

RESEARCH ARTICLE

Relation between Ki-67, ER, PR, Her2/neu, p21, EGFR, and TOP II- α Expression in Invasive Ductal Breast Cancer Patients and Correlations with Prognosis

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Abstract

The aim of the present study was to investigate the expression of the transcription factor Ki-67, ER, PR, Her2/neu, p21, EGFR, and TOP II- α in the tumor tissue of patients with invasive ductal carcinoma (IDC); in addition, we examined correlations between these markers. Two hundred and sixteen IDC patients, who were not previously been treated with chemo- or radiotherapy, were included in the study. All tumors were grade I-III. Expression of molecular markers was determined by immunohistochemical analysis on paraffin-embedded tissue sections. Follow-up data were collected for 3 months to 10 years and analyzed for tumor recurrence, survival time, and prognostic risk factors. We determined Ki-67 expression correlates with the expression of ER, PR, HER-2, EGFR, and TOP- α , as well as lymph node involvement, high tumor grade, lymphovascular invasion, high tumor stage, and high TNM stage in IDC. Positive Ki-67 expression was a risk factor for rapid tumor recurrence and may help tumor progression, leading to poor prognosis in IDC. Ki-67 was directly correlated with EGFR, TOP II- α , lymph node involvement, high tumor grade, lymphovascular invasion, high tumor stage, and high TNM stage in the hormone receptor subtypes of breast cancer. In triple negative breast cancer, Ki-67 correlated with TOP II- α . Expression of Ki-67 correlated with that of ER, PR, HER-2, EGFR, TOP II- α , and p21. In addition, the biomarker Ki-67 has a role as a prognostic factor and indicates a poor prognosis in IDC.

Keywords: Ki-67 - invasive ductal breast cancer - prognosis

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Introduction

Breast cancer (BC) is the most frequent malignancy in women, and the main cause of death in women aged 35-55 years old. It is a great burden to society. Despite the improvement of diagnostic methods and chemotherapeutic regimens, the 5-year overall survival (OS) rates of patients significantly depend on the stage of BC and were 56.2% in Ukraine and 88.0% in the USA from 2000-2005 (Cancer in Ukraine, 2010). Clinical characteristics such as age, tumor size, menstrual status, morphology, and lymph node status of the tumor are traditionally the most important prognostic factors. However, research on the tumor's molecular features led to a greatly improved prognosis of the disease course. Most molecular markers, such as Ki-67, p21, EGFR, and TOP II- α , determine the ability of cells to grow malignantly (Hanahan, 2000). For BC, the estrogen and progesterone receptors (ER and PR) were the first predictive molecular markers. Patients who are determined to be positive for these steroid

hormone receptors (HRs) generally have high sensitivity to hormone therapy. The next marker included in clinical practice was HER-2. A positive HER-2 status in patients with BC correlates with high sensitivity to targeted therapy with trastuzumab. These biomarkers play an important role in clinical practice by individualizing treatment and determining adequate chemotherapeutic schemes for patients. However, 25-50% of ER and PR positive tumors are resistant to hormone therapy (Klijn, 1991), and tumors with HER-2 overexpression do not always respond to trastuzumab therapy. In addition, about 20% of patients with BC are negative for all three markers mentioned above, and their tumors are more resistant to traditional therapy schemes.

Ki-67 is a nuclear non-histone protein, and a proliferation marker expressed in the G1, S, G2, and M-phases of the cell cycle, but not the G0 phase. Ki-67 is a large nuclear protein that is thought to be involved in cellular functions such as cell cycle regulation, ribosomal RNA processing, and organizing DNA, or to

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have a structural role within the nucleus (Szelachowska et al., 2012). Patients with Ki67 expression have a worse outcome in a wide variety of malignancies such as BC (Pathmanathan et al., 2013), sarcomas (Sorbye et al., 2012), lymphomas (Kanavaros et al., 2001), and gliomas (Arshad et al., 2010). Multiple published studies have observed a significant association between Ki-67 expression and clinical outcome for BC (Agboola et al., 2013; Meattini et al., 2013; Inwald et al., 2013); although, opposite findings are also reported. Cell cycle progression is regulated by cyclin-dependent kinases and their specific inhibitors (Sherr et al., 1999), including p21WAF1/CIP1 (p21), which is a universal inhibitor of cyclin-dependent kinases (Xiong et al., 1993). It halts cell cycle progression in response to various stimuli (Bartek et al., 2007). A p21 deficiency may increase carcinogenesis in combination with other oncogenic mutations (Martín-Caballero et al., 2001; Poole et al., 2004; Carnero et al., 2004; Yang W et al., 2005). Some studies indicate that p21 can negatively modify the activity of other positive regulators of the cell cycle (CDKs), and the increasing expression of p21 in cancer is consistent with the theory that abnormal cells increase p21 expression in an attempt to “brake” the process of cellular proliferation at the G1 checkpoint (Thor et al., 2000). Ashrafi found that expression of cyclin D1 and p21 was increased in BC tissues compared to normal mammary glands (Ashrafi et al., 2012). TOP2 α (Top II- α) reduces DNA supercoiling and twisting and is important for chromosome segregation and condensation in dividing cells. Its expression is highest in the G2/M phase of exponentially growing cells, and some studies found that overexpression predicts shorter disease free survival (DFS) and OS (Midura-Nowaczek et al., 2013). High Top II- α RNA levels are significantly associated with shorter metastasis-free survival in node-negative BC, but associated with a high frequency of pathological complete response in anthracycline-treated patients (Mrklič et al., 2013).

It has been confirmed that the expression of Ki-67, p21, EGFR, and TOP II were altered in BC tissues compared to normal mammary glands, and this correlated with prognosis. It is still unclear whether a correlation exists between these markers and ER, PR, and Her2/neu status. Our study aimed to investigate expression of Ki-67, ER, PR, Her2/neu, p21, EGFR, and TOP II- α in the tumor tissue of patients with invasive ductal carcinoma (IDC), as well as to analyze correlations between these markers. Our results indicated that Ki-67 expression correlates with ER, PR, HER-2, EGFR, TOP II- α , lymph node involvement, high tumor grade, lymphovascular invasion, high tumor stage, and high TNM stage in IDC. Positive Ki-67 expression was a risk factor for rapid tumor recurrence and may help tumor progression leading to a poor prognosis in IDC.

Materials and Methods

Patient selection and data collection

Specimens were obtained from 216 patients with IDC in The Third Affiliated Hospital of Southern Medical University from September 2005 to September 2010.

Patients who received chemotherapy or radiotherapy before surgery and those who underwent surgery for other types of carcinomas were excluded. The clinicopathological features of these patients were assessed. This study was approved by the Institutional Review Board of the Key Lab of Breast Disease Research in Guangdong Province. All patients provided written informed consent.

Immunohistochemical and image analysis

IDC samples and paired paracancerous tissues from 216 patients were analyzed by immunohistochemistry and relative optical density (OD) calculations, along with normal control tissues. Briefly, formalin-fixed and paraffin-embedded sections (4 μ m thick) were cut and mounted on 3-aminopropyltriethoxysilane-treated slides. Slides were routinely deparaffinized with xylene and rehydrated in ethanol washes. Non-enzymatic antigen retrieval was performed by microwave heat treatment 3 times for 7 min each in 0.01 M sodium citrate buffer (pH 6.0). Endogenous peroxidase activity and nonspecific background staining were blocked by incubating the slides in methanol containing 0.3% H₂O₂ for 30 min. The slides were subsequently washed with PBS for 15 min and then incubated with primary antibody (anti-Ki-67, anti-p21, anti-EGFR, anti-TOP II-, In vitrogen, Camarillo, CA) for 1 h at 37°C. The working dilution of the primary antibody was 1:50. The sections were rinsed with PBS for 15 min and then incubated with ENVISION+ rabbit/horseradish peroxidase for 45 min. After washing with PBS for 15 min, the final products were visualized using the 3-amino-9-ethylcarbazole substrate system, and the sections were counterstained with Mayer's hematoxylin for 20 s before mounting. Both positive and negative controls were performed for each section. Normal rabbit serum IgG was used in place of the primary antibody as a negative control. All experiments were performed in duplicate.

Result analysis

The stained sections were observed under a light microscope (400 \times) (Olympus, Japan), and digital images were analyzed with the HPIAS-2000 multimedia colored pathology image analysis system. To determine the OD and the percentage of positive protein in the staining area, 10 complete sections without overlapping fields were randomly selected for quantitative analysis. Each section was evaluated by 3 different researchers, and the mean value of each slice was calculated.

Follow-up

After surgery, patients were regularly followed at the outpatient clinics. Every 2 to 3 months patients were monitored by abdominal and chest CT and ultrasound in the first two postoperative years, and then every 5 to 6 months in the following years. In the final years, an annual abdominal CT scan was performed. CT or MR imaging of the abdomen, pelvis, and chest was performed and a bone scan was used as monitoring method to define suspicious lesions demonstrated on CT. Survival time was calculated starting with the month the surgical intervention took place to the month of final follow-up. Complete data were recorded for patients that died from IDC during this

period; while, cases of survival and loss to follow-up were recorded as censored data.

Statistical analysis

The data are presented as the means \pm S.D. Student's t-test and Pearson's chi-square (χ^2) test were used for statistical analyses. The logistic regression model was used to identify independent risk factors. The survival rate of IDC patients was analyzed with Kaplan-Meier survival curves and the log-rank (Mantel-Cox) statistical method. A multivariate Cox regression model was used to identify the risk factors for DFS. Correlation analysis was used to determine the correlation between Ki-67 OD and the prognosis of IDC patients. SPSS 19.0 for Windows was used for the statistical analyses. A value of $p < 0.05$ was considered statistically significant.

Results

Clinical, pathological, and immunohistochemical results

A total of 216 female patients with a mean age of 47.21 ± 0.75 years (range: 21 to 75 years) and invasive ductal BC were involved in this study. A high TNM stage (T3, T4), according to the international clinical criteria of TNM staging, was assigned to 32.6% ($n=70$) of cases and a high histological grade (grade III) was found in 45.6% ($n=103$). Eight cases with bone metastases; six cases with lung metastases; three cases with bone and lung metastases; two cases with lung and liver metastases; and one case of bone, lung, and liver metastases were also found. Of the cases, 26% ($n=56$) had vascular invasion, 42 had tumors with diameters > 5 cm, 18 had chest wall and skin invasion, and 53% ($n=92$) were lymph node positive. Immunohistochemically, 62.8% ($n=136$) were ER positive, 53.7% ($n=116$) were PR positive, 41.4% ($n=89$) were HER-2 positive, 62.5% ($n=135$) were Ki-67 positive, 27.3% ($n=59$) were EGFR positive, 57.8% ($n=125$) were p21 positive, and 63.7% ($n=138$) were TOP II- α positive (Figure 1).

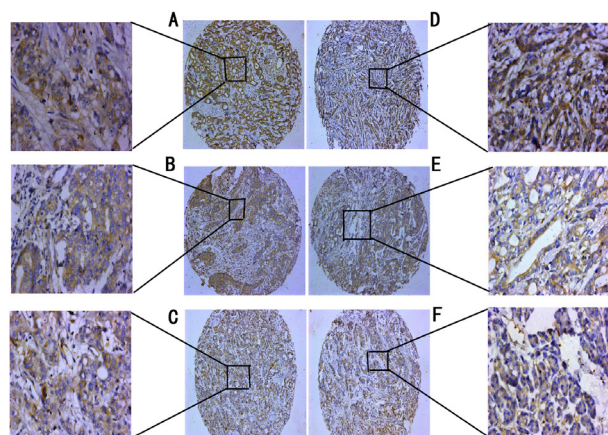


Figure 1. Microscopic Immunohistochemistry Pictures of Tissue Microarrays of IDC Tissues Representing Expression of Ki-67, ER, PR, Her2/neu, p21, and TOP II- α . A) Ki-67 positive; B) ER positive; C) PR positive; D) Her2/neu positive; E) p21 positive; F) TOP II- α positive

Table 1. The Relation between Ki-67 Positive and Other Clinico-pathological Factors

Variables	Ki-67 (+) (n=135)	Ki-67 (-) (n=81)	P	R
ER Status				
Positive	80	76	<0.01	-0.263
Negative	55	5		
PR Status				
Positive	51	67	<0.01	-0.274
Negative	84	14		
HER-2 Status				
Positive	67	17	<0.01	0.214
Negative	68	64		
EGFR Status				
Positive	43	2	<0.01	0.368
Negative	92	79		
P21				
Positive	97	42	0.057	0.128
Negative	38	39		
TOP II- α Status				
Positive	128	15	<0.01	0.547
Negative	7	66		
Lymph Node				
Positive	92	31	<0.01	0.219
Negative	43	50		
Tumor Grade				
High	94	16	<0.01	0.342
Low	41	65		
Lymphovascular Invasion				
Positive	47	3	<0.01	0.233
Negative	88	78		
Tumor Stage				
High (T3,T4)	17	4	<0.01	0.148
Low (T1,T2)	118	77		
TNM Stage				
High (Stage 3,4)	65	5	<0.01	0.211
Low (Stage 1,2)	70	76		

Table 2. Distribution of Original 216 Invasive Ductal Breast Cancer Cases into four Groups According to Hormone Receptor and HER-2 Status

Group	Ki-67	
	Positive (%)	Negative (%)
Group A ($n=95$) (HR positive and HER-2 negative)	41 (43.6%)	54 (56.4%)
Group B ($n=56$) (HR positive and HER-2 positive)	37 (65.2%)	19 (34.8%)
Group C ($n=37$) (HR negative and HER-2 positive)	33 (82.9%)	4 (17.1%)
Group D ($n=28$) (HR negative and HER-2 negative)	21 (73.7%)	7 (26.3%)

*HR: hormone receptor; HR positive: ER and/or PR positive

Analysis of Ki-67 and its correlation with other clinicopathological factors

The Ki-67 status could be determined in 135 (62.5%) of the original 216 patients, which correlated with other clinicopathological factors (Table 3). There was an inverse correlation between Ki-67 positive expression and ER and PR positive expression ($p < 0.01$; $r = -0.263$ and $p < 0.01$; $r = -0.274$, respectively). As shown in Table 1, there was a direct correlation between Ki-67 and HER-2, EGFR, and TOP II- α positive expression, as well as lymph node

positive, high grade tumor, lymphovascular invasion, high tumor stage (T3,T4), and high TNM (stage 3,4) (all $p < 0.01$; $r = 0.214, 0.368, 0.547, 0.219, 0.342, 0.233, 0.148, 0.211$, respectively). No correlation was found between Ki-67 positive expression and P53 positive status ($p = 0.057$; $r = 0.128$).

Comparison of Ki-67 positive rates between different groups

According to the HR and HER-2 status, the original 216 cases of invasive ductal BC were divided into four groups (Figure 2) to compare the positive rates of Ki-67 between different groups. Patients who were HR positive (ER and/or PR positive) and HER-2 negative were in Group A (n=95). Patients who were HR positive and HER-2 positive were in Group B (n=56). Patients who were HR negative (ER and PR negative) and HER-2 positive made up Group C (n=37). Patients who were HR negative and HER-2 negative made up Group D (n=28) (Table 2).

The expression of Ki-67 was higher in the HER-2 positive group (group C) (82.9%) than the other 4 groups: 73.7% in the triple negative group (group D), 65.2% in group B (HR positive, Her-2 positive), and 43.6% in group A (HR positive) (Table 4). There were significant differences in the Ki-67 positive rates in group A vs B, A vs

C, A vs D, and B vs C (all $p < 0.05$) (Table 3). There were no significant comparisons in group B vs D and group C vs D ($p > 0.05$). After comparing positive rates of Ki-67 between groups, only the HR positive group (group A) showed significant differences with all the groups.

Expression of Ki-67 in different groups and their relationship with other clinicopathological factors

In the HR positive group (Group A), there were direct correlations between Ki-67 positive rates and expression of EGFR and TOP II- α , lymph node involvement, high grade tumor, lymphovascular invasion, and high TNM stage (all $p < 0.01$; $r = 0.264, 0.519, 0.236, 0.313, 0.356$, and 0.273 , respectively) (Table 4). There was no relationship between expression of Ki-67 and p21 and high tumor stage in this group ($p = 0.057$ and 0.098 ; $r = 0.181$ and 0.164 , respectively). In the HR positive and HER-2 positive group (Group B), the Ki-67 positive status directly correlated with TOP II- α , lymph node involvement, and high tumor grade ($p < 0.01$, $r = 0.629$; $p < 0.05$, $r = 0.283$; $p < 0.05$, $r = 0.273$, respectively) (Table 4). There were no relationships between expression of Ki-67 and p21, EGFR, lymphovascular invasion, high tumor stage, and high TNM in this group.

The expression of biomarker Ki-67 was not related with other clinicopathological characteristics (p21, EGFR, TOP II- α , lymph node involvement, high grade tumor, lymphovascular invasion, high tumor stage, and high TNM stage) in the HER-2 positive group (Group C). Only TOP II- α had a significant correlation with Ki-67 positive status in the HR negative and HER-2 negative (triple

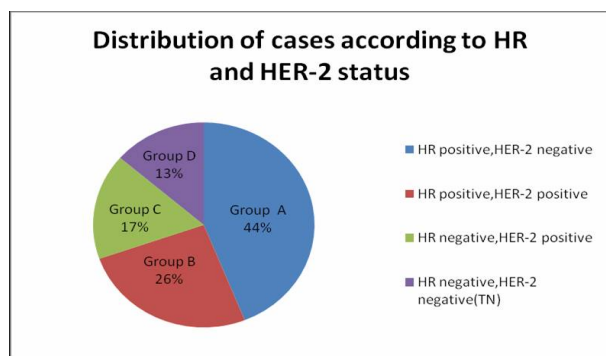


Figure 2. Distribution of Cases into Four Groups According to Hormone Receptor and HER-2 Status. HR, hormone receptor; HR positive, ER and/or PR positive; HR negative, ER and PR negative; TN, triple negative

Table 3. Comparison of Ki-67 Positive Rates between Groups

	χ^2	P
Group A vs B	8.869	<0.01
Group A vs C	27.26	<0.01
Group A vs D	16.596	<0.01
Group B vs C	8.873	<0.05
Group B vs D	3.051	0.173
Group C vs D	1.037	0.421

Table 4. Relationship of Ki-67 with Other Clinicopathological Factors in Different Groups

	P21	EGFR	TOP II-a	LN	High grade	LVI	High T stage	High TNM
Group A								
Ki-67 positive(n=41)	28(68.3%)	6(15.7%)	32(78.3%)	26(-63.50%)	24(-57.50%)	15(-37.70%)	7(-16.10%)	15(36.5%)
R	0.181	0.264	0.519	0.236	0.313	0.356	0.164	0.273
P	0.067	<0.01	<0.01	<0.01	<0.01	<0.01	0.097	<0.01
Group B								
Ki-67 positive(n=37)	22(-58.60%)	9(-25.10%)	32(-87.40%)	27(73.2%)	22(-60.50%)	17(-45.20%)	12(31.7%)	20(52.8%)
R	0.107	1.186	0.629	0.283	0.273	0.158	0.119	0.087
P	0.368	0.151	<0.01	<0.05	<0.05	0.241	0.437	0.482
Group C								
Ki-67 positive(n=33)	21(62.3%)	18(54.3%)	27(-81.70%)	22(65.2%)	20(-60.60%)	12(37.5%)	11(32.1%)	16(49.8%)
R	-0.002	0.213	0.155	0.117	0.251	-0.107	0.058	0.092
P	1	0.252	0.371	0.467	0.131	0.467	1	0.709
Group D								
Ki-67 positive(n=21)	14(67.6%)	16(78.5%)	19(88.7%)	8(38.3%)	17(82.8%)	5(25.2%)	4(-21.20%)	6(-27.20%)
R	-0.124	0.281	0.383	0.264	0.318	0.158	0.253	0.273
P	0.695	0.112	<0.05	0.118	0.091	0.6759	0.182	0.175

*LN, lymph node; LVI, lymphovascular invasion

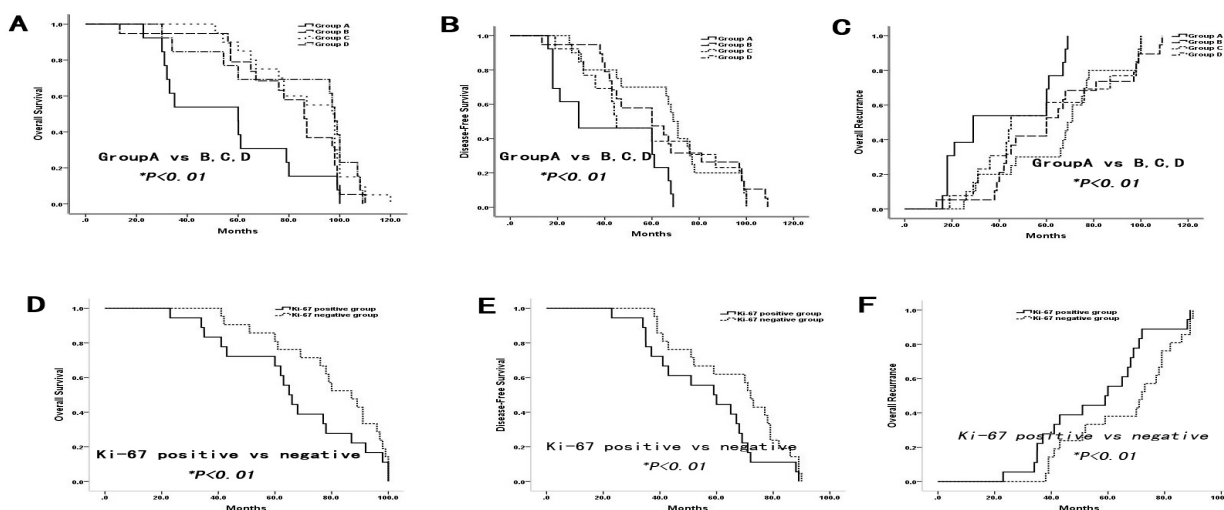


Figure 3. Overall Survival, Disease-free Survival, and Overall Occurrence Curves for IDC Patients with Different ER, PR, HER-2, and Ki-67 Expression Statuses. (A-C) Group A had a shorter OS and DFS, and higher OR than groups B, C, and D (all $p < 0.01$). (D-F) The Ki-67 positive group had a shorter OS and DFS, and higher OR than the Ki-67 negative group (all $p < 0.01$)

negative) tumor group (Group D) ($p < 0.05$, $r = 0.383$).

Patient demographics and outcomes

To address whether HR, HER-2 status, and Ki-67 positive rates were associated with the patients' IDC progression, we analyzed the relevant clinical information and correlated the data between the four groups. As shown in Figure 3A-C, there was a significant inverse correlation between HR and HER-2 status and the OS, DFS, and overall recurrence (OR) for patients in the four groups ($p = 0.001$). After comparing OS, DFS, and OR between the four groups, we found that only the HR positive group (group A) showed significant comparisons with all the groups. Group A had a shorter OS and DFS (median, OS: 60 months; DFS: 37.1 months), and higher OR than group B (median, OS: 85 months; DFS: 59.5 months), group C (median, OS: 96 months; DFS: 68 months), and group D (median, OS: 97 months; DFS: 51 months) (all $p < 0.01$).

We further analyzed the relationship between Ki-67 expression and IDC patients' prognoses. The results indicated there was a significant inverse correlation between Ki-67 expression and patients' OS, DFS, and OR between the Ki-67 positive group ($n = 135$) and Ki-67 negative group ($n = 81$). The Ki-67 positive group had shorter OS and DFS (median, OS: 65 months; DFS: 58.5 months), and a higher OR than the Ki-67 negative group (median, OS: 81 months; DFS: 71.5 months) as shown in Figure 3D-F.

Discussion

Ki-67, a nuclear non-histone protein, is a nuclear antigen expressed in the G1, S, G2, and M-phases of the cell cycle, but not the G0 phase. Ki-67 is a large nuclear protein (395kDa) and has been hypothesized to be involved in several different cellular functions such as cell cycle regulation, ribosomal RNA processing, and organizing DNA, or to have a structural role within the nucleus (Hendrayani et al., 2013). Ki-67 has been

considered a proliferative marker associated with tumor invasiveness and recurrence (Salehi et al., 2009). Several studies have investigated and considered Ki-67 as a prognostic factor for BC (Tarhan et al., 2013; Inwald et al., 2013; Mrklič et al., 2013; Eom et al., 2013). Many previous studies have demonstrated Ki-67 expression is associated with poor differentiation of tumors and large tumor size in BC (Ryu et al., 2012; Loehberg et al., 2013; Synnestvedt et al., 2013).

In this study, we investigated the expression of Ki-67 and its relationship with other clinical and pathological parameters and the expression of other molecular markers in 216 female invasive ductal BC patients with a mean age of 47.21 ± 0.75 years (range: 21 to 75 years). Patients were from a single academic tertiary care center and the clinical significance of Ki-67 as a prognostic marker of BC was evaluated. Of these cancers, 135 were Ki-67 positive using immunohistochemical techniques, which directly correlated with poor prognostic features such as lymph node involvement, high grade tumor, lymphovascular invasion, high tumor stage, and high TNM stage, and had statistically significant value in identifying high risk women with invasive ductal BC. Previous studies reported that high expression of Ki-67, detected by immunohistochemistry, is the strongest individual prognostic factor of tumor recurrence or death in high tumor stage diseases (Chan et al., 2012; Kamineni et al., 2013; Ferguson et al., 2013).

This study showed an inverse correlation between expression of the HRs (ER and PR) and Ki-67, but a direct correlation between Ki-67 and HER-2 expression. Numerous studies have shown that expression of Ki-67 had a negative correlation with ER expression (Dede et al., 2013; Galea et al., 2013; Ibrahim et al., 2013; Bjerre et al., 2013). A recent study analyzed 356 BC samples retrospectively and evaluated the association between expression of Ki-67 and some clinicopathological parameters. They found that Ki-67 expression was correlated with tumor size, grade, and lymph node

metastases, but was inversely correlated with HR expression, which supports the findings from this study.

The patients were divided into four groups according to HR and HER-2 status. Patients who were HR positive (ER and/or PR positive) and HER-2 negative formed Group A. Patients who were HR positive and HER-2 positive were assigned to Group B. Patients who were HR negative (ER and PR negative) and HER-2 positive became Group C. Patients who were HR negative and HER-2 negative were in Group D. The expression levels of Ki-67 were compared between the different groups and then with the other clinical and pathological parameters, to analyze their role in the progression of invasive ductal BC. The highest expression of Ki-67 was in the HER-2 positive Group C.

Our study supports molecular subtype of BC by gene expression profiles, in which BC cases are classified into ER positive (Luminal A and B) and ER negative (HER-2 positive, triple negative) groups. Different types of tumors vary in their biology, disease outcome, and responses to therapy. In triple negative and non-triple negative BC, the ER status may play a more important role than HER-2 status to predict the prognosis. HER-2 positive tumors that are HR negative are traditionally associated with a poor prognosis. However, recent studies reported that treatment with trastuzumab may eliminate the disparity in disease outcomes (Chan et al., 2013; Lemieux et al., 2013; Perissinotti et al., 2013). In the HR positive tumor group, Ki-67 expression directly correlated with lymph node metastases, lymphovascular invasion, high tumor grade, and high TNM stage in this study. These findings are in agreement with other reports that also revealed Ki-67 was associated with lymph node metastases frequently in all types of BC (Agboola et al., 2011; Chen et al., 2013; Tawfik et al., 2013). In this study, lymph node metastases and a high tumor grade also correlated with expression of Ki-67 directly in HR positive and HER-2 positive groups.

To address whether positive expression of the HRs, HER-2, and Ki-67 were associated with progression in IDC patients, this study analyzed relevant clinical information and correlated the data between four groups. We found there was a significant inverse correlation between the HR and HER-2 statuses and the OS, DFS, and OR of patients in all four groups. After comparing the positive rates of Ki-67 expression between groups, only the HR positive group (group A) was significantly different than all the groups (Table 3). The results of Kaplan-Meier survival curves also showed that only group A had significantly shorter OS and DFS, and higher OR than the other groups. These findings indicate that the Ki-67 positive rates correlate with progression in IDC patients. A specific therapy for triple negative BC is lacking; although specific treatment strategies may be developed as further biological characterizations occur. In this study, 13.6% (n=29) of cases were triple negative BC; among them, 76.7% were Ki-67 positive, which directly correlated with expression of TOP II- α .

In conclusion, Ki-67 expression correlates with that of ER, PR, HER-2, EGFR, and TOP II- α , as well as lymph node involvement, high tumor grade, lymphovascular invasion, high tumor stage, and high TNM stage in IDC.

Positive Ki-67 expression is a risk factor for rapid tumor recurrence and may lead to tumor progression with a poor prognosis in IDC. Ki-67 directly correlates with EGFR and TOP II- α expression, lymph node involvement, high tumor grade, lymphovascular invasion, high tumor stage, and high TNM stage in the HR subtype of BC. In triple negative BC, Ki-67 correlates with TOP II- α .

Acknowledgements

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