

# CORRELATIONS IN SURVIVIN EXPRESSION WITH THE EXPRESSION OF p53 AND BCL-2 IN INVASIVE DUCTAL CARCINOMA OF THE BREAST

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**Abstract.** This work studied the correlations between survivin, bcl-2 and p53 in infiltrating ductal carcinoma of the breast. A total number of 382 cases were collected from 3 hospitals in northeastern Malaysia. Survivin, bcl-2 and p53 were detected by immunohistochemistry on samples prepared from tissue blocks. Significant correlations were found between tumor histological grades and tumor size and lymph node involvement. Highly significant statistical correlations ( $p < 0.001$ ) were found in expression of the markers under study. It is concluded that such significant correlations may imply that the alterations in the expression take place in a concerted fashion, implying that many of these cases may share common abnormalities.

## INTRODUCTION

Survivin and bcl-2 are inhibitors of apoptosis while mutant p53 is a tumor suppressor protein with lost functional ability (Sakaguchi *et al*, 1998; Sharma and Srikani, 1998; Shi *et al*, 1999; Taylor *et al*, 1999). Accumulated p53 represents the mutated type and not the wild type in cancer (Lu *et al*, 1998; Norberg *et al*, 2001; Ostrakovitch and Cherian, 2004). Although cancer is a disease of uncontrolled proliferation, the mechanism of proliferation of its cells is similar to that of normal cells (Andreef *et al*, 2000). Both p53 and bcl-2 play a role in determining tumor growth by their effects on apoptosis and cell proliferation (Linjawi *et al*, 2004). Hence, tumor growth is the net result of cell proliferation and cell death (Siziopikou and Schnitt, 2000). In gastric carcinomas, a significant correlation be-

tween survivin and p53, or bcl-2 expression has been reported (Lu *et al*, 1998). This finding is important in demonstrating the link between these two anti-apoptosis proteins and the role of mutant p53 in cancer. A more recent finding suggests the expression of survivin and its processing depends on p53 (Vegran *et al*, 2007). In breast cancer, bcl-2 has a significant inverse correlation with mutant p53 (Barbareschi *et al*, 1996; Hori *et al*, 1997; Gursan *et al*, 2001; Tsutsui, *et al*, 2006). Survivin is correlated with bcl-2 but not with p53 in breast carcinoma. Nevertheless, survivin is correlated weakly with p53 in intraductal epithelial neoplasia (Kavasalcuk *et al*, 2004).

The correlations of expression among survivin, bcl-2 and p53 in infiltrating ductal carcinoma (IDC) of the breast were studied. The study utilized the findings in 382 cases, collected from 3 hospitals in northeastern Malaysia. Clinical correlations with the histological grades of tumors were established. Highly significant correlations were established in the expression of the markers under study.

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## PATIENTS AND METHODS

### Patients

Data and tissue samples from a total of 382 patients with invasive ductal carcinoma of the breast were obtained from three general hospitals in two northeastern states 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 from 2001 to 2003 (n= 37), and Hospital Kuala Terengganu (HKT), Kuala Terengganu, Terengganu State, from 2001-2004 (n= 79). Ethical approval was obtained from the School of Medical Sciences, University of Science of Malaysia in September 2001, and patient consent was obtained prior to the study. Data obtained from the records included age, tumor size, histological grade, estrogen receptor status (ER: 282 cases), progesterone receptor status (PR: 259 cases), lymph node involvement, and the histopathology diagnoses.

### Collection, preservation and immunohistochemistry (IHC) of the tumor masses

Fresh samples of breast cancer tissue were fixed in 10% formalin. Old samples in wax blocks were obtained from the pathology departments of the three hospitals. Survivin, p53 and bcl-2 were detected by immunohistochemistry. For survivin, an EDTA antigen retrieval buffer pH 9 was used, and the IHC assay utilized a primary rabbit antibody raised against oligonucleotides representing the C- and N-termini of the survivin molecule (Al-Joudi *et al*, 2005). The p53 assay utilized a citrate buffer pH 6, and a mouse anti-human p53 primary antibody (DO-7; Dako). The bcl-2 assay utilized citrate buffer pH 6 and a mouse monoclonal anti-human bcl-2 primary antibody (clone 124; DAKO). A relevant HRP-conjugated secondary antibody was used for each antigen, and the reaction was visualized using a standard avidin-biotin-peroxidase complex/diaminobenzidine (DAB) using an

Avidin-Biotin Complex kit (DAKO).

### Statistical analyses

The Pearson chi-square test (Pearson  $\chi^2$ ) 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

Following processing of tissue sections on slides, they were examined microscopically for the expression of survivin, p53 and bcl-2 (Figs 1-3)

The distribution of age, tumour size, survivin, p53, bcl-2, estrogen receptor (ER), progesterone receptor (PR) and lymph node metastasis according to tumor histological grade were investigated (Table 1). Grade III tumors were associated more with patients under 50 years old (30.9%) compared to those above 50 years (16.2%), although the difference was not significant ( $p>0.05$ ). There was a significant difference ( $p<0.05$ ) between each group of tumor size grouping. With survivin expression, it was demonstrated that the highest percentage of survivin positive staining was among patients of grade III tumors (31.9%) compared to other tumor grades although the difference was not significant ( $p>0.05$ ). With p53 expression, the highest positive staining for p53 was among grade III tumors compared to other grades with a significant difference ( $p<0.05$ ). Similarly, with bcl-2 expression, the highest positive expression of bcl-2 was among grade III tumors (20.9%) compared to grade I tumors (6%) and grade II tumors (16.7%) but with no statistical significance ( $p>0.05$ ).

### Correlation between survivin and p53 expression

There was a significant inverse correlation between survivin expression and p53 expression among the study cases (Pearson chi-square test= 55.24,  $p<0.001$ , Spearman cor-

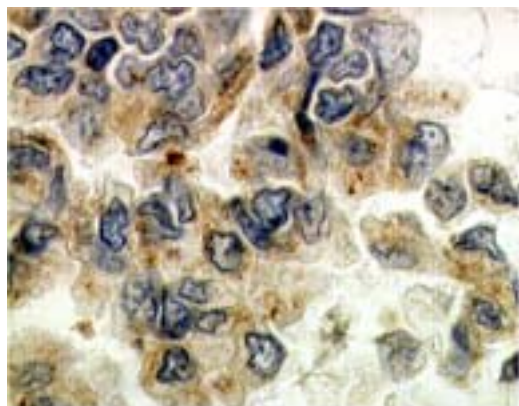


Fig 1—A micrograph showing the cytoplasmic staining of survivin in a paraffin-embedded IDC of the breast section (Original magnification x400).

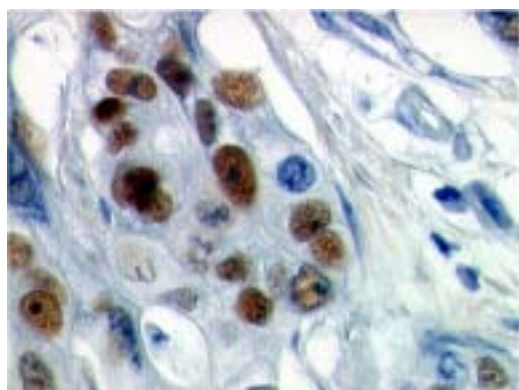


Fig 2—A micrograph showing positive nuclear immunostaining of p53 in a paraffin-embedded IDC of the breast section (Original magnification x400).

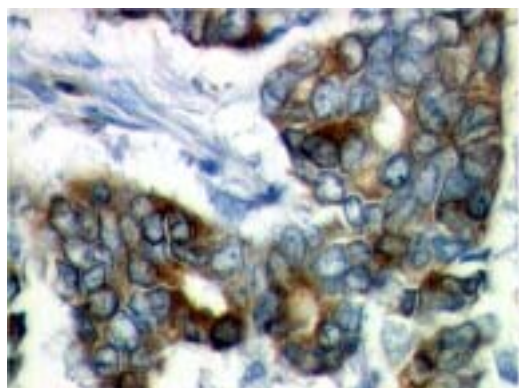


Fig 3—A micrograph showing positive cytoplasmic immunostaining of bcl-2 in a paraffin-embedded tissue section of IDC of the breast (Original magnification x400).

relation= -0.380). The expression of survivin was higher in the p53 negative cases (56%) than its expression in the p53 positive cases (12%) (Table 2).

#### Correlation between survivin and bcl-2 expression

Analysis of the data obtained showed that 48.1% of the study cases expressed cytoplasmic bcl-2. The expression of survivin showed a significant strong positive correlation with the expression of bcl-2 among the subjects studied (Pearson chi-square =39.296,  $p < 0.001$ , Spearman correlation =0.321). The positive expression of survivin increased proportionally with the positive expression of bcl-2 (Table 3).

#### Correlation between p53 and bcl-2 expression

A significant inverse correlation between p53 and bcl-2 expression was found (Pearson chi-square= 23.39,  $p < 0.001$ , Spearman correlation= -0.247) among the study subjects (Table 4). The highest p53 positive expression was 22.3% in negative expression of bcl-2 among the patients studied.

## DISCUSSION

Survivin is considered new to the community of biomarkers or tumor markers whereas p53 and bcl-2 are considered established tumor markers. Therefore, in this study, survivin expression was studied simultaneously with p53 and bcl-2 to examine their correlations and interactions in infiltrating ductal carcinoma of the breast. The information for p53 and bcl-2 expression in breast carcinomas was validated and proven by numerous studies. However, in spite of the scarcity of literature regarding survivin, it is opening new research tracks. Survivin may act simultaneously with the bcl-2 family proteins, although it has a different apoptosis inhibitory mechanism (Mirza *et al*, 2002; Zhou *et al*, 2002). New light is being shed on the role of p53 in regulating the expression of survivin,

Table 1  
The distribution of age, tumor size, survivin, p53, bcl-2, ER, PR, and lymph node metastasis according to tumour histological grade.

	Histological tumor grading			
	I Number (%)	II Number (%)	III Number (%)	
Age (n=382)				
≤ 50	31 (8.1)	109 (28.5)	118 (30.9)	ns
> 50	14 (3.7)	48 (12.6)	62 (16.2)	
Tumor size (n=382)				Pearson $\chi^2 = 132.7$ df=8, p< 0.001
<1 cm	0 (0)	0 (0)	1 (0.3)	
1-2 cm	6 (1.6)	4 (1)	1 (0.3)	
2.1-5 cm	31 (8.1)	33 (8.6)	10 (2.6)	
5.1-10 cm	4 (1)	64 (16.7)	60 (15.7)	
≥ 10 cm	4 (1)	56 (14.6)	108 (28.3)	
Survivin (n=382)				ns
Positive	30 (7.8)	108 (28.2)	122 (31.9)	
Negative	15 (3.9)	49 (12.8)	58 (15.2)	
p53 (n=382)				Pearson $\chi^2=6.150$ , df=2, p=0.046
Positive	8 (2.1)	42 (11)	63 (16.5)	
Negative	37 (9.7)	115 (30.1)	117 (30.1)	
bcl-2 (n=382)				ns
Positive	23 (6)	64 (16.7)	80 (20.9)	
Negative	22 (5.8)	93 (24.3)	100 (26.2)	
ER (n=282)				ns
Negative	18 (6.4)	84 (29.8)	85 (30.1)	
Positive	11 (3.9)	40 (14.2)	44 (15.6)	
PR (n=259)				ns
Negative	16 (6.2)	88 (34)	89 (34.4)	
Positive	9 (3.5)	30 (11.6)	27 (10.4)	
Lymph node metastasis (n=382)				Pearson $\chi^2 = 30.279$ df=2, p< 0.001
Node +	12 (3.1)	98 (25.6)	128 (33.5)	
Node -	33 (8.6)	59 (15.4)	52 (13.6)	

ns= not significant, ER= Estrogen receptor, PR = Progesteron receptor,  $\chi^2$  = Chi-square, df= degree of freedom, p = significant value

since p53 mutations have been reported to be significantly associated with an increased expression of survivin and, in particular, its antiapoptotic splice variants (survivin-DeltaEx3 and survivin-3B) (Vegran *et al*, 2007). Conversely, the transcription of survivin has been shown to be directly repressed by wild-type p53 (Mirza *et al*, 2002).

The intrinsic apoptotic pathway is medi-

ated by the mitochondrial release of cytochrome c (Pruschy *et al*, 2001; Kiechle and Zhang, 2002). It is mainly activated when damaged DNA is not sensed and repaired by checkpoint genes. Initiation of apoptosis may occur immediately or it may be delayed following DNA damage. The response may or may not be dependent on the presence of the nuclear transcription factor, p53. When p53

Table 2

The correlation between survivin and p53 in infiltrating ductal carcinoma of the breast.

	Survivin		
	Positive	Negative	
P53			Pearson $\chi^2$ test =55.240
Positive	12%(n=46)	17.5%(n=67)	p=0.000
Negative	56.0%(n=214)	14.4%(n=55)	df=1
			Spearman correlation = - 0.380

Table 3

The correlation between survivin and bcl-2 in infiltrating ductal carcinoma of breast patients.

	Survivin		
	Positive	Negative	
Bcl-2			Pearson $\chi^2$ test =39.296
Positive	37.2%(n=142)	6.5%(n=25)	p=0.000
Negative	30.9%(n=118)	25.4%(n=97)	df=1
			Spearman correlation = 0.321

Table 4

The correlation between p53 and bcl-2 in infiltrating ductal carcinoma of breast patients.

	P53		
	Positive	Negative	
Bcl-2			Pearson $\chi^2$ test = 23.39
Positive	7.3%(n=28)	36.4%(n=139)	p=0.000
Negative	22.3%(n=85)	34.0%(n=130)	df=1
			Spearman correlation = -0.247

is upregulated, it is activated by the phosphorylation of serine 46 by the homeodomain-interacting protein kinase-2, and the two proteins cooperate in the activation of p53-dependent transcription (Levine, 1997). However, p53 that is accumulated in transformed cells has been reported to have lost its functional activity. Furthermore, IAPs of both cellular and viral origin have been identified to be intrinsic cellular suppressors of apoptosis that block the apoptotic program in response to viral infection or other forms of stress (Shu *et al*,

1997; Li and Li, 2000; Thomas, 2000; Pruschy *et al*, 2001).

The significant correlations found may imply that the alterations in the expression mostly take place together in a concerted pattern, implying that many of these cases possibly share common abnormalities. Similar correlations were reported between p53 and bcl-2 in breast cancer and in gastric carcinoma (Lu *et al*, 1998), to further suggest a common mechanism(s) between the two cancer types. Signaling through the mammalian

extrinsic and intrinsic pathways of apoptosis (Coultas and Strasser, 2003) can be modulated by IAPs (inhibitors of apoptosis proteins) such as bcl-2 and survivin, and may change the fate of transformed cells, to adopt apoptotic pathways equivalent to those of cytotoxic agents (Al-Joudi *et al*, 2005; Rahman *et al*, 2006). Survivin and bcl-2 selectively inhibit the activation and functional activity of various caspases (Deveraux and Reed, 1999; Reed, 1999; Campora *et al*, 2000; Kaufmann and Earnshaw, 2000; Parton *et al*, 2001; Sanna *et al*, 2002).

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