Int J Clin Exp Pathol 2016;9(12):12797-12806
www.ijcep.com /ISSN:1936-2625/IJCEP0037888

*Original Article*

# High expression of PIK3R1 (p85α) correlates with poor survival in patients with metastatic breast cancer

Yumei Yang[1], Mei Li[2], Yanshuang Li[1]

[1]*Department of Operating Room, The Second Affiliated Hospital of Harbin Medical University, Harbin, China;*
[2]*Department of Operating Room, The Hong Qi Hospital of Mudanjiang Medical University, Mudanjiang, China*

Received August 10, 2016; Accepted October 10, 2016; Epub December 1, 2016; Published December 15, 2016

**Abstract:** The phosphatidyl inositol-3 kinase (PI3K) pathway regulates downstream cellular processes including cell growth, cell survival, and cell migration. The expression of the PI3KR1 gene, encoding the regulatory subunit (p85α) of PI3K, and its effect on prognosis remains unclear in breast cancer patients with or without metastasis. Tissues from 186 cases of breast cancer were subjected to immunohistochemistry for PI3KR1. The relationship between PI3KR1 expression level to clinicopathologic variables including patients overall survival and metastasis-free survival was investigated. PI3KR1 was highly expressed in hormone-receptor negative breast cancer patients, and metastasis-free survival was significantly shorter in patients with metastasis than in patients without metastasis. Univariate and multivariate analyses confirmed that high PI3KR1 expression was an independent and significant factor predicting poor prognosis in patients with metastatic breast cancer. High PI3KR1 expression correlates with poor survival in patients with metastatic breast cancer.

**Keywords:** Metastatic Breast cancer, PI3K/AKT pathway, PI3KR1, overall survival, metastasis-free survival

## Introduction

Breast cancer is second most common cancer behind lung cancer, with more than 1,300,000 cases and 450,000 deaths reported each year worldwide [1, 2]. The prognosis of breast cancer worsens with cancer cell spread or metastasis to other sites. Metastasis inhibitors could prevent cancer cell metastasis thereby saving millions of lives worldwide [3].

The phosphatidylinositol-3-kinase (PI3K) pathway has been recognized as a key player in tumor progression and as the most commonly activated signaling pathway in human tumors [4, 5]. The PI3K pathway is initiated by extracellular signals triggering either growth-factor receptor or integrin pathways. Once activated, PI3K kinase produces phosphatidylinositol-3,4,5-trisphosphate by phosphorylating inositol lipids, which in turn activate the serine/threonine kinase AKT. Many signaling pathways are regulated by AKT, including the mammalian target of rapamycin (mTOR) pathway which controls cell apoptosis, proliferation, motility, and adhesion. Activation of the mTOR pathway may also result in endocrine or chemotherapy resistance in several tumors and worsen cancer-specific survival [6-15]. PI3Ks are divided (based on structural characteristics and substrate specificity) into Class I, II, and III PI3Ks [16-19]. Class IA PI3Ks regulating cell growth consist of a p110 catalytic subunit encoded by the PI3KCA gene and a p85 regulatory subunit encoded by the PIK3R1 gene [20]. The Class I PI3K heterodimer is activated upon association of the p85 subunit with upstream adaptor proteins or receptor tyrosine kinases. These interactions reduce the inhibitory activity of p85 on p110, thus allowing p110 to phosphorylate its lipid substrates and subsequently induce activation of downstream effector molecules. PI3KCA is frequently activated in many malignant tumors [21-26]. Studies on the PI3K pathway have mainly focused on PI3KCA, while the contribution of PI3KR1 to breast cancer development has not been fully elucidated.

In the present study, we assessed the relationships between immunohistochemically deter-

mined PI3KR1 expression and clinicopathological features, between PI3KR1 expression and clinicopathologic and biological variables in breast cancer patients with or without metastasis, and between PI3KR1 aberrant expression and overall survival (OS) and metastasis-free survival (MFS). To our knowledge, the present study is the first to investigate the immunohistochemical expression of PI3KR1 in relation to clinicopathological features and survival in breast cancer patients. Collectively, our findings may suggest a new therapy (i.e., PI3R1 inhibitors) that targets breast cancer through inhibition of the PI3K pathway.

## Materials and methods

### *Patients*

All participants provided their written informed consent to participate in this study. The study protocol was approved by the Ethics Committee of Harbin Medical University. Totally, 301 patients with primary breast cancer treated at the Second hospital affiliated to Harbin Medical University and the Hong Qi Hospital of Mudanjiang Medical University from January 1, 2006 to December 31, 2006 were enrolled in this study. Clinical data (including age, stage of primary breast cancer at diagnosis according to the sixth edition of the American Joint Committee on Cancer TNM Classification of Malignant Tumors, treatment history [including local and systemic therapies in adjuvant and metastatic settings], recurrence, and vital status) were available for 186 patients.

### *Breast cancer tissue samples*

All samples were collected from January 1, 2006 to December 31, 2006 at the Department of Pathology of the above two Hospitals. Each sample was analyzed immunohistochemically for expression of ER, PR, KI-67, P53, and HER-2 (rabbit polyclonal antibodies; Maxim Biotech, Fuzhou, China). Positivity was defined as staining of 10% of the nuclei in the invasive component of the tumor [27]. HER2 was scored according to current American Society of Clinical Oncology ASCO)/College of American Pathologists (CAP) criteria. Staining intensity was graded 0 and 1 (negative), 2 (indeterminate), and 3 (positive). Fluorescence in situ hybridization (FISH) was performed on all grade 2 samples. Samples with less than 2-fold-change in expression were regarded as negative and those with more than 2-fold increase were regarded as positive for gene amplification [28].

### *Construction of breast cancer tissue microarray (TMA)*

Briefly, a tissue arraying instrument was used to create holes in a receptive paraffin block and to acquire tissue cores from donor tissue blocks. A thin-walled needle (inner diameter, 2 mm) held in an X-Y precision guide was used for acquisition of each core sample. The core samples were retrieved from selected regions of the donor tissue blocks and extruded directly into the receptive tissue block at defined array coordinates. A solid steel wire was inserted into each needle to facilitate transfer of tissue cores into the receptive block. Sections (4-μm thickness) of the constructed array block were cut with a microtome. Blocks of samples from 186 surgical patients with breast invasive ductal carcinoma paired with samples from the corresponding normal areas of breast tissue were arrayed in triplicate (spots, 2 mm in diameter) in tissue microarrays and consecutive sections were placed in order on a set of slides.

### *Immunohistochemistry (IHC)*

The tissue sections were dried at 70°C for 3 h. After deparaffinization and hydration, sections were washed in phosphate-buffered saline (PBS; 3×3 min), treated with 3% $H_2O_2$ in the dark for 5-20 min to block endogenous peroxidase, washed in distilled water, washed in PBS (3×5 min), cooked in citrate buffer (pH 6.0) for antigen retrieval, treated with 300-500 ml of p85-alpha rabbit monoclonal antibody (diluted 1:200; Abcam, Hong Kong) solution at 4° overnight, washed in PBS (3×5 min), incubated with 300-500 ml of secondary antibody at room temperature for 30 min, washed in PBS (3×5 min), treated with 300-500 ml of diaminobenzidine (DAB) working solution at room temperature for 3-10 min, and then washed in distilled water.

### *Evaluation of PI3KR1 expression by IHC*

PI3KR1 protein expression was assessed by evaluating the average proportion of stained cells and intensity of staining in a series of 10

**Table 1.** Correlation between PIK3R1 expression and clinicopathological features

| Variables | No. of case | PIK3R1 | | |
|---|---|---|---|---|
| | | *High expression* | *Low expression* | *p value* |
| Age | | | | 0.661 |
| <50 | 106 | 35 | 71 | |
| ≥50 | 80 | 24 | 56 | |
| Tumor size | | | | 0.323 |
| pT1 | 66 | 22 | 44 | |
| pT2 | 113 | 34 | 79 | |
| pT3/pT4 | 7 | 4 | 3 | |
| Histology Grade | | | | 0.939 |
| G1 | 14 | 5 | 9 | |
| G2 | 52 | 16 | 36 | |
| G3 | 120 | 38 | 82 | |
| Lymph node status | | | | 0.802 |
| pN0 | 78 | 26 | 52 | |
| pN1 | 50 | 14 | 36 | |
| pN2/pN3 | 58 | 19 | 39 | |
| ER status | | | | 0.023 |
| Positive | 89 | 21 | 68 | |
| Negative | 97 | 38 | 59 | |
| PR status | | | | 0.147 |
| Positive | 118 | 33 | 85 | |
| Negative | 68 | 26 | 42 | |
| Ki67 status | | | | 0.358 |
| ≤15% | 89 | 30 | 59 | |
| 16%-30% | 64 | 22 | 42 | |
| >30% | 33 | 7 | 26 | |
| P53 status | | | | 0.190 |
| Positive | 152 | 45 | 107 | |
| Negative | 34 | 14 | 20 | |

randomly selected high-power fields (400× magnification). The proportion of positively stained tumor cells in a field was scored as 0 (none); 1 (<10%); 2 (10-50%); and 3 (>50%). The staining intensity in a field was scored as 0 (no staining); 1 (weak staining appearing as light yellow); 2 (moderate staining appearing as yellowish-brown); and 3 (strong staining appearing as brown). The staining index (SI) was calculated as: (averaged staining intensity score) × (proportion score). The log-rank test with regard to OS was used to determine the best cut-off value for PI3KR1 staining. Accordingly, an SI of 4 (a cut-off point) was used to distinguish between low (≤4) and high (>4) expression of PI3KR1. The staining of each sample was scored independently by two investigators without knowledge of the clinicopathological findings. Cases with discrepancies were re-evaluated by the original two pathologists and a senior pathologist until a consensus was reached.

*Identification of breast cancer subtypes*

Breast cancer subtype was defined by IHC receptor status using 2013 St. Gallen rational guidelines as follows: luminal subtype A (ER+/HER2-/Ki-67+, ≤14%, and PR+, >20%), HER-2 negative luminal subtype B (ER+/HER2-/Ki-67+, >14%, and PR+, ≤20%), HER-2 positive luminal subtype B (ER+/HER2+/Ki-67+ and PR+, ≤20%), HER-2 over-expressing subtype (ER-/HER2+ and PR-), and basal-like subtype (ER-/HER2- and PR-) [29, 30].

*Follow-up*

Clinical records were obtained from the follow-up clinic of the hospital. Clinical and pathological records of all patients in the study were reviewed periodically. Patients were followed up for history, physical examination, and routine laboratory and imaging investigations (every 3 months for the first 2 years, every 6 months for the next 1-2 years, and every year thereafter) at the Third Affiliated Hospital of the Harbin Medical University.

*Statistical analysis*

Statistical analysis was performed using SPSS 13.0 (SPSS, Chicago, IL, USA). OS was defined as the time from diagnosis of primary breast cancer to death or last follow-up; MFS was calculated from the date diagnosis to the date of metastasis. The correlations between PI3KR1 expression and patients' clinicopathological variables were analyzed by the $X^2$ test or Fisher's exact test. The Kaplan-Meier method was used to estimate OS. MFS differences according to PI3KR1 expression were analyzed using the log-rank test. The influence of variables on MFS was assessed using Cox univariate and multivariate regression analysis. The risk ratio and its 95% confidence interval were recorded for each variable. *P* values of <0.05 were considered statistically significant in all of the analyses.

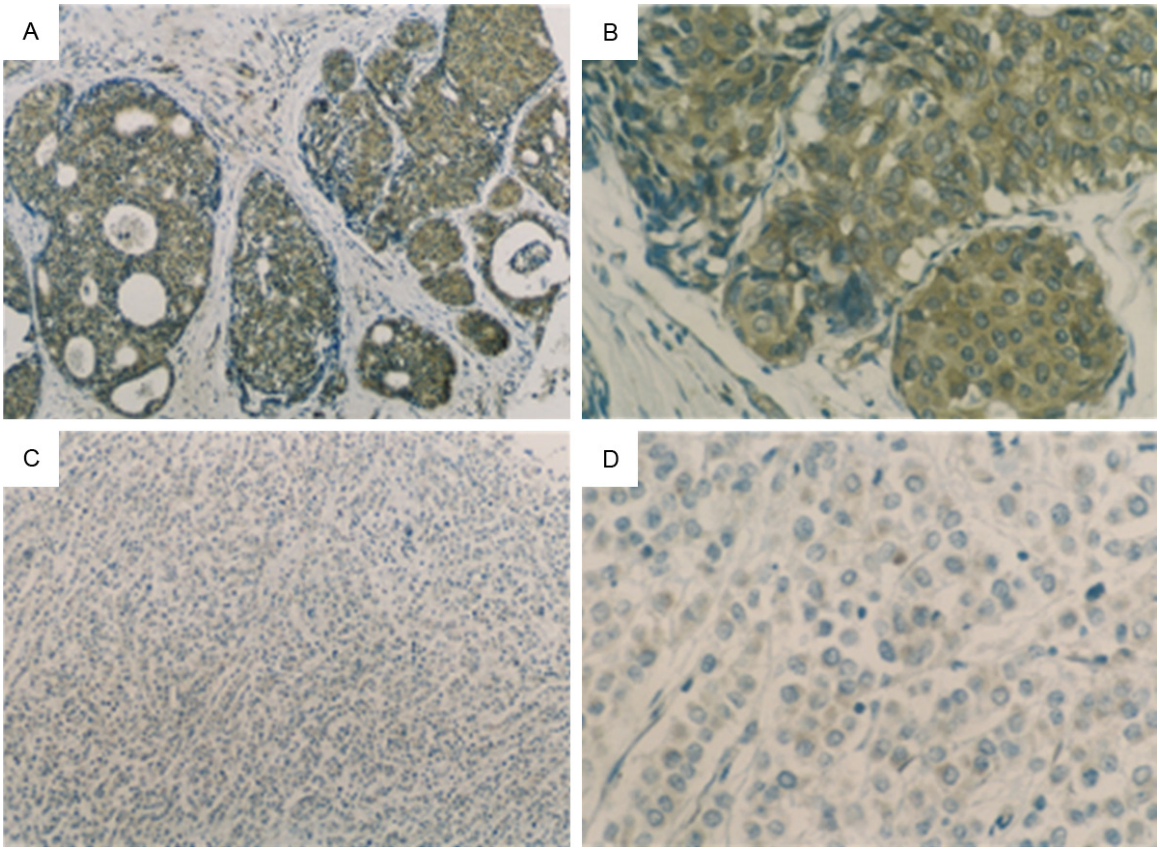


**Figure 1.** Immunohistochemical pattern of PIK3R1 expression in breast carcinoma. A: High PIK3R1expression specimen (×200). B: High PIK3R1expression specimen (×400). C: Low PIK3R1expression specimen (×200). D: Low PIK3R1expression specimen (×400).

## Results

### *Patient and tumor characteristics*

Among the 301 patients with primary breast cancer included in this study, 42 (14.0%) failed to complete the regular follow-up, 52 (17.3%) were lost to follow up because of a changed telephone number, and 21 (7.0%) had incomplete medical records. Our analysis of the factors associated with PI3KR1 expression was therefore conducted on the remaining 186 patients, including 66 (35.5%) patients with metastasis to the lung (n=32), bone (n=34), liver (n=5), and brain (n=2). The median age at diagnosis of primary breast cancer was 49 years (range, 31 to 75 years). At the time of study completion, 48 patients (25.8%) had died and 138 patients (74.2%) were still alive. All patients were followed until death or the study closing date (May 15, 2013). The primary lesions were classified as pT1 in 66 of the 186 patients (35.5%), pT2 in 113 (60.8%) patients, pT3/pT4 in 7 patients (3.8%), grade I in 14 patients (7.5%), grade II in 52 patients (28.0%), and grade III in 120 patients (64.5%). Lymph node metastasis was present in 108 patients (58.1%). The clinical characteristics of the study population are presented in **Table 1**.

### *PI3KR1 expression and its correlations with clinicopathological features*

PI3KR1 was located exclusively in the cytoplasm of tumor cells. PI3KR1 expression was high in 59 patients (**Figure 1A**, **1B**; **Table 1**) and low in 127 patients (**Figure 1C**, **1D**). Correlations between PI3KR1 expression and various clinicopathological features are summarized in **Table 1**. High PIK3R1 expression was found in 1 of 4 ductal carcinomas specimen in situ, 1 of 3 mucinous carcinomas, and 1 of 1 papillary carcinoma (**Table 4**). PIK3R1 expression was significantly correlated with hormone-receptor status but not with age, tumor size, histological grade, lymph node status, PR status, Ki67 status, and P53 status, and significantly higher in estrogen receptor (ER)-negative tumors than in ER-positive tumors (P=0.023; **Table 1**).

**Table 2.** Cox proportional hazards model of OS and MFS in 186 breast cancer patients

| variables | HR* | OS Univariate 95% CI | *p* value | HR* | OS Multivariate 95% CI | *p* value | HR* | MFS Univariate 95% CI | *p* value | HR* | MFS Multivariate 95% CI | *p* value |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Age | 1.024 | 0.995-1.053 | 0.102 | 1.025 | 0.994-1.057 | 0.112 | 1.000 | 0.976-1.025 | 0.988 | 1.005 | 0.979-1.031 | 0.707 |
| ER status | 0.878 | 0.495-1.558 | 0.657 | 1.192 | 0.536-2.650 | 0.666 | 0.864 | 0.535-1.379 | 0.552 | 1.214 | 0.620-2.378 | 0.572 |
| Her-2 status | 1.148 | 0.535-2.463 | 0.723 | 0.701 | 0.289-1.701 | 0.432 | 0.772 | 0.345-1.512 | 0.388 | 0.479 | 0.210-1.093 | 0.080 |
| PR status | 0.776 | 0.428-1.371 | 0.369 | 0..669 | 0.229-2.132 | 0.530 | 0.725 | 0.446-1.178 | 0.194 | 0.656 | 0.250-1.720 | 0.391 |
| P53 Status | 0.785 | 0.390-1.580 | 0.498 | 1.134 | 0.505-2.544 | 0.761 | 1.171 | 0.613-2.235 | 0.633 | 1.429 | 0.704-2.900 | 0.323 |
| KI67 Status | 1.455 | 0.820-2.582 | 0.200 | 1.312 | 0.690-2.492 | 0.408 | 1.127 | 0.697-1.821 | 0.626 | 1.058 | 0.619-1.808 | 0.836 |
| Tumor size | 2.667 | 1.531-4.646 | 0.001 | 2.465 | 1.342-4.527 | 0.004 | 1.690 | 1.064-2.684 | 0.026 | 1.760 | 1.050-2.949 | 0.032 |
| LN status | 2.392 | 1.644-3.480 | 0.000 | 2.368 | 1.594-3.519 | 0.000 | 1.626 | 1.219-2.168 | 0.001 | 2.037 | 1.493-2.779 | 0.000 |
| Gene subtype | 1.078 | 0.851-1.364 | 0.534 | 0.994 | 0.620-1.593 | 0.979 | 1.139 | 0.937-1.384 | 0.191 | 1.073 | 0.772-1.593 | 0.728 |
| Histology Grade | 1.492 | 0.889-2.503 | 0.129 | 1.304 | 0.748-2.273 | 0.394 | 1.586 | 1.026-2.451 | 0.038 | 1.412 | 0.898-2.221 | 0.135 |
| PIK3R1 status | 1.174 | 0.641-2.151 | 0.603 | 1.214 | 0.615-2.399 | 0.576 | 1.332 | 0.802-2.179 | 0.273 | 1.551 | 0.889-2.703 | 0.122 |

CI: confidence interval. *Hazard ratio of age (>50) versus age (≤50), ER positive versus negative, Her-2 positive versus negative, PR positive versus negative, P53 positive versus negative, KI67: (15%-30% & >30%) versus KI67 (≤15%), tumor size: pT2-4 & versus pT1, LN status pN2-3 versus pN1, Gene subtype: luminal B & Her-2 & triple negative versus luminal A, Histology Grade: G2 & G3 versus G1, high PIK3R1 expression versus low PIK3RI expression.

**Table 3.** Cox proportional hazards model of OS and MFS in 66 metastasis breast cancer patients

| Variables | HR* | OS Univariate 95% CI | *p* value | HR* | OS Multivariate 95% CI | *p* value | HR* | MFS Univariate 95% CI | *p* value | HR* | MFS Multivariate 95% CI | *p* value |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Age | 1.017 | 0.987-1.049 | 0.259 | 1.004 | 0.965-1.045 | 0.848 | 1.002 | 0.977-1.027 | 0.893 | 1.012 | 0.982-1.042 | 0.439 |
| ER status | 0.784 | 0.419-1.467 | 0.446 | 1.473 | 0.559-3.380 | 0.434 | 0.849 | 0.517-1.392 | 0.516 | 0.897 | 0.410-1.963 | 0.786 |
| Her-2 status | 1.431 | 0.618-3.314 | 0.402 | 2.227 | 0.668-7.417 | 0.192 | 0.834 | 0.389-1.785 | 0.640 | 1.044 | 0.390-2.798 | 0.932 |
| PR status | 0.794 | 0.419-1.506 | 0.480 | 0.976 | 0.271-3.512 | 0.971 | 0.926 | 0.554-1.547 | 0.768 | 0.540 | 0.197-1.480 | 0.231 |
| P53 Status | 0.891 | 0.405-1.961 | 0.774 | 0.777 | 0.254-2.379 | 0.658 | 1.159 | 0.559-2.242 | 0.661 | 1.382 | 0.597-3.202 | 0.450 |
| KI67 Status | 1.854 | 0.998-3.480 | 0.055 | 1.104 | 0.546-2.234 | 0.783 | 1.998 | 1.208-3.304 | 0.007 | 1.857 | 1.032-3.340 | 0.039 |
| Tumor size | 2.262 | 1.082-4.730 | 0.030 | 1.393 | 0.613-3.169 | 0.429 | 1.363 | 0.807-2.300 | 0.247 | 1.204 | 0.660-2.197 | 0.545 |
| LN status | 2.214 | 1.434-3.418 | 0.000 | 2.074 | 1.277-3.370 | 0.003 | 1.981 | 1.429-2.746 | 0.000 | 2.178 | 1.455-3.260 | 0.000 |
| Gene subtype | 1.123 | 0.874-1.444 | 0.365 | 1.220 | 0.684-2.177 | 0.501 | 1.072 | 0.876-1.313 | 0.499 | 0.825 | 0.515-1.322 | 0.424 |
| Histology Grade | 1.321 | 0.787-2.217 | 0.293 | 1.377 | 0.737-2.572 | 0.316 | 1.502 | 0.992-2.73 | 0.055 | 1.315 | 0.820-2.108 | 0.256 |
| PIK3R1 status | 2.561 | 1.272-5.516 | 0.008 | 1.576 | 0.753-3.301 | 0.228 | 2.897 | 1.607-5.223 | 0.000 | 2.996 | 1.549-5.681 | 0.001 |

CI: confidence interval. *Hazard ratio of age (>50) versus age (≤50), ER positive versus negative, Her-2 positive versus negative, PR positive versus negative, P53 positive versus negative, KI67: (15%-30% & >30%) versus KI67 (≤15%), tumor size: pT2-4 & versus pT1, LN status pN2-3 versus pN1, Gene subtype: luminal B & Her-2 & triple negative versus luminal A, Histology Grade: G2 & G3 versus G1, high PIK3R1 expression versus low PIK3RI expression.

**Table 4.** PIK3R1 expression and histologic type of breast cancer

| Histologic type | High PIK3R1 expression (%) |
|---|---|
| Ductal carcinoma in situ | 1 of 4 (25) |
| Invasive carcinoma | 58 of 182 (31.9) |
| Invasive ductal carcinoma | 56 of 180 (31.1) |
| Mucinous carcinoma | 1 of 3 (33.3) |
| Papillary carcinoma | 1 of 1 (100) |

*Univariate and multivariate analyses of PI3KR1 expression and clinicopathological variables*

The result of univariate analysis and multivariate analysis of OS and MFS using Cox regression models is shown in **Tables 2**, **3**. Tumor size and lymph node metastasis were considered significant prognostic predictors of poor OS and MFS in 186 patients (**Table 2**), while PI3KR1 expression and other clinicopathological factors had no significant effect on OS and MFS. In 66 patients with metastasis, univariate analysis and multivariate analysis identified lymph node metastasis (P=0.000, 0.003, respectively) as a significant prognostic predictor of OS and PI3KR1 expression (P=0.000, 0.001, respectively), lymph node status (P=0.000, 0.000, respectively), and KI67 status (P=0.007, 0.039, respectively) as significant prognostic predictors of MFS (**Table 3**).

*Kaplan-Meier survival analysis*

Of the 186 study patients, OS and MFS might be shorter in patients with high PI3KR1 expres-

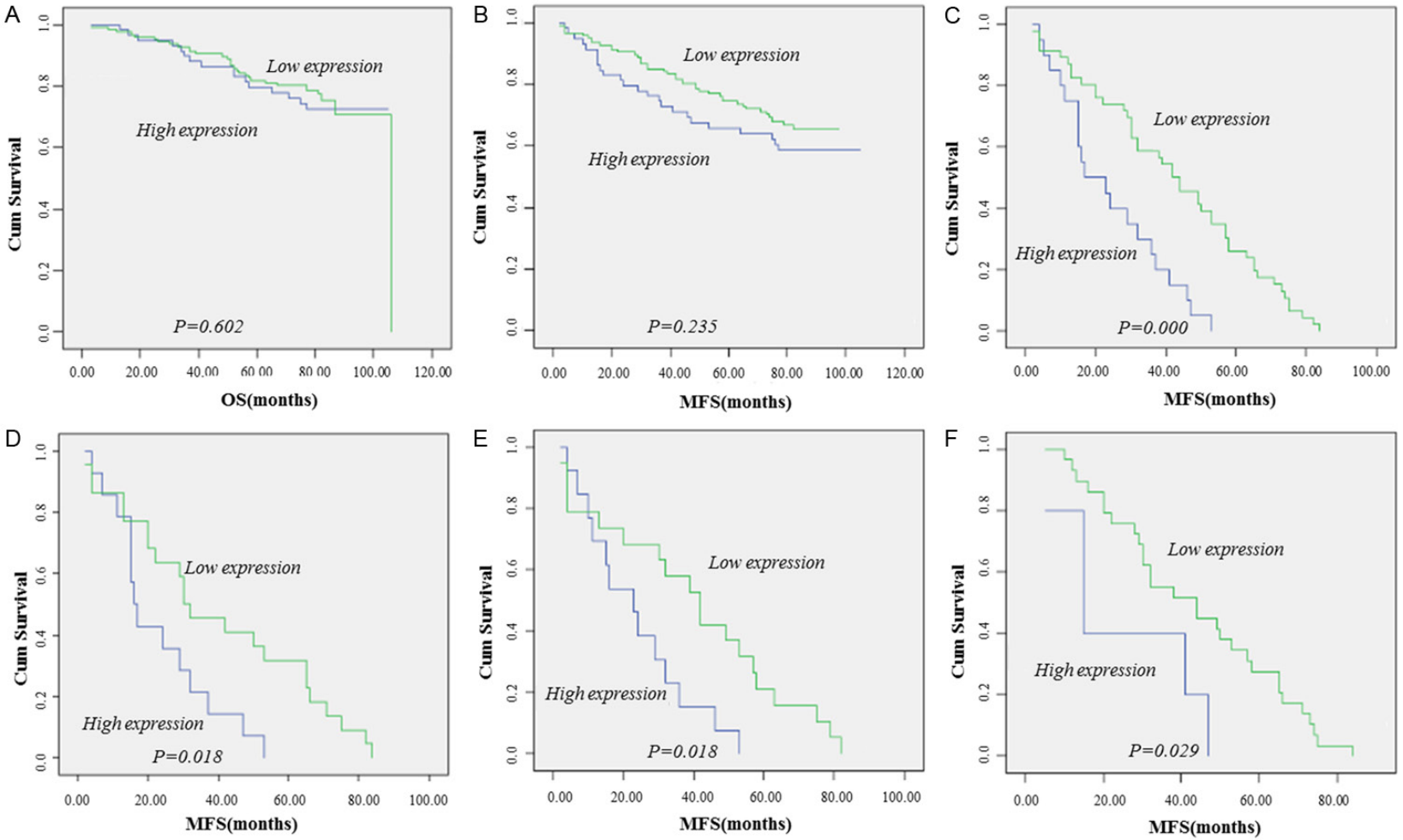


**Figure 2.** Survival status of breast cancer patients. A: OS of patients in high PIK3R1 expression group vs. low PIK3R1 expression group. B: MFS of patients in high PIK3R1 expression group vs. low PIK3R1expression group. C: MFS of metastasis patients in high PIK3R1 expression group vs. low PIK3R1 expression group. D: MFS of patients with ER negative and metastasis in high PIK3R1 expression group vs. low PIK3R1 expression group. E: MFS of lung metastasis patients in high PIK3R1 expression group vs. low PIK3R1 expression group. F: MFS of bone metastasis patients in high PIK3R1 expression group vs. low PIK3R1 expression group.

sion tumors than those with low PI3KR1 expression tumors (P=0.602, 0.235, respectively; **Figure 2A**, **2B**). MFS of metastasis patients with high PIK3R1 expression was shorter than those with low PI3KR1 expression (P=0.000; 0.018, 0.018, 0.029, respectively; **Figure 2C-F**).

## Discussion

The function of PI3KCA has been well studied in various solid tumors, including breast tumor. However, the role of PI3KR1 over-expression and its significance in primary breast cancer remains unknown. To clarify this issue, the expression of PI3KR1 protein was assessed immunohistochemically in tumor specimens from 186 breast cancer patients with or without metastasis. We found that in breast cancer patients with metastasis, MFS was significantly shorter in patients with high PI3KR1 expression tumors than in those with low PI3KR1 expression tumors, and that high PI3KR1 expression was an independent factor indicating poor survival in patients with metastasis.

High PI3KR1 expression as a marker for poor prognosis in patients with breast cancer could be confirmed from this observation. PI3KR1 expression was high in 59 of 186 (31.7%) breast carcinoma patients. It has been reported that PI3KCA activation in the PI3K pathway may lead to p110 protein over-expression, resulting in poor prognosis in patients with malignant breast and colon tumors [31-35]. Interestingly, our investigation found that OS and MFS tended to be poorer in patients with high PI3KR1 expression (P=0.602, 0.235, respectively; **Figure 2A**, **2B**). Although the difference between these two groups was not statistically significant, a study with a larger cohort and longer follow-up time is warranted to clarify this finding. On the other hand, in the subgroup of 66 patients with metastatic breast cancer, MFS rates were significantly shorter in patients with high PI3KR1 expression tumors (P=0.000, **Figure 2C**). In the 66 metastasis patients with ER-negative, survival was significantly shorter in patients with high PI3KR1 expression (P=0.018; **Figure 2D**). The poorer prognosis of patients with ER-negative disease relative to patients with ER-positive disease may partly be due to the activation of the PI3K/AKT pathway caused by PI3KR1 over-expression. MFS was also shorter in patients with high PI3KR1 expression lung metastasis or bone metastasis (P=0.018, 0.029, respectively; **Figure 2E**, **2F**), suggesting that PI3KR1 over-expression plays a positive role in metastasis development in breast cancer, especially in ER negative breast cancer. Considering its association with unfavorable prognosis, PI3KR1 may be a potential target to inhibit activation of the PI3K/AKT pathway in patients with advanced breast cancer.

PI3K pathway activation depends on a critical molecular balance between the regulatory p85 and catalytic subunits p110. Complete depletion of p85 results in significantly increased apoptosis due to reduced PI3K signaling, whereas haploin sufficiency of PI3KR1 can result in increased PI3K signaling [36, 37]. The p85 regulatory subunit exerts both positive and negative effects on PI3K signaling in tumorigenesis. Decreased PI3KR1 expression in human solid tumors such as colon and liver cancers indicates that p85 has tumor suppressor properties [38]. However, in the present study, the presence of the p85 regulatory subunit increased signaling. The main function of the p85 subunit is to bind and stabilize the p110 catalytic subunit and inhibit its activity in the PI3K pathway [39, 40]. Oncogenic mutations of p85 reduce the inhibition of p110, which leads to unchecked constitutive activity of the PI3K/AKT pathway and thereby oncogenesis [41, 42]. In this way, over-expression of PI3KR1 can account for the poorer prognosis of patients with tumors.

In the present study, our analysis of the correlation between PI3KR1 expression and various clinicopathological features showed that PI3KR1 expression was significantly associated with ER-negative breast cancer. We also found that PI3KR1 expression was higher in ER-negative breast cancer than ER-positive breast cancer (**Table 1**; P=0.023). The data indicate that PI3KR1 might be a useful marker for classifying breast cancer into prognostic subtypes. PI3KR1 expression was not associated with other clinicopathological factors including the new gene subtype described in the 2013 St. Gallen Expert Consensus Panel Recommendation on breast cancer. Why PI3KR1 expression is higher in ER-negative breast cancer is unexplained and deserves further investigation. The number of patients with histologic types of PI3KR1 expressing breast

tumors other than ductal carcinomas was small in the present study. PIK3R1 expression was high in 1 of 4 ductal carcinomas in situ, 1 of 3 mucinous carcinomas, and 1 of 1 papillary carcinoma (**Table 4**). These results indicate that PI3KR1 is not only expressed in ductal carcinomas but also in other types of breast cancers. PI3KR1 was also overexpressed in 20 patients with histological grade G3, 29 with G2, and 10 with G1, indicating that PI3KR1 expression is high mainly in low and moderately differentiated tumors.

The main prognostic factors identified by univariate and multivariate analysis were tumor size, lymph node metastasis, and histological grade in our 186 patients (**Table 2**) and PI3KR1 expression, tumor size, lymph node metastasis, and high Ki-67 expression in our patients with metastatic breast cancer (**Table 3**). These results strongly confirm that PI3KR1 expression status can be used as a biological marker of poor survival in patients with breast cancer (especially metastatic breast cancer). Other studies identified androgen receptor, HER-2, and other biological markers as prognostic factors of breast cancer [43, 44]. However, these factors are less important than traditional prognostic parameters such as tumor size and lymph node metastasis and have a supportive role clinically. Therefore, to improve the effectiveness of traditional prognostic parameters in patients with breast cancer, pathological and biological features including PI3KR1 expression should be evaluated together in future research.

Some limitations of our study need to be mentioned. Our study's follow-up rate was low and cohort size was small. Outcome as a function of PI3KR1 expression may have been influenced by the number of participants in this study. Second, patients were followed up for less than 10 years, which may lead to bias in mortality estimates. Further studies with additional patients and a longer follow-up time will be needed to obtain more definitive results.

In conclusion, our study suggests that high PI3KR1 expression is associated with poor survival outcome in breast cancer patients with metastasis. PI3KR1, involved in PI3K/AKT signaling, might be an attractive new target of breast cancer treatment. A large cohort study focused on the mechanisms underlying the effect of PI3KR1 expression on tumor behavior should be conducted.

## Disclosure of conflict of interest

None.

**Address correspondence to:** Yanshuang Li, Department of Operating Room, The Second Affiliated Hospital of Harbin Medical University, 246 Xuefu Road, Harbin, China. Tel: 86 (451) 86605361; Fax: 86 (451) 86605361; E-mail: wuhuei112@163.com

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