The Prognostic Value of P53 Immunostaining in Node-Negative Breast Carcinoma

Prinya Soontrapornchai MD*, Apinop Chanvitan MD*,
Sittichai Koontongkaew PhD**, Somkiat Sunpaweravong MD*

** Department of Surgery, Faculty of Medicine, Prince of Songkla University, Hat Yai, Songkhla*
*** Department of Dentistry, Faculty of Dentistry, Thammasat University, Bangkok*

Background: *P53 plays a key role in cell cycle arrest, apoptosis, DNA repair, and angiogenesis. Although some studies have reported as prognostic factor for poor survival in node-positive breast cancer, controversy about possible prognostic index for node-negative still exists.*
Objectives: *To look for correlations between the expression of the p53 protein and clinicopathological parameters, and to assess its prognostic value in node-negative invasive ductal breast carcinoma.*
Material and Method: *Immunohistochemistry using formalin-fixed, paraffin-embedded sections from 71 node-negative breast carcinomas in Songklanagarind Hospital. Data were analyzed with respect to tumor size, estrogen receptor, and survival.*
Results: *P53 mutations were found in 12 patients (17%). Expression of p53 was not associated with tumor size, estrogen receptor, and overall survival. Mean follow-up time was 164.4 months (median 163 months).*
Conclusion: *P53 expression was not a significant prognostic factor for survival in node-negative breast carcinoma.*

Keywords: *Breast carcinoma, P53, Prognostic factor*

J Med Assoc Thai 2007; 90 (9): 1833-8
Full text. e-Journal: *http://www.medassocthai.org/journal*

Breast carcinoma is the most common cancer worldwide in women. It is a heterogeneous disease with variable clinical behavior. Although the lymph node status is a major prognostic parameter[1,2], 30% of patients with node-negative breast carcinoma are estimated to die of their disease without adjuvant treatment[3]. Despite the application of valuable prognostic parameters such as tumor size and histological grade, it is not always feasible to predict the outcome of the disease. Therefore, additional prognostic parameters are needed to identify those patients with node-negative breast carcinoma who are more likely to relapse and who might benefit from adjuvant treatment.

The tumor suppressor gene p53 is critical for cell cycle control and apoptosis. Mutations of p53 are one of the most common known defects in human cancer[4]. Its status has been described as a prognostic factor in a variety of malignancies. P53 mutations have been found in about 20 to 30% of primary breast cancers and these mutations are more commonly found in node-positive tumors[5]. Although numerous studies have examined potential prognostic factors for poor survival in patients with invasive breast cancer, relatively few have dealt specifically with node-negative patients. Interpretation of existing studies has been limited by heterogeneity of experimental methods, small sample size, and limited follow-up times. The purpose of the present study was to investigate the prognostic value of p53 protein in node-negative breast cancer.

Correspondence to : Sunpaweravong S, Department of Surgery, Faculty of Medicine, Prince of Songkla University, Songkhla 90110, Thailand. Phone: 074-451-401, Fax: 074-429-384, E-mail: susomkia@medicine.psu.ac.th

Material and Method

The case records of 71 consecutive patients with T1-T3, N0, M0 invasive ductal breast carcinoma who had been treated at Songklanagarind Hospital from 1988-1994 were evaluated. The clinical records were reviewed for data regarding tumor size, hormone

receptors, adjuvant treatment (chemotherapy, hormonal, or radiotherapy), and outcome parameters, which is death and overall survival. The project was approved by the ethical committee of Prince of Songkla University.

Immunohistochemistry (IHC)

Formalin-fixed, paraffin-wax-embedded, 5 □m thick sections were dewaxed by xylene, rehydrated in graded alcohols, and processed using the streptavidin-biotin-immunoperoxidase method. Briefly, sections were submitted to antigen retrieval by microwave oven treatment for 10 minutes in 0.01 mol/L citric acid at pH 6.0. The sections were incubated with 1% hydrogen peroxide for 15 minutes, to block endogenous peroxidase activity, and subsequently with 1% bovine serum albumin diluted in Tris buffered saline (TBS) at pH 7.6 for 20 minutes, to block non-specific binding. The slides were wiped and incubated overnight at 4□C in a humid chamber with appropriately diluted primary antibody. The antibody used was anti-p53 protein (DO-7) mouse monoclonal antibody (NCL-p53-DO; Novocastra Laboratories Ltd, Newcastle, UK; 1/50 dilution). The slides were then rinsed three times in TBS and incubated with the reagents of the StrAvigen Multilink HRP concentrated detection kit. After three washes with TBS, the peroxidase reaction was developed in freshly prepared 0.025% diaminobenzidine/0.1% hydrogen peroxide in TBS. Finally, the sections were counterstained with haematoxylin. Tissues previously known to be positive for p53 were used as a positive control. Sections prepared with substitution of the primary antibody by TBS were used as negative control. IHC staining > 10% was considered positive.

Statistical analysis

The associations between p53, tumor size and estrogen receptor were examined by Chi-square tests or Fishers' Exact test where appropriate. The effect of p53 on a patient's survival was determined by log rank test using the Kaplan-Meier method. Survival was measured in months starting from the date of the first pathological diagnosis. P-values ≤ 0.05 were regarded as statistically significant. Data analysis was performed using SPSS software (version 11.0; SPSS, Inc., Chicago IL).

Results

The clinical and histopathological data of the patients are summarized in Table 1. Forty-two patients receiving some form of adjuvant systemic therapy were not omitted from analysis. The mean follow up time was 164.4 months and the median follow up time was 163 months. Mutant p53 proteins were found in 12 patients (17%). Expression of p53 was not associated with tumor size, estrogen receptor, age, and adjuvant therapy. By a univariate analysis of the 59 patients with p53-negative tumors compared to the 12 patients with p53-positive tumors, there was no significant

Table 1. Clinicopathological parameters in relation to P53 expression in breast carcinoma (n = 71)

Parameter	P53-positive [n, (%)]	P53-negative [n, (%)]	Total [n, (%)]	p-value
Total	12 (17)	59 (83)	71 (100)	
Age (years)				NS
≤ 50	6 (50)	38 (54)	44 (62)	
> 50	6 (50)	21 (46)	27 (38)	
T status				NS
I	3 (25)	12 (20)	15 (21)	
II	7 (58)	39 (66)	46 (65)	
III	2 (17)	8 (14)	10 (14)	
ER status				NS
Positive	1 (8)	15 (25)	16 (13)	
Negative	11 (92)	44 (75)	55 (87)	
Adjuvant therapy				NS
Chemotherapy	5	13	18	
Hormonal therapy	4	20	24	
Radiotherapy	0	3	3	

NS = not significant

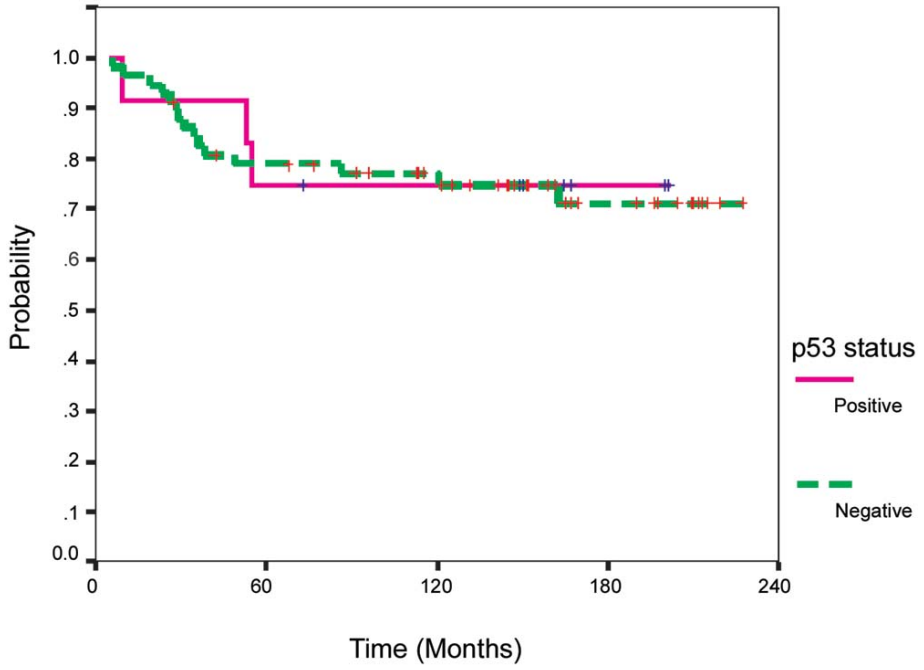

Fig. 1 Survival of node-negative breast carcinoma by p53 status

correlation with overall survival. The survival curve is shown in Fig. 1.

Discussion

The P53 gene, located on chromosome 17, was the first gene to be identified as a mutant in tumors, in 1979[6]. P53 is a nuclear phosphor-protein important in cell cycle regulation, repairing DNA damage, apoptosis to eliminate and inhibit the proliferation of abnormal cells, and inhibition of angiogenesis (Fig. 2)[7].

Two different methods have been used to assess p53 alterations: DNA sequencing and IHC. Most p53 alterations found in breast cancers are point mutations leading to the synthesis of a stable, malfunctioning and non-degradable protein that accumulates in tumor cells, and thus can be detected by IHC[8].

The incidence of p53 mutations varies among populations and stages. Mutations in p53 occur in 20-30% of sporadic breast cancers[5]. The incidence in node-negative patients is lower than that in node-positive patients, and large tumors have a higher incidence of mutations than small tumors. The prevalence of p53 mutations in recurrent tumors is higher than that in primaries, and younger patients seem to have a higher frequency[9].

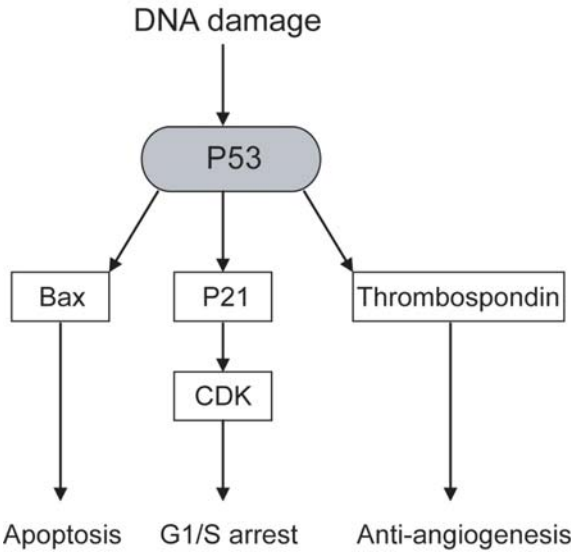

Fig. 2 The role of p53 as a tumor suppressor gene. P53 induces apoptosis via the Bax molecule, mediates cell cycle arrest for DNA repair via the Bax molecule, mediates cell cycle arrest for DNA repair via p21 and cyclin-dependent kinase (CDK), and inhibits angiogenesis through thrombospondin

Breast cancer patients with negative lymph nodes have a favorable prognosis, but still approximately 30% of these patients will die of their cancer. The identification of prognostic factors associated with either the metastatic or growth potential of breast cancer would assist physicians in determining which node-negative patients would benefit from adjuvant therapy. The long-term prognosis for clinically node-negative women with very small tumors (< 1 cm) is excellent, with a 10 year disease-free survival rate of 88%, and 75% of patients showing no evidence of disease at 30 years[3]. Due to significant toxicities associated with the chemotherapy, routine adjuvant therapy in this group would be difficult to justify.

The present series showed no correlation with the unfavorable prognostic factors of tumor size, estrogen receptor and positive p53 protein expression. Moreover, p53 status showed no significant correlation with overall survival.

In a review by Mirza AN et al[10] of sixteen large studies with long follow-up periods in node-negative patients, the useful prognostic factors were found to be tumor size, tumor grade, cathepsin-D, Ki-67, S-phase fraction, mitotic index, and vascular invasion. However, HER2/neu and DNA ploidy showed only limited association with survival in this patient group, whereas ER and p53 showed mixed results[11-26].

In conclusion, the authors have found that accumulation of p53 measured by IHC is not associated with poor outcome in node-negative breast cancer patients. However, further investigations with larger sample sizes and extended follow-up periods will be needed to determine the role of p53 as a prognostic factor in breast cancer.

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โปรตีน P53 ในการพยากรณ์ผู้ป่วยมะเร็งเต้านมที่ยังไม่กระจายไปต่อมน้ำเหลือง

ปริญญา สุนทราภรณ์ชัย, อภิณพ จันทร์วิทัน, สิทธิชัย ขุนทองแก้ว, สมเกียรติ สรรพวีรวงศ์

ภูมิหลัง: *โปรตีน P53 มีความสำคัญในกระบวนการหยุดพัก วัฏจักรของเซลล์ การเสื่อมสลายของเซลล์ การซ่อมแซม DNA และการสร้างหลอดเลือดใหม่ มีรายงานว่าสามารถใช้พยากรณ์โรคมะเร็งเต้านมที่มีการกระจายไปต่อมน้ำเหลือง แต่ในส่วนโรคมะเร็งเต้านมระยะที่ยังไม่มีการกระจายไปต่อมน้ำเหลืองนั้น ยังมีการศึกษาน้อยและยังไม่มีข้อสรุป*

วัตถุประสงค์: *ดูความสัมพันธ์ของการแสดงออกโปรตีน P53 ในผู้ป่วยมะเร็งเต้านมที่ยังไม่มีการกระจายไปต่อมน้ำเหลือง*

วัสดุและวิธีการ: *ตรวจย้อมด้วยวิธีทางอิมมูโนฮิสโตเคมีในผู้ป่วยจำนวน 71 ราย จากโรงพยาบาลสงขลานครินทร์ โดยเก็บข้อมูลศึกษาขนาดก้อนมะเร็ง, ตัวรับฮอร์โมนเอสโทรเจนและการรอดชีวิต*

ผลการศึกษา: *พบการแสดงออกของโปรตีน P53 จำนวน 12 ใน 71 ราย (ร้อยละ 17) โดยไม่มีความสัมพันธ์กับขนาดก้อนมะเร็ง ตัวรับฮอร์โมนเอสโทรเจน และระยะรอดชีวิต ระยะเวลาเฉลี่ยติดตามผู้ป่วย 164.4 เดือน (มัธยฐาน 163 เดือน)*

สรุป: *การแสดงออกของโปรตีน P53 ไม่สามารถพยากรณ์การอยู่รอดของผู้ป่วยมะเร็งเต้านมที่ยังไม่แพร่กระจายไปต่อมน้ำเหลือง*