ORIGINAL ARTICLE

The Expression of p53 in Invasive Ductal Carcinoma of the Breast: A Study in the North-East States of Malaysia

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SUMMARY

The p53 gene is a tumour suppressor gene that encodes a 393-amino-acid nuclear DNA-binding phosphoprotein. The significance of p53 detection is that p53 mutation is linked with chemo-resistance and transformation to more aggressive disease in a large number of tumour types and it was confirmed that mutant p53 is involved in neoplastic transformations. In addition, the expression of p53 has been closely correlated with clinicopathological findings. Since breast cancer has been reported as one of the most frequent malignancies in women in Malaysia, the expression of p53 was studied in 382 cases of invasive ductal carcinoma of the breast, obtained from three major hospitals in the North-East States of Malaysia. The study utilized an enzyme immunohistochemistry assay for the detection of p53. It was found that p53 was expressed in 29.6% of all the study cases. Furthermore, its expression was significantly correlated with the age and the clinical grading of the disease. No significant statistical correlations were depicted with lymph node status, tumour size, side of tumour, and expression of estrogen and progesterone receptors. Nevertheless, knowledge of the p53 status may be valuable in making clinical decisions regarding diagnosis, prognosis and therapy.

KEY WORDS:
Breast cancer, p53 expression, Clinical correlations

INTRODUCTION

The p53 gene is a tumour suppressor gene that encodes a 393-amino-acid nuclear DNA-binding phosphoprotein[1]. In normal cells, p53 is kept at low concentrations by its relatively short half-life, not exceeding 30 min [2,3]. The cell either undergoes cell cycle arrest or apoptosis after p53 activation, following cellular stress, particularly that induced by DNA damage [4,5]. In cancer cells that bear a mutant p53, this protein is no longer able to control cell proliferation, resulting in inefficient DNA repair and the emergence of genetically unstable cells[6]. Preserving and the persistence of genomic damage in the presence of mutant p53 could potentially lead to neoplasia [7, 8]. Mutation rates vary in different tumour types, occurring in 25-30% of breast carcinomas and up to 70% of poorly differentiated ovarian, colorectal, and head and neck tumours. Careful studies with microdissected tumour material have shown that p53 mutations may occur in ductal carcinoma in situ (DCIS) before the development of invasive breast cancer, and that the frequency increases from around zero in low-grade DCIS to 30-40% in high grade DCIS[9,10,11]. These results emphasize the important role of p53 alterations early in the carcinogenic process of the breast [12].

The aim of this work was to investigate the expression of p53 in infiltrating ductal carcinoma of the breast in the North-East States of Malaysia as well as to study the clinicopathological associations with p53 expression.

MATERIALS AND METHODS

A total of 382 records of patients with infiltrating ductal carcinoma of the breast were obtained from three general hospitals in the North-East coast of Malaysia: Hospital of The University of Science of Malaysia (HUSM), Kota Bharu, Kelantan, from 1992 to 2004 (n= 266), Hospital Kota Bharu (HKB), Kota Bharu, Kelantan State, from 2001 to 2003 (n= 37), and Hospital Kuala Terengganu (HKT), Kuala Terengganu, Terengganu State, from 2001 to 2004 (n= 79). The clinical data obtained from the records and the histopathology reports included the final diagnosis, lymph node status, tumour size, side of tumour, estrogen receptor status (282 cases only) and progesterone receptor status (259 cases only). Ethical approvals were obtained at the School of Medical Sciences, University of Science of Malaysia in September 2001, in addition to consents from patients which were obtained prior to the start of the work.

Fresh samples of breast cancer tissue obtained from the operations theatre were fixed in 10% formalin within 13 hours at room temperature. Older tissue samples in paraffin wax blocks were obtained from the Departments of Pathology of the three hospitals.

For the tissue detection of p53, 4 µm breast cancer tissue sections were deparaffinized and rehydrated. The sections were heated in a microwave three times at 900 W for a total of 15 min in 0.01 M sodium citrate buffer, pH 6.0. A mouse anti-human p53 antibody (DO-7; DAKO), diluted 1:50 with phosphate-buffered saline (PBS), was added and incubated for one hour. A biotinylated rabbit anti-mouse IgG (DAKO) was diluted 1:100 with PBS, added and incubated for one hour. The detection used a standard avidin-biotin-peroxidase complex/DAB using ABComplex kit (DAKO). A positive control for p53 staining was obtained from breast cancer tissues. Negative controls included those treated in the

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absence of a primary antibody, and normal breast tissue. All the experiments were performed at room temperature. Nuclear staining marked the positive expression of p53 (Fig. 1).

The Pearson Chi-square test (Pearson χ^2) and Spearman rank correlation were measured using the Statistical Package for Social Sciences (SPSS version 11.0 software package for Macintosh, SPSS Inc., Chicago, IL).

RESULTS

The overall expression of p53 in 382 cases of infiltrating ductal carcinoma of the breast was 29.6% (n=113 out of 382). There was 17% (n=65) of p53 expression in the patients ≤ 50 years of age, compared to 12.6% (n=48) in patients > 50 years of age (Fig. 2). The correlation between p53 expression and patients ages was statistically significant.

A correlation with the histological grade of tumours was sought. It was found that 16.5% (n=63) of p53 positive expression were of the histological grade III, compared to 11% (n=42) and 2.1% (n=8) in the histological grades II and I, respectively (Fig. 3). The correlation of p53 expression with the histological grade was found to be statistically significant.

Among the p53 positive cases, 19.6% (n=75) were associated with lymph node involvement whereas 9.9% (n=38) of the cases had no lymph node involvement. There was no significant correlation between lymph node involvement and p53 expression (p>0.05). With the tumour size parameter, the highest percentage of p53 positive cases was observed in the tumour size range > 10cm (12.8%, n= 49) compared to other tumour size ranges. The percentage of p53 positive cases was found to be 14.7% (n=56) at the left side, 14.1% (n=54) at right side and 0.8% (n=3) bilateral. Out of 282 cases, the percentage of p53 positive expression was higher than in estrogen receptor negative cases (19.5%, n=55) than in the estrogen receptor positive (12%, n= 34) cases. Similarly, out of 259 cases, p53 was higher among the progesterone receptor negative cases (23.5%, n=61) compared to progesterone receptor positive cases (8.5%, n=22). However, the correlation of p53 expression with lymph nodes involvements, tumour size, side of tumour, and with hormone receptors, were statistically not significant (Table I).

DISCUSSION

Knowledge of the p53 status in a given tumour can aid in the diagnosis and guide in the therapeutic decision process in addition to predicting prognosis [7, 11, 14-18]. Previous reports indicated that mutant p53 expression has a significant positive correlation with high proliferation index (MIB1)[19], increased grade values [9, 20], and that it shows a negative correlation with the steroid receptors status [11, 21, 22]. Furthermore, other investigations reported that over-expression of an activated form of the p53 protein may be involved in neoplastic transformations[3,23-24]. However, it was later confirmed that mutant p53 is involved in neoplastic transformations, not the wild type [1]. Furthermore, detailed knowledge of the p53 mutation status and its expression signature predicts patients' survival [25], response to therapy [26], and suggests p53 as a potential target for gene therapy [27]. In addition, p53 is significantly associated with lower age, larger tumour size, ductal morphology, and high tumour grade[28]. Although such correlations were not statistically significant in the current study, the differences in the results may be attributable to genetic, environmental, and possibly social factors. These results point at the important role of p53 alterations early in the carcinogenic process of the breast [12-13].

The overall reported expression of p53 in breast cancer ranged from 9% to 69% [12]. In our work, p53 was detected in 29.6% of the cases. These results were comparable with previous reports especially regarding the correlations with histologic grading and the hormonal status. The relatively high expression of p53 may be attributed to genetic and

Table I: Correlations between clinicopathologic factors and expression of p53 in breast cancer

	P53 expression		
	Positive (number of patients)	Negative (number of patients)	p-value
Lymph node metastasis (n=382)			
Node +	75	163	n.s.
Node -	38	106	p=0.288
Tumour size (cm) (n=382)			
< 1 cm	0	1	n.s.
1-2 cm	1	10	p=0.554
2.1 - 5 cm	22	52	
5.1 – 10 cm	41	87	
≥ 10 cm	49	119	
Tumour side (n=382)			
Right	54	130	n.s.
Left	56	131	p=0.978
Bilateral	3	8	
Estrogen receptor status (n=282)			
Negative	55	132	n.s.
Positive	34	61	p=0.276
Progesterone receptor status (n=259)			
Negative	61	132	n.s.
Positive	22	44	p=0.795

All analyses were tested using Pearson Chi-square test (Pearson χ^2) and Spearman rank correlation, p< 0.05 is considered significant, n.s. = not significant, n= number of patients

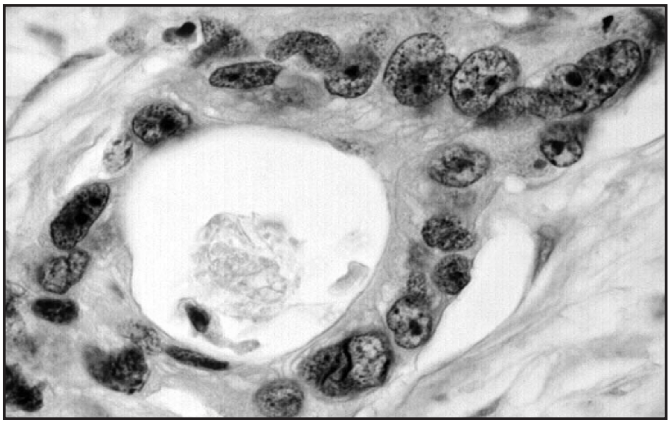

Fig. 1: A micrograph showing the nuclear positive immunostaining of p53 in breast cancer (Original magnification x400).

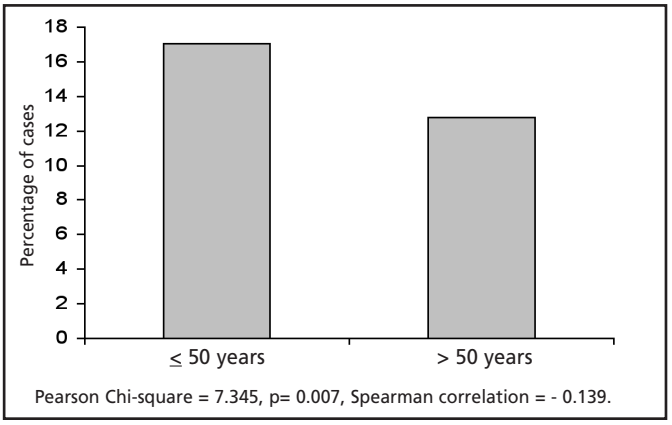

Fig. 2: The correlation of p53 expression with age, categorized as ≤50 years and >50 years.

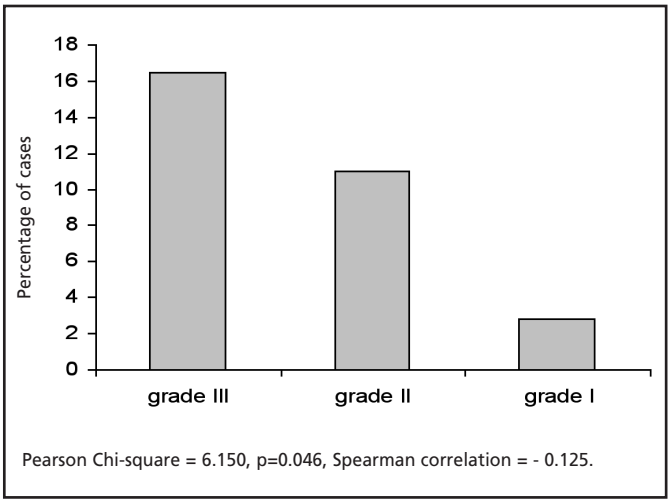

Fig. 3: The correlation between p53 expression and the histological grading.

environmental factors that dictate the p53 mutation type. However, mechanisms that enhance the accumulation of p53, such as increased production or increased half life of p53, cannot be elucidated from the methodology used. Nevertheless, at first diagnosis, most of the cases would be at relatively advanced stages of the disease [29] and the relatively elevated expression of p53 in this study may further reflect bad prognoses among the study cases since the presence of mutated p53 can lead to poor outcomes [11, 30-32]. Moreover, p53 may aid in the prediction of local recurrences[14]. It was also reported that the detection of p53 in the sera of breast cancer patients correlated directly with the extent of accumulation in tissues[33]. Mutant p53 may itself be a candidate for tumour therapy since the down regulation of p53 can result in reduction in tumour aggressiveness [27].

In conclusion, this study showed that p53 detection was significantly associated with higher grades of tumours, and may thus serve in directing clinical decisions regarding diagnosis, therapy, and prognosis. The introduction of p53 detection into routine clinical diagnosis may serve these purposes.

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