EVALUATION OF THE EFFECTS OF BIOLOGICAL PROGNOSTIC AND PREDICTIVE FACTORS ON SURVIVAL OF BREAST CANCER PATIENTS

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Abstract- The molecular basis of metastatic potential of human breast carcinoma cells can be useful information to determine the practical implications in the diagnosis, determining prognosis and treatment of breast cancer. The aim of this study was to identify predictors of aggressive biological behavior and metastatic potential in breast carcinoma among a number of intrinsic biomarkers of tumor cells. We used routine formalin fixed, paraffin embedded tumor samples; sections were stained immunohistochemically to determine the expression of estrogen receptor (ER), progesterone receptor (PR), HER2/neu, Ki67, p53 and cathepsin D in 66 breast carcinoma patients. The result of the quantitative immunohistochemical assays were correlated with clinical and histological data such as patient age, tumor size, axillary lymph node status, tumor grade, the therapeutic regimens and survival rates. Univariate analysis revealed a statistically significant relation between tumor size and overexpression of p53, and between tumor grade and PR status, p53 status and Ki67. In multivariate analysis the independent factors predicting for tumor grade were Ki67 and PR status. Among patients with ER expression, negative p53 or Ki67 status, tumors with lower grades and negative axillary lymph nodes (or < 4 involved lymph nodes), there was a higher survival rate (either disease free or overall); however, relationship was not statistically significant, most probably due to the low number of studied patients. In conclusion, Ki67 was an independent factor to predict tumor grade in our study; the use of this proliferation activity marker in routine approach to patients with breast cancer is recommended, at least to evaluate the accuracy of tumor grading by mitotic count.

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Key words: Breast carcinoma, immunohistochemistry, prognostic factor, predictive factor, Ki67

INTRODUCTION

The mortality reported for human breast carcinoma is mainly from metastatic breast carcinomas (1) and due to this, a more accurate knowledge on the cells could be beneficial in the practical implications of the diagnosis of breast cancer and also determining the prognosis and treatment of metastasis ones.

The process of tumor spread is a dynamic process, progressing through many intracellular molecular changes. These changes may results in genetic alterations and overexpression of genes which normally regulate cell proliferation and differentiation, such as hormone receptors, growth factors, oncoproteins and tumor suppressor genes.

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These intrinsic metastatic potentials of tumor cells may be the reason for the failure in determining the prognosis of breast cancer in some individuals based solely upon risk factors such as that tumor size, grade and axillary lymph node.

During the past several years an increasing number of biologic factors have been identified in patients with breast cancer (2) but the results reported about them vary widely. Many of them were reported to interfere with the mechanisms of proliferation and differentiation of tumor cells and so promote tumor growth. Others have been reported to reflect the aggressiveness of tumor (3-7). Only some of these factors were listed in a recent classification of prognostic and predictive factors of breast cancer (2) and are considered in the routine examination of breast cancer patients; the role of the others is still controversial (1, 4) and needs more studies to be done.

In this study we assessed the interrelationship of the expression of hormone receptors (ER, PR), oncogene products (HER2/neu), proliferation marker (Ki67), tumor suppressor gene product (p53) and cathepsin D in a series of invasive breast cancers to elucidate the role of these factors in tumor spread.

MATERIALS AND METHODS

We used the follow up data of a series of patients with breast carcinomas who underwent surgery along with adjuvant treatment in the Cancer Institute, Imam Khomeini Hospital, between 1991 and 1998. These patients were participants of an international multi-centre trial evaluating the effectiveness of the combinations of adjuvant hormonal therapy and chemotherapy (ABC trial).

We intended to use the follow-up data of all of the 110 patients who had previously entered the mentioned trial but due to some deficiencies in the data (unavailability of appropriate paraffin blocks for IHC examination, lost to follow-up patients, etc.) only 66 patients matched our inclusion criteria to enter the present study. The date of the primary diagnosis was considered as the start of the follow-up period and patients were followed up until breast cancer related death or last clinical contact in 2004.

All patients had been treated by lumpectomy or modified radical mastectomy and also received adjuvant chemotherapy, ovarian suppression and/or radiotherapy. The allocation of the patients to different treatment options were done randomly, taking into account their individual parameters. However, all of them received tamoxifen.

At the preliminary stages of the trial (1991-1993), enrollment was performed without any specific attempt to determine ER status, but following the routine use of ER to prescribe hormonal treatment in the patients. The trialist, in the first few years of the study, decided that only ER+ patients should be included in the trial. In the present study, ER status was determined blindly without any knowledge of the patients' previous ER test.

To undertake the analysis, the patients were divided by age ($\leq$ 50 years and > 50 years), tumor size ($\leq$ 2 cm and > 2 cm in diameter) and the axillary lymph node status. Based on the number of positive axillary lymph nodes, the patients were divided into three groups: a) Negative, b) one to three positive nodes and c) four or more positive nodes. To determine tumor grade The Nottingham modification (9) of the Bloom-Richardson grading system was used.

IHC examination on formalin fixed paraffin embedded blocks samples were used to determine the status of the biologic markers and therefore, 3µm sections were cut for immunohistochemical staining. In the IHC test, the following monoclonal antibodies, each from Zymed laboratories Inc. were also obtained and used:

1. Monoclonal mouse antiER, Clone: 1D5, Isotype: IgG1-Kappa
2. Monoclonal mouse antiPR, Clone: PR-2C5, Isotype: IgG1-Kappa
3. Mouse antihuman cellular phosphoprotein P53, clone: BP53.12, Isotype: IgG2a, Kappa
4. Monoclonal mouse anti-C-erbB2, Clone: CB11, Isotype: IgG,
5. Monoclonal mouse anti-Ki67, Clone / PAD: 7311, Isotype IgG1
6. Cathepsin D mouse monoclonal antibody, clone 1C11, Isotype: IgG1.

The immunostaining results were interpreted by a pathologist using a light microscope. Immunoreactivity for ER and PR was graded as

negative and positive according to the H-Score (8). For HER-2/neu (9), tumors were considered positive when at least 10 percent of tumor cells had partial or complete membranous staining. For p53 and Ki67 (1) a cutoff score of 10% of cells with nuclear staining, and for cathepsin D (10) a cutoff score of 10% of cells with coarse granular cytoplasmic staining were used. Known positive breast carcinoma samples were used as positive control.

In statistical analysis, overall survival time was calculated as the interval from the date of diagnosis to the last clinical control or death; disease free survival time was calculated as the interval between the date of diagnosis to the metastasis and/or recurrence or last clinical contact. For univariate analysis, Kaplan-Meier method and log-rank test was used to analyze the differences between groups. For multivariate analysis, regression method was used to examine several parameters simultaneously. *P* values < 0.05 were considered significant.

RESULTS

Table 1 shows the association between traditional prognostic factors and immunohistochemically determined expression of hormone receptors, p53, Ki67, HER2/neu and cathepsin D in each tumor. p53 status was clearly associated with tumor size (P = 0.045) and there was a statistically significant correlation between tumor grade and PR (P = 0.042), Ki67 (P = 0.001) and p53 (P = 0.008). Table 2 summarizes the clinical, histopathological and immunohistochemical data of the 66 breast cancer patients as well as the influence of the studied data on patient survival rates in univariate analysis.

Among 66 studied patients, 21.2% died with a median survival time of 44.4 month (range 12 to 98 months), 78.8% of the patients had withdrawn alive from follow-up and had a maximum survival of 117.5 months. Most of the patients were older than 50 years (69.7%).

From the studied tumors, 28.8% were less than 2 cm in diameter; 30.3% of the patients were identified as axillary lymph node negative and 69.7% as node positive. With regards to histological grade, there were 34.8% low grade, 59.1% intermediate grade and 6.1% high grade tumors. Hormone receptor assessment by IHC study showed that there was 40.9% ER negative and 59.1% ER positive tumors. PR status was equally distributed. HER2/neu negative tumors were 60.6% and HER2 positive were 39.4%; 72.2% were p53 negative and 27.3% were p53 positive. A high proliferation rate as measured by Ki67 expression was found in 54.5% of tumors while the proliferation index was low in 45.5% of cases. Table 2 shows no statistically significant relationship between any studied biological markers and survival rates, but Figures 1 to 4 show a clear correlation between some of these investigated marker and survival rates (either disease free or overall), specially about ER, PR, p53, Ki67 , lymph node status and tumor grade.

In multivariate regression model for relationship between tumor grade and PR, Ki67 and p53 status, PR (P=0.048) and Ki67 (P=0.007) were independent factors to predict tumor grade.

Table 1. Association between traditional prognostic factors and biologic tumor features (P values for Chi²)

Characteristics	ER	PR	HER2	Ki67	p53	Cath. D
Age (<50, >50)	0.583	0.592	0.630	0.625	0.785	0.985
Tumor size (<2, >2)	0.769	0.238	0.739	0.382	0.045	0.597
Grade (high, low)	0.119	0.042	0.393	0.001	0.008	0.071
Axillary lymph node (1-3, > 4)	0.351	0.400	0.428	0.074	0.207	0.780

Abbreviations: ER, estrogen receptor; PR, progesterone; Cath D, cathepsin D.

Table 2. Data on 66 patients with invasive breast carcinoma, with log-rank test differences in survival time among groups of patients for studied predictors

Factors	Percent	Disease free survival (*P* value)	Overall survival (*P* value)
Age			
< 50	69.7%	0.688	0.739
> 50	30.3%		
Axillary lymph node status			
0	30.3%	0.301	0.349
1-3	33.3%		
>4	36.4%		
Grade			
I	34.8%	0.401	0.264
II	59.1%		
III	6.1%		
Tumor size			
< 2 cm	28.8%	0.307	0.281
> 2 cm	71.2%		
PR			
-	50%	0.868	0.886
+	50%		
ER			
-	40.9%	0.340	0.327
+	59.1%		
HER2/neu			
-	60.6%	0.989	0.925
+	39.4%		
p53			
-	72.2%	0.391	0.248
+	27.3%		
Ki67			
-	45.5%	0.410	0.378
+	54.5%		
Cathepsin D			
-	27.3%	0.704	0.696
+	72.7%		
Menopause			
Pre	75.8%	0.357	0.339
Post	24.2%		
Chemotherapy			
-	19.7%	0.341	0.293
+	80.3%		
Ovarian suppression			
-	72.7%	0.443	0.470
+	27.3%		
Radiotherapy			
-	53%	0.410	0.391
+	47%		

Abbreviations: ER, estrogen receptor; PR, progesterone

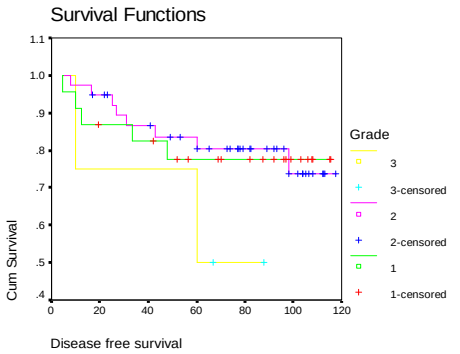

Fig. 1. Relation between tumor grade and survival rate.

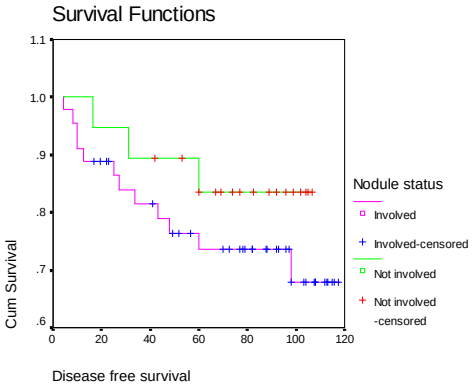

Fig. 2. Relation between ER status and survival rate.

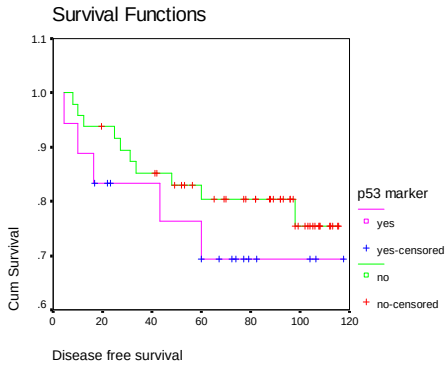

Fig. 3. Relation between P53 status and survival rate.

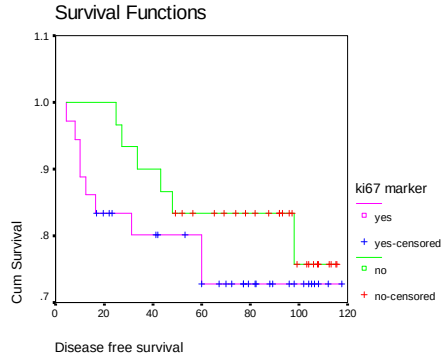

Fig. 4. Relation between Ki-67 status and survival rate.

DISCUSSION

Nowadays, the key factors in determining the prognosis and treatment for breast cancer are still pathological parameters such as tumor size, grade and lymph node involvement. These three parameters are considered as the gold standards in clinical practice and can provide very important prognostic information. However, they are not sufficiently accurate in predicting the prognosis and optimal therapeutic management in individual breast carcinoma patients. Therefore, the purpose of this study was to examine the role of a number of histological and molecular factors of malignant invasive breast carcinoma in determining the prognosis of the patients and to establish the correlation between the traditional prognostic factors and these biological factors.

In the present study we found a statistically significant relation between tumor size and p53 status and between higher grades of tumor and negative PR, overexpression of p53 and Ki67; for the latter, after multivariate analysis, only Ki67 and PR were independent factors to predict tumor grade. Tan *et al.* too showed increased Ki67 protein expression correlating with high histologic grade, mitotic score and estrogen receptor immunonegativity (11). Genes related to apoptosis and cell death (bcl2, MAP2K4, TNF10) were noted to be downregulated in tumors that disclosed > 40% Ki67 immunostaining. Another study published by Mylonas *et al.* demonstrated significantly different expression patterns of Ki67 in breast in-situ versus invasive ductal carcinomas (12).

Chow *et al.* used Ki67 as a marker of proliferative activity in their trial of a new therapeutic regimen and found a novel relationship between COX-2, Ki67, and p53 expression of breast invasive ductal carcinomas (13). These studies are all in accordance with our findings. In contrast, a study attempting to characterize the relationship of the proliferation marker Ki67 with response to systemic treatment in early breast cancer found Ki67 unlikely to be useful as a predictive marker for therapeutic response (14).

In the survival analysis, we did not achieve any statistically significant relationship between each of the investigated factors and survival rates, but there was a clear difference between various groups in relation with survival rates. This failure to achieve statistically relationship is probably due to the low number of studied patients. Based upon the Nottingham grading system, proliferation activity of tumor cells is measured by mitotic count, but the results of this measurement is quite variable between different persons counting these figures. In this study, Ki67 was an independent factor to predict tumor grade; thus the use of this marker of proliferation activity in routine approach to patients with breast cancer is recommended, at least to evaluate the accuracy of the mitotic count.

Conflict of interests

The authors declare that they have no competing interests.

REFERENCES

1. Hlupic L, Jakic-Razumovic J, Bozikov J, Coric M, Belev B, Vrbanec D. Prognostic value of different factors in breast carcinoma. Tumori. 2004 Jan-Feb; 90(1):112-119.
2. Fitzgibbons PL, Page DL, Weaver D, Thor AD, Allred DC, Clark GM, Ruby SG, O'Malley F, Simpson JF, Connolly JL, Hayes DF, Edge SB, Lichter A, Schnitt SJ. Prognostic factors in breast cancer. College of American Pathologists Consensus Statement 1999. Arch Pathol Lab Med. 2000 Jul; 124(7):966-978.
3. Winer EP, Morrow M, Osborne CK, Harris JR. Malignant tumors of the breast. In: DeVita VT, Hellman S, Rosenberg SA, editors. Principles and practice of oncology. 6th ed. Philadelphia: Lippincott Williams and Wilkins; 2001. P. 1651–1717.
4. Overgaard J, Yilmaz M, Guldberg P, Hansen LL, Alsner J. TP53 mutation is an independent prognostic marker for poor outcome in both node-negative and node-positive breast cancer. Acta Oncol. 2000; 39(3):327-333.
5. Bhatavdekar JM, Patel DD, Shah NG, Vora HH, Suthar TP, Chikhlikar PR, Ghosh N, Trivedi TI. Prognostic significance of immunohistochemically localized biomarkers in stage II and stage III breast cancer: a multivariate analysis. Ann Surg Oncol. 2000 May; 7(4):305-311.

6. Zellars RC, Hilsenbeck SG, Clark GM, Allred DC, Herman TS, Chamness GC, Elledge RM. Prognostic value of p53 for local failure in mastectomy-treated breast cancer patients. J Clin Oncol. 2000 May; 18(9):1906-1913.
7. Amornmarn R, Bui MM, Prempree TB, Masood S. Molecular predictive factors for local recurrence and distant metastasis of breast cancer after lumpectomy with postoperative radiation therapy. Ann Clin Lab Sci. 2000 Jan; 30(1):33-40.
8. Tavassoli FA. Pathology of the breast. 2nd edition. Norwalk: Appleton & Lange; 1999. P. 53.
9. Rosai J. Rosai and Ackerman's surgical pathology. 9th edition. New York: Mosby; 2004. P. 1822-1827.
10. Castiglioni T, Merino MJ, Elsner B, Lah TT, Sloane BF, Emmert-Buck MR. Immunohistochemical analysis of cathepsins D, B, and L in human breast cancer. Hum Pathol. 1994 Sep; 25(9):857-862.
11. Tan PH, Bay BH, Yip G, Selvarajan S, Tan P, Wu J, Lee CH, Li KB. Immunohistochemical detection of Ki67 in breast cancer correlates with transcriptional regulation of genes related to apoptosis and cell death. Mod Pathol. 2005 Mar; 18(3):374-381.
12. Mylonas I, Makovitzky J, Jeschke U, Briese V, Friese K, Gerber B. Expression of Her2/neu, steroid receptors (ER and PR), Ki67 and p53 in invasive mammary ductal carcinoma associated with ductal carcinoma In Situ (DCIS) Versus invasive breast cancer alone. Anticancer Res. 2005 May-Jun; 25(3A):1719-1723.
13. Chow LW, Loo WT, Wai CC, Lui EL, Zhu L, Toi M. Study of COX-2, Ki67, and p53 expression to predict effectiveness of 5-flurouracil, epirubicin and cyclophosphamide with celecoxib treatment in breast cancer patients. Biomed Pharmacother. 2005 Oct; 59 Suppl 2:S298-301.
14. Assersohn L, Salter J, Powles TJ, A'hern R, Makris A, Gregory RK, Chang J, Dowsett M. Studies of the potential utility of Ki67 as a predictive molecular marker of clinical response in primary breast cancer. Breast Cancer Res Treat. 2003 Nov; 82(2):113-123.