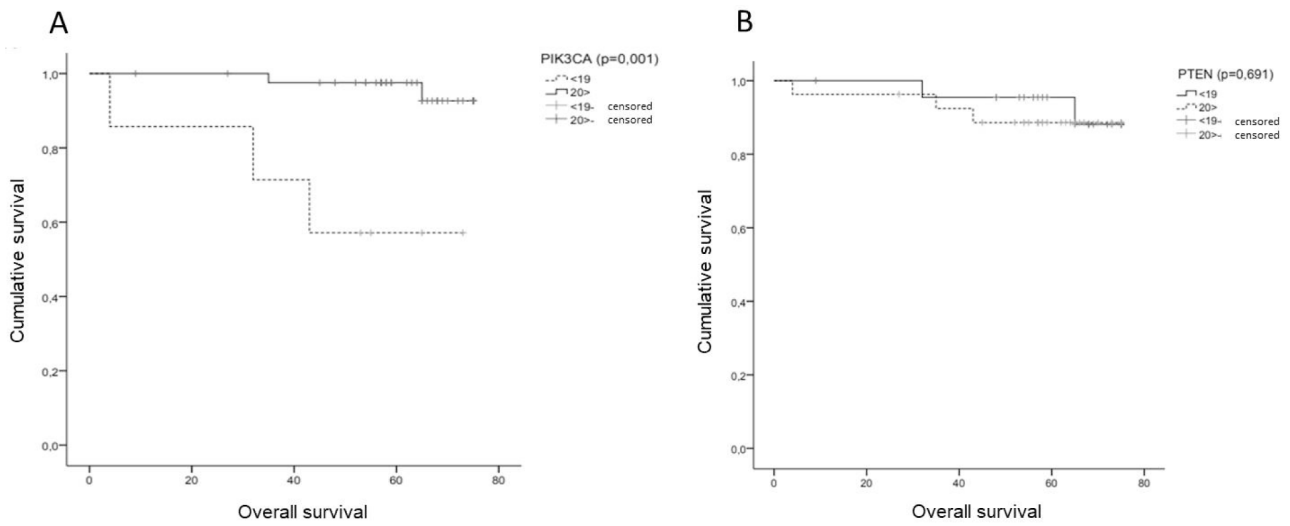

Figure 2. OS curve in months of breast cancer patients. Association between PIK3CA expression and overall survival **(A)** and association between PTEN expression and overall survival **(B)**.

the variables histological grade, staging, PR, Ki67 and relapse, no statistically significant correlations were observed (Table 2).

We found significant associations between the lowest expression of *PTEN* and the positivity of ER (p=0.006) and highest expression of *PIK3CA* and the lowest number of deaths (p=0.016) (Table 2). The overall survival analysis showed that patients with higher expression of *PIK3CA* had longer survival (p=0.001). The expression of *PTEN* had no impact on the lifetime of patients (Figure 2).

Discussion

Breast cancer has a very heterogeneous pathology of high biological complexity and variability in molecular and clinical patterns [3]. Currently there are four major groups, or molecular phenotypes which can classify the breast cancer: luminal A, luminal B, *HER2*-positive and basal-like. Each one of these classes comprises its own treatments and prognoses [12]. The subtype studied here, *HER2*-positive, has indication for therapy with the target drug trastuzumab, which has shown progressive therapeutic failures, for which there is still no substitute treatment or conclusive markers [5]. Some authors believe that *PTEN* deficiency and increased expression of *PIK3CA* may predict resistance to therapy for *HER2*-positive breast cancer by increasing the phosphorylation of *PI3K/AKT* which blocks the antiproliferative effect of trastuzumab [7,13,14].

In this study, lower expression of *PTEN* was observed in 46% of the cases. Loss of *PTEN* was observed in previous studies ranging from 19.2 to 43% [15-19]. However, there is much disparity between the methodology adopted in relation to the definition of lesser expression or loss of IHC expression of *PTEN*, making direct comparison of results difficult. In relation to *PIK3CA*, we found an increase in the expression in 86% of cases. Previous molecular studies report mutation frequencies in the gene ranging from 12 to 40% [16,18-23]. In addition, several studies comprise specific subgroups of breast cancer, such as metastatic breast carcinoma, or triple negative [17,18]. Few authors have examined the expression of *PIK3CA* in breast cancer by the IHC technique [11], and some have analyzed the marker expression in other types of neoplasms, such as esophageal, gastric cancer and lymphoma [25,26,27].

Lindberg et al (2011) and Fu et al (2014) observed that reduction of *PTEN* expression is associated with PIK/AKT signaling activation and ER expression reduction in breast cancer [28]. In our study, most patients with decreased *PTEN* expression were positive for ER. However, the parameters used to define loss of IHC expression were not the same. Moreover, according to Lazaridis et al (2019), who studied 1265 patients with *HER2*-positive breast cancer at an early stage, the investigation of *PTEN* expression loss had predictive power in breast cancer when analyzed by molecular methods. The authors also say that IHC does not completely translate the gene expression status and suggest that this could not be the best method for this analysis [19].

Regarding *PIK3CA*, our study showed a correlation between higher IHC expression and longer survival time of patients. The molecular study by Saikia et al (2018), found longer survival time in patients with mutated *PIK3CA* treated with traztuzumab and ER-positive [20], unlike Jensen et al

(2012), who found an inverse association between survival time and the presence of mutations in *PIK-3CA*. In both studies, no associations were observed between the molecular status of the gene and clinicopathological characteristics of the patients [16]. Papaxoinis et al (2015), Karakas et al (2013) and Saal et al (2005) found no significant association between *PIK3CA* mutational status and patient survival [8,22,23].

No immunohistochemical analysis of *PIK3CA* and survival time were found in samples similar to those studied.

The present study demonstrated a relationship between IHC overexpression of *PIK3CA* and the OS time of patients with *HER2*-positive breast cancer. Controversies in accessing [OR ASSESSING] the expression and function of *PTEN* and *PIK3CA*, as well as in the sample size, point to the need for methodological standardization and greater reproducibility in future research.

Conflict of interests

The authors declare no conflict of interests.

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