

The potential of ki67 and p53 assessment in development of individualized targeted therapy in breast cancer patients

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Key words

Breast cancer • P53 • Ki67

Summary

Introduction. Despite the improvement of diagnostic methods and chemotherapeutic regimens in breast cancer, overall 5-year survival significantly depends on the stage of the disease. Over expression of tumor suppressor gene p53 and the marker for cellular proliferation Ki67 in breast cancer may have prognostic significance.

Methods. We evaluated 675 patients diagnosed with breast cancer at UF Health Jacksonville between January 2000 and June 2007 with up to 5-year follow up. The aim of the study was to determine whether immunohistochemical (IHC) assessment of Ki67 and p53 may predict outcome, the ‘hazard’ of dying. Cox’s proportional hazards models were used to control for age (< 50 vs. ≥ 50), race (white vs. other), lymph node group (negative vs. positive), ER (estrogen receptor) group (negative vs. positive), PR (progesterone receptor) group (negative vs. positive), and tumor type.

Results. When only p53 was considered in the model, the hazard of dying was significantly higher for p53 positive compared to p53 negative (HR = 1.32, 95 % CI 1.02, 1.70, $p = 0.036$). When only ki67 was considered in the model, the hazard of dying was significantly higher for ki67 positive compared to ki67 negative (Hazard ratio = 1.64, 95 % CI 1.08, 2.49, $p = 0.021$). Neither of the two markers, nor their interaction was significant when all variables were considered in the model.

Discussion. This study confirms the expression of p53 and Ki67 as strong individual indicators of patient outcome. However, when controlling for the other variables, the two markers are not independent predictors. Future studies that will include these markers might help design targeted therapy.

Introduction

Breast cancer is a major public health problem for women across the globe, being the most common type of cancer in women. Despite the improvement of diagnostic methods and chemotherapeutic regimens, overall 5-year survival of patients significantly depends on the stage of the disease. However, over the last decades, understanding of the tumor biology has been greatly improved by molecular research that allows application of a tumor’s molecular features for selection of personalized therapy and prediction of short and long term therapy¹.

Traditionally, there has been a division of breast cancer into hormone receptor positive and negative groups to guide treatment with endocrine and chemotherapy, re-

spectively. However, within the group of hormone receptor positive patients, a subgroup of patients appeared to respond poorer to this somewhat targeted therapy, with early relapses and poorer survival². Studies have shown that Ki-67 can be used for subtyping and distinguishing breast cancer and is therefore of greatest importance, considering the implications for treatment protocols, especially when combined with other markers such as p53^{2,3}. In addition, utilizing immunohistochemistry as surrogate marker for molecular testing (gene profiling) to sub-classify breast cancer is an important factor in terms of cost containment.

Ki67 is an immunohistochemical marker of proliferating cells which is widely used in various types of malignancy to assess proliferation rate. It is a fairly large

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nuclear protein (395kD) that is present during all active cell cycle phases, except for G0⁴. Naturally, proliferation status correlates tightly with tumor aggressiveness, and therefore, Ki67 labeling index is a commonly used prognostic indicator in breast cancer. In DCIS (Ductal Carcinoma in Situ), Ki67 positivity is associated with a higher risk of developing DCIS local recurrence after breast conserving therapy.

P53 is a tumor suppressor gene which regulates cell cycle progression in response to various stimuli; alteration of p53 function is seen in tumor development and progression in various organ systems. Positivity for these markers generally infers a worse prognosis, greater probability of failure with endocrine therapy in hormone receptor positive patients and poorer survival^{5,6}.

An Iranian study suggested that Ki67 may have more significant prognostic strength in terms of survival than p53; however, this study was fairly small, and a larger scale study is needed to substantiate these findings⁷.

There is also an urgent need to improve prognostic classifiers in breast cancer. Most recent decisions for breast cancer patients are made on the basis of prognostic and predictive factors. Molecular studies of breast cancer continue to unveil the biological heterogeneity of the disease which has opened new perspectives for personalized therapy. Overexpression of tumor suppressor gene p53 and the marker for cellular proliferation Ki67 in breast cancer may have prognostic significance.

The aim of our study was to investigate the correlation between Ki67 and p53 positivity with patient outcome in a large retrospective study of 675 patients with 5 year follow up, to potentially predict patient outcome and open new horizons in the development of individualized targeted therapy.

Materials and methods

We evaluated 675 patients diagnosed with breast cancer with 5 year follow up at UF Health Jacksonville between January 2000 and June 2007. The expression of Ki67 and stability of p53 were determined by immunohistochemistry. The aim of the study was to determine whether immunohistochemical (IHC) assessment of Ki67 and p53 may predict outcome.

The primary outcome variable was the 'hazard' of dying. Hazard is the instantaneous probability of dying given that patients have survived up to a given point in time or the risk for death at that moment. First, three Cox's proportional hazards models were fit: one with p53, ki67, and interaction p53*ki67 as predictors, one with only p53 predictor, and one with only ki67 as predictor. Second, models were fit to determine the joint effects of p53 group and ki67 group of patients, controlling for age (< 50 vs. ≥ 50), race (white vs. other), lymph node group (negative vs. positive), ER (estrogen receptor) group (negative vs. positive), PR (progesterone receptor) group (negative vs. positive), and tumor type (DCIS group vs. other, Lobular carcinoma vs. other, Infiltrating ductal carcinoma vs.

other, and other tumors vs. DCIS+Lobular carcinoma + Infiltrating ductal carcinoma). The best subset selection method was performed to assess the best predictive model using all the other variables. The criterion used to determine the "best" subset is based on the global score chi-square statistic. For two different models, each having the same number of explanatory variables, the model with the higher score chi-square statistic is considered to be better. Then, each of the markers p53 and ki67 were added in the best model previously found.

Results

Patients' demographic and baseline characteristics are listed on Table I. Seventy percent of the patients were 50 years or older, and 53 % were white (53 %). P53 was negative for 74 % of the sample, and Ki67 was negative for 43 % of the patients. Assessment of positive results was based on the standard of practice (for p53 scoring >10 % nuclear staining was considered positive, for Ki67 > 20 % is positive, 10 to 20 % borderline and 1 to 9 % negative)^{8,9}. Figure 1 presents patterns of staining for Ki67 and p53.

First, the joint effect of p53, ki67, and the interaction between p53 and ki67 on the probability of survival was assessed. The reference level for both markers was chosen to be the "negative" level. None of the two markers, nor their interaction was significant ($p = 0.050$, $p = 0.242$, and $p = 0.252$, respectively). When only p53 was considered in the model, the hazard of dying was significantly higher for p53 positive compared to p53 negative (HR = 1.32, 95 % CI 1.02, 1.70, $p = 0.036$). Figure 2 is the survival plot that contains two curves, one for p53 positive and one for p53 negative. It can be seen that, overall, the probability of survival is higher for p53 negative compared to p53 positive. When only ki67 was considered in the model, the hazard of dying

Tab. I. Patients' demographic and characteristics (N = 675).

Variable	Category	Count (%)
Age	50 years or older	515 (76)
Race	White	355 (53)
	Hispanic	18 (3)
P53	Positive	175 (26)
Ki67	Negative	292 (43)
	Borderline	90 (14)
	Positive	293 (43)
Tumor	DCIS	86 (13)
	IDC	533 (79)
	Lobular carcinoma	34 (5)
	Other	22 (3)
	Non-emergent	211 (33)
ER	Negative	166 (30)
PR	Negative	213 (39)
ER and PR	Negative	163 (30)

DCIS = Ductal Carcinoma in Situ, IDC = Invasive Ductal Carcinoma

Fig. 1. Immunohistochemistry Ki67 and p53 staining pattern in negative and positive breast cancer.

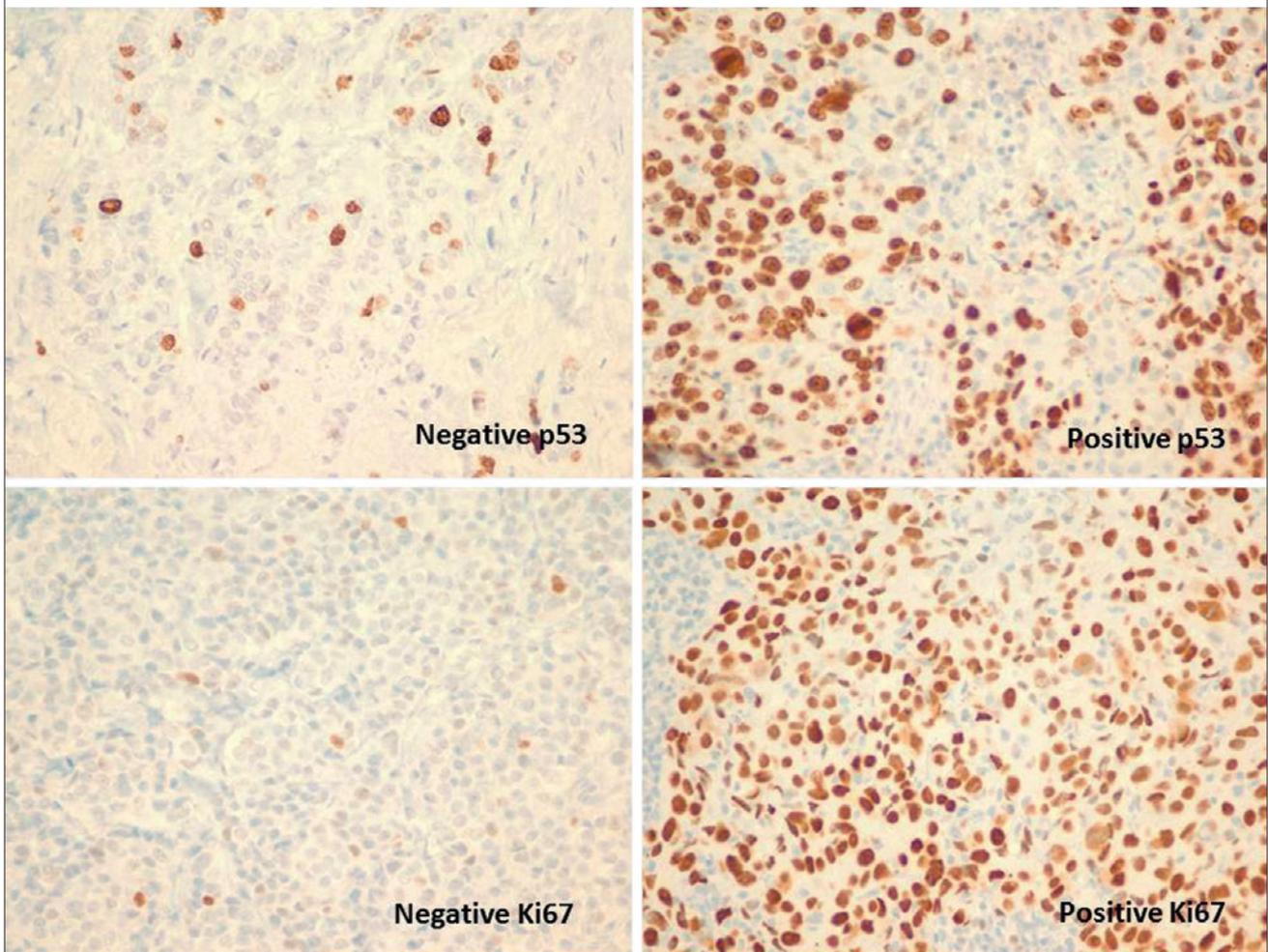


Fig. 2. Survival curves for p53 group.

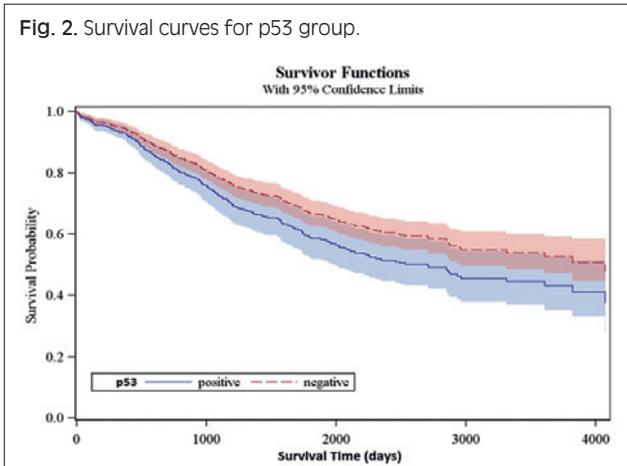
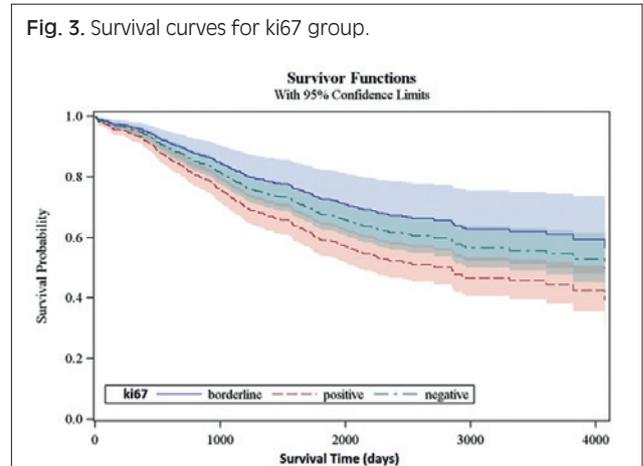


Fig. 3. Survival curves for ki67 group.



was significantly higher for ki67 positive compared to ki67 negative (Hazard ratio = 1.64, 95 % CI 1.08, 2.49, $p = 0.21$). There was no difference between ki67 borderline compared to ki67 negative (Hazard ratio = 0.82, 95 % CI 0.53, 1.26). Figure 3 presents the survival curves for ki67 groups.

Using the best subset selection method, the “best” model selected was the model including race ($p = 0.14$), lymph node group ($p = 0.013$), PR group ($p = 0.18$), DCIS group ($p = 0.06$), and LC group ($p = 0.22$) as significant predictors. When entered in the model described above, p53 ($p = 9.65$) and ki67 ($p = 5.77$) are no longer signifi-

cant. The hazard ratio of dying was 1.00 (95 % CI 0.72, 1.41) for p53 positive compared to p53 negative, and 1.22 (95 % CI 0.76, 1.96) for ki67 positive compared to ki67 negative.

Discussion

Several studies have shown the cumulative value of including p53 and Ki67 immunohistochemical staining when assessing breast cancer prognosis and risk-stratifying patients. Positivity for these markers generally infers a worse prognosis (e.g. greater probability of failure with endocrine therapy in hormone receptor positive patients)^{2,10}. Sarode et al. reported high Ki67 and p53 overexpression were characteristic of HER2 and triple negative subtypes of breast cancer¹¹. While Ki67 has been established as an important prognostic indicator, there seems to be a lack of uniformity in reporting Ki67 values, with different reasons, such as different techniques, cut off values, etc¹². In addition, intratumoral heterogeneity of proliferation can make it very difficult to assess an accurate Ki67 proliferation rate, depending on adequate tissue sampling¹². Standardization of Ki67 reporting values may help strengthen the value of Ki67 as a prognostic factor.

An Iranian study suggested that Ki67 may have a more significant prognostic strength in terms of survival than p53⁶; however, this study was fairly small, and a larger scale study would be indicated to substantiate these findings. Other studies demonstrated that the combination of p53 and Ki67 is more accurate than Ki67 alone in predicting the prognosis for patients with hormone receptor positive and Her2-negative breast cancer⁴.

Our results confirm that both Ki67 and p53 are significantly associated with an adverse clinical outcome in terms of reduced overall survival. When adjusting for clinic-pathological parameters such as race, lymph node status, ER and PR status, the association between outcome and p53 /Ki-67 status is no longer significant, indicating that these variables are confounders. This correlates with a study from Cheang et al. reporting that Ki67 was an independent prognostic factor for luminal B type of breast carcinoma, advocating the use of a surrogate IHC panel including ER/PR/HER2 and Ki67 for biological subtyping, independently of standard clinic-pathologic parameters such as age, lymph node status, tumor size and grade¹³. Our results indicate that both Ki67 and p53 are single prognostic indicators that may be evaluated separately from other clinic-pathological parameters.

Individually, Ki-67 and p53 are valuable prognostic factors in the setting of breast cancer that can also aid in subtyping different types of breast cancer. However, in our study, they are no longer significant predictors when both markers were entered in the same model, emphasizing their independence as prognostic indicators and

indicating that they should be evaluated separately when assessing a panel of immunohistochemical stains as surrogate markers for tumor biology.

Conclusions

This study confirms the expression of p53 and Ki67 as strong single predictors of patient outcome and survival. This in the future might help to design studies for targeted therapy development and improving patient care.

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