

Expression of survivin and its clinicopathological correlations in invasive ductal carcinoma of the breast

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ABSTRACT

Introduction: Survivin is a 16.5-kDa intracellular protein that inhibits apoptosis and regulates cell division, and belongs to the inhibitors of apoptosis gene family. It appears to have an important role in regulating apoptosis at the cell cycle checkpoints. Survivin has been found to have a differential distribution in cancer compared to normal tissue, as it is over-expressed in malignant tumours.

Methods: In addition to the demographical analysis of the disease, data from 382 women with invasive ductal carcinoma of the breast were collected from three hospitals in Northeast Malaysia, and analysed for survivin expression by immunohistochemistry.

Results: Invasive ductal carcinoma of the breast was found to be the most prevalent breast cancer type. Survivin was detected in 260 (68.1 percent) study cases. In addition, significant correlations have been shown between survivin expression on one hand, and tumour size and lymph node involvement on the other hand (p-value is less than 0.05). However, no significant correlations were found with other clinicopathological factors, such as tumour histological grade, tumour side, oestrogen and progesterone receptors. Nuclear expression of survivin was detected in 16.5 percent of the study cases, cytoplasmic expression was detected in 24.1 percent, and 27.5 percent of the cases expressed survivin in both nuclear and cytoplasmic locations simultaneously. The subcellular localisation of survivin was significantly correlated (p is less than 0.001) with the lymph node involvement

its value in predicting the aggressiveness of tumour cells, since it increases the resistance to apoptosis and promotes cell proliferation.

Conclusion: This is the first known report on survivin expression in cancer in West Malaysia and Southeast Asia. It emphasises the importance of the detection of survivin in breast cancer to aid in diagnosis, confirm malignancy, and to assess the disease progress and response to therapy.

Keywords: breast cancer, invasive ductal breast carcinoma, survivin expression

Singapore Med J 2007; 48(7):607-614

INTRODUCTION

Survivin is a 16.5 kDa protein, also known as AP14 or BIRC5.⁽¹⁾ It is an intracellular protein that inhibits apoptosis and regulates cell division, and belongs to the inhibitors of apoptosis gene family.^(1,2) Survivin over-expression in vivo increases cell resistance to apoptosis⁽³⁾ and appears to have an important role in regulating apoptosis at the cell cycle checkpoint(s). Its expression is highly cell cycle regulated, and is detectable in the nucleus selectively at the G2/M phase.⁽⁴⁾ However, the correlation of nuclear expression with the aggressiveness of tumours has not been conclusive, although it has been suggested that nuclear expression in breast cancer correlated with favourable prognosis,⁽⁵⁾ while cytoplasmic expression correlated with unfavourable prognosis.⁽⁶⁾ Furthermore, when treated with chemotherapeutic drugs, cultured breast cancer cells were found to increase the expression of survivin, in an attempt to resist apoptosis.⁽⁷⁾ Transcription of survivin has been shown to be directly repressed by wild-type p53, another cell cycle checkpoint-regulating protein that induces apoptosis.⁽⁸⁾ On the contrary, survivin expression was suppressed when ErbB2 was selectively knocked down.⁽⁹⁾ Moreover, one of the most significant

features of survivin is its differential distribution in cancer compared to normal tissue. Over-expression of survivin has been demonstrated in tumours of the lung, breast,⁽¹⁰⁾ oesophagus, pancreas, bladder, uterus, cervix, ovary,⁽¹¹⁻¹³⁾ large-cell non-Hodgkin's lymphoma and leukaemia,⁽¹⁴⁻¹⁷⁾ neuroblastomas, melanomas, gastric tumours,⁽¹⁸⁻²⁰⁾ colon cancer,⁽²¹⁻²²⁾ stomach and liver cancers,⁽³⁾ oral cancers,⁽²³⁻²⁵⁾ thyroid tumours,⁽²⁵⁾ laryngeal squamous cell carcinoma,⁽²⁶⁾ osteosarcoma,⁽²⁷⁾ and prostatic cancer.⁽²⁸⁾ Despite its role in mitosis, it is clear that over-expression of survivin in cancer does not simply reflect the presence of a higher number of proliferating cells. In melanoma, survivin expression was indistinguishable in cases with low or high mitotic index.⁽³⁾ In addition, the fact that survivin is typically observed in nearly all tumour cells, and not just in the mitotic fraction, suggests that expression of the survivin gene is deregulated in cancer, albeit still retaining cell-cycle periodicity in mitosis.⁽²⁹⁻³¹⁾ Survivin is thought to shield tumour cells from the physiological process of cell death and to promote tumour cell proliferation. Due to almost exclusive expression in cancer and not in normal tissues, survivin is potentially an ideal diagnostic tool. This concept has been validated in bladder cancer, where the presence of survivin in urine may help identify, with considerable accuracy, patients carrying the disease or those at risk of recurrence after treatment. This may also be applicable in breast cancer, especially where high-risk individuals need to be monitored. The role of survivin in many cancers has been reported in numerous publications, but little has been published about the role of survivin in breast cancer, where its expression in breast cancer ranged from 60% to 72% of the cases examined.^(5,6,10,32,33)

In Malaysia, cancer has become increasingly important as a public health concern.⁽³⁴⁾ The estimated population of Malaysia in 1999 was about 22.8 million. In the two neighbouring northeastern states of Kelantan and Terengganu, the female populations were 762,600 and 499,300, respectively.⁽³⁵⁾ The commonest cancer in women registered in Malaysian hospitals in 1995 was breast cancer.⁽³⁶⁾ Furthermore, invasive ductal carcinoma of the breast is the most common among Malaysian women, and this is similar to the worldwide trend where it contributes to 80% of breast cancer cases.⁽³⁷⁾ It was reported that invasive ductal carcinoma comprised 85.1% (57/67) of all breast cancer cases registered in Penang Hospital, in the west coast of Malaysia in 2003,⁽³⁸⁾ and 93% (256) of cases registered in the Hospital of the University of Science of Malaysia in Kelantan state, from 1992 to 2003.⁽³⁹⁾

The current study represents the first report of survivin expression in cancer in West Malaysia, where it was found to be expressed in 68.1% of cases of invasive ductal carcinoma. Significant correlations have been discovered between survivin expression, and tumour size

and lymph node involvement.

METHODS

Clinical data from 382 women with invasive ductal carcinoma of the breast were obtained from three general hospitals in the northeastern part of West Malaysia: Hospital Universiti Sains Malaysia, Kota Bharu, Kelantan from 1992 to 2004 (n = 266), Hospital Kota Bharu, Kota Bharu, Kelantan from 2001 to 2003 (n = 37), and Hospital Kuala Terengganu, Kuala Terengganu, Terengganu, from 2001 to 2004 (n = 79). Ethical approval was obtained at the School of Medical Sciences, University of Science of Malaysia in September 2001, and consent was obtained from the patients prior to the start of the study. Clinical information obtained from the records and the histopathology reports included the age, first diagnosis, tumour side, tumour size and grade, oestrogen-receptor (ER) and progesterone-receptor (PR) status, and lymph nodal involvement. Fresh samples of breast cancer tissue were obtained from the operating theatre and fixed in 10% formalin within 13 hours at room temperature. Old samples preserved in wax blocks were obtained from the pathology departments of the three participating hospitals.

The immunohistochemistry (IHC) assay was performed as described previously.⁽⁴⁰⁾ Tissues were subjected to a series of processing steps, which included fixation, dehydration with ethanol, clearing with xylene, and wax impregnation with paraffin in Tissue Tek[®], an automated closed system (Sakura Finetechnical, Tokyo, Japan). Glass slides were dipped in the poly-L-lysine 0.01% (Dako, Glostrup, Denmark) for two minutes and dried to prevent the peeling off of tissues from the slides during the IHC staining. Tissues were embedded in paraffin as the final process of making tissue blocks, which were then trimmed and sectioned with a microtome (Leica, Wetzlar, Germany) at room temperature to obtain 4 µm sections. The ribbons of sections were floated in a 50°C water bath (TissuePrep[™] Flotation Bath Model 135, Fisher Scientific, Shah Alam, Malaysia), and "fished" and mounted onto the poly-L-lysine-treated glass slides. The sections were then deparaffinised on a 60°C hot plate. This was followed by a hydration process, which included immersion in xylene for two minutes and a series of steps of decreasing ethanol concentrations beginning with absolute ethanol (2 min), 95% ethanol (2 min), and 80% ethanol (2 min). The slides were then dipped in 3% hydrogen peroxide for 15 minutes.

Used as positive controls, the slides with sections from the colon tissue were put through the antigen retrieval process using the pressure cooker method at 120°C for 20 minutes in Tris-EDTA buffer, pH 9.⁽⁴⁰⁾ They were then cooled down in cold water for 15 minutes. Bovine serum albumin was added, followed by the primary antibody

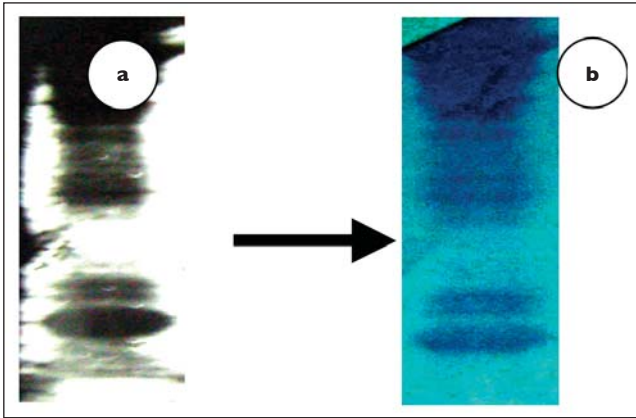


Fig. 1 Photographs show breast cancer tissue lysate. (a) The SDS-PAGE of whole lysate (Coomassie blue); and (b) the proteins after Western blotting onto a PVDF membrane and stained with Amido Black.
SDS-PAGE: sodium dodecyl sulfate polyacrylamide gel electrophoresis.

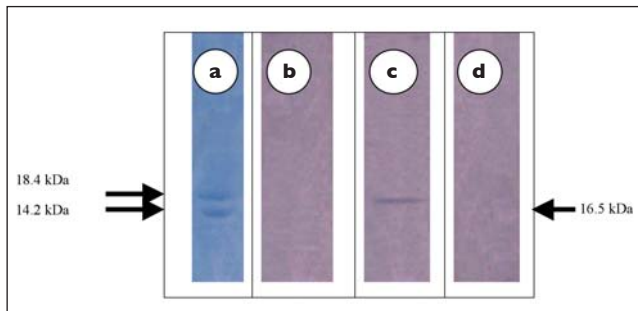


Fig. 2 Immunoblots show the specific immunoreactivity of antibodies to breast cancer tissue lysate on PVDF membrane. (a) Molecular markers bands (arrows) stained with Amido Black; (b) preimmunised serum with no immunoreactivity; (c) SUR12A-CFI shows reactivity to a single band of protein at approximately 16.5 kd, consistent with the molecular weight of survivin; and (d) SUR12A-CFI against normal breast tissue lysate showing no immunoreactivity.

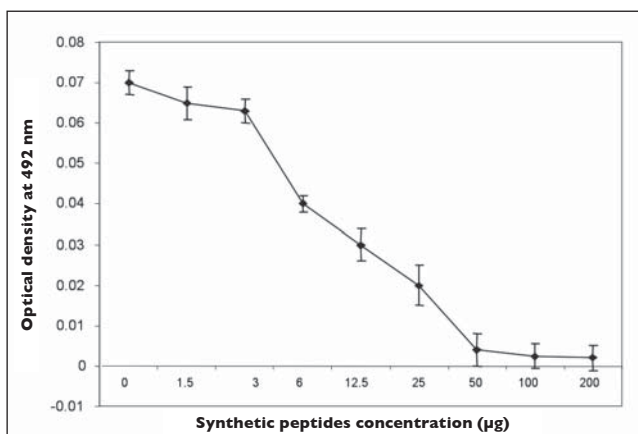


Fig. 3 Graph shows a gradual reduction in the reactivity of the SUR12A-CFI antibody following preabsorption with an increasing concentration of the preabsorption antigens, the synthetic survivin oligopeptides.

which was incubated overnight at 4°C. The primary anti-survivin antibody was prepared in the research laboratory at the Chemical Pathology Department, School of Medical Sciences, Universiti Sains Malaysia, by hyperimmunisation of rabbits with oligopeptides with sequences representing the C- and N-termini of the survivin amino acid sequence. These antibodies were tested for specificity to survivin by preabsorption tests in competition ELISA and by immunoblotting (Figs. 1–3). These sera were used in the IHC assay and were further tested and validated using colon cancer tissues and breast cancer tissues.⁽⁴⁰⁾ The primary antibody SUR12A-CFI was diluted 1:1280 in antibody diluent (50 mM Tris, 150 mM NaCl, 0.5% BSA, 15 mM sodium azide and 0.05% Tween 20, pH 7.6) and added. After washing, the diluted biotinylated secondary antibody that was conjugated with horseradish peroxidase (Dako, Glostrup, Denmark), was added at a dilution ratio of 1:160. Immunoreactive survivin was visualised by the avidin-biotin-enhanced horseradish peroxidase method (Dako, Glostrup, Denmark) using diaminobenzidine (Sigma, Moreton-in-Marsh, UK) as the substrate or chromogen to form an insoluble brown product after the reaction with peroxidase. This was followed by a light-blue nuclear counterstain with Gill's haematoxylin. Finally the sections were dehydrated by immersion in increasing concentrations of alcohol beginning with 90% ethanol (2 min), 95% ethanol, then in increasing concentrations of xylene. Specificity controls included an internal negative control incubated with normal rabbit serum. Normal breast tissue was used as external negative control and colon cancer tissue was used as the positive control.

Survivin expression was quantified in the various samples examined using a scoring method utilised previously.^(6,19) A mean percentage of positive tumour cells was determined in at least five areas at a magnification of 400×, and assigned to one of the five following categories: (a) 0, < 5%; (b) 1, 5%–20%; (c) 2, 21%–50%; (d) 3, 51%–75%; and (e) 4, > 75%. The immunostaining intensity of survivin was scored as follows: (a) weak, 1+; (b) moderate, 2+; and (c) intense, 3+. For tumours that showed heterogeneous staining, the predominant pattern was taken into account for scoring. The percentage of positive cells and the staining intensity were multiplied to produce a weighted score for each case. Cases with weighted scores of < 1 were considered negative, and those with scores of ≥ 1 were considered positive. The background staining was also evaluated and put in brackets with the final score. Microscopy for immunoreactivity was evaluated by two separate observers.

The statistical analyses utilised the Pearson chi-square test (Pearson χ^2) and Spearman rank correlation, which were measured using the Statistical Package for Social Sciences for Windows version 11.0 (SPSS Inc, Chicago, IL, USA).

RESULTS

The patients' ages ranged from 24 to 87 years (mean age \pm SD, 47.10 ± 11.16 years). The median age was 46 years. The predominant age group with the highest incidence of invasive ductal carcinoma of the breast was 40–49 years (40%, 153/382 patients), followed by the age group of 50–59 years (22%, 83/382 patients). The younger age group of 20–29 years (3.1%, 12/382 patients) made up a distant third place. Only 1.3% (5/382) of the patients in this study were very old women within the age group of 80–89 years. Ethnically, Malays comprised the predominant ethnic group in this study population, followed by Chinese, Indians, and others.

Looking at the tumour size at first diagnosis, it was found that the tumour size exceeding 10 cm (44%, $n = 168$) was the predominant tumour size among the invasive ductal carcinoma of the breast patients. There was only one case (0.26%) with a tumour size of less than 1 cm. Regarding the distribution of the disease according to the tumour side, it was almost equal on the right and left sides, with a few cases having bilateral involvement. There was no significant difference between the right and the left sides among the patients ($p > 0.05$). With lymph node involvement, 62.3% ($n = 238$) of patients had histological evidence of lymph node involvement, whereas the remaining 37.7% ($n = 144$) had no evidence of lymph node involvement. Furthermore, histological grade III predominated with the highest incidence among the invasive ductal carcinoma of the breast patients (47.1%, $n = 180$) compared to histological grade II (41.1%, $n = 157$) and histological grade I (11.8%, $n = 45$). Statistically, the difference between the cases with histological grades II and III was not significant ($p > 0.05$).

IHC staining revealed that anti-survivin polyclonal antibodies SUR12A-CFI specifically reacted with survivin in breast cancer cells. Positive staining revealed that survivin was mostly expressed in the cytoplasm. Survivin expression was not observed in normal tissues, except for some non-specific, background staining adjacent to cancer cells (Fig. 4). Survivin was also detected in both cytoplasmic and nuclear locations ($n = 30$) (Fig. 5). The intensity of survivin staining was generally homogenous, but the number of positive tumour cells stained by the anti-survivin polyclonal antibodies varied from 5% to 100% among the cases investigated.

It was found that the expression of survivin in all subjects was 68.1% (260/382 cases). The expression of survivin was correlated with age, histological grade, lymph node involvement, tumour size, tumour side, ER and PR status. Significant correlations were established only with the lymph node involvement ($p = 0.023$), and with tumour size ($p = 0.031$). The remaining clinicopathological characteristics did not correlate significantly with survivin expression (Table I).

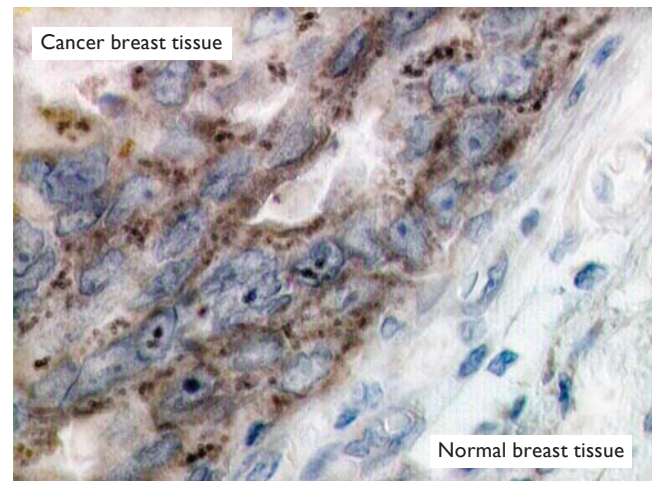


Fig. 4 Photomicrograph shows survivin staining in a formalin-fixed paraffin-embedded breast cancer tissue section scoring +++ (+) with mostly cytoplasmic staining and scanty nuclear positivity in tumour cells. In the IHC assay, SUR12A-CFI was used as the primary antibody at a dilution of 1:1280, and Tris-EDTA pH9 was utilised for antigen retrieval. No survivin staining is seen in the normal breast tissue on the right side although some non-specific background staining appears ($\times 400$).

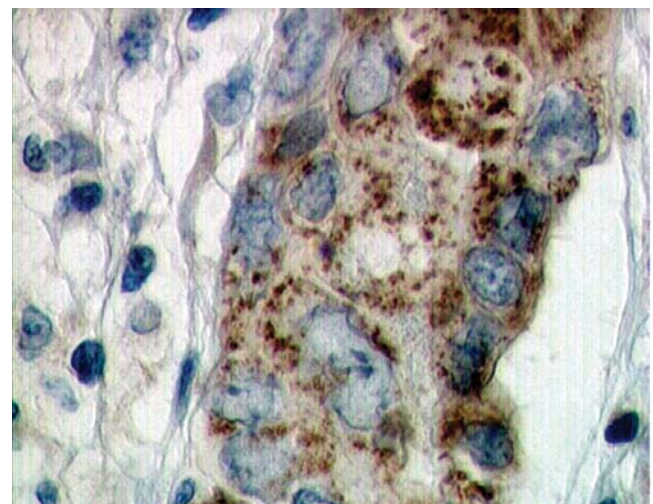


Fig. 5 Photomicrograph shows the expression of survivin in paraffin-embedded breast cancer tissue sections stained by IHC for survivin detection ($\times 400$).

It was found that, among patients with invasive ductal carcinoma of the breast, survivin-positive nuclear staining accounted for only 16.5% ($n = 63$), while positive cytoplasmic staining accounted for 24.1% ($n = 92$). 105 patients demonstrated positive staining results for both nuclear and cytoplasmic staining, making up 27.5% of the total (Fig. 6). Both nuclear and cytoplasmic staining of survivin were found to be higher in tumour sizes exceeding 10 cm (14.1%) compared to other tumour sizes. The data demonstrated a positive correlation between tumour size and survivin expression in both nuclear and cytoplasmic sites in cancer cells. (Chi-square test with

Table I. The correlation between clinicopathological characteristics and expression of survivin in breast cancer.

Characteristics (n = 382)	Survivin expression (no. of patients)			
	Positive	Negative	p-value	Statistical analyses
Age (years)				
≤ 50	179	79	NS 0.426	
> 50	81	43		
Histological grade				
I	30	15	NS 0.958	
II	108	49		
III	122	58		
Lymph node metastasis				
Node +	172	66	0.023	$\chi^2 = 5.138$ df = 1 Spearman correlation = 0.116
Node -	88	56		
Tumour size (cm)				
< 1	1	0	0.031	$\chi^2 = 10.625$ df = 4 Spearman correlation = - 0.102
1–2	10	1		
> 2–5	42	32		
> 5–10	83	45		
10	124	44		
Tumour side				
Right	127	57	NS 0.854	
Left	125	62		
Bilateral	8	3		
Estrogen receptor status				
Negative	130	57	NS 0.713	
Positive	64	31		
Progesterone receptor status				
Negative	134	59	NS 0.968	
Positive	46	20		

All analyses were tested using Pearson chi-square test (χ^2) and Spearman rank correlation; $p < 0.05$ is considered significant; NS: not significant; df: degree of freedom.

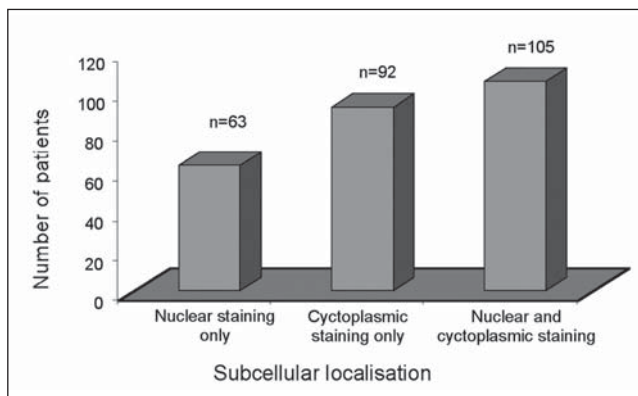


Fig. 6 Bar chart shows the subcellular localisation of survivin in survivin positive tissues among the invasive ductal carcinoma of the breast patients.

likelihood ratio 21.787; $p < 0.05$; Cramer's $V = 0.135$) (Table II).

The subcellular localisation of survivin was independent of the tumour grade ($p > 0.05$). Nevertheless, it is interesting to note that grade III tumours demonstrated the highest percentage (13.1%) of survivin-positive staining in both nuclear and cytoplasmic tests (Table III). In a comparison between tumour side and subcellular localisation of survivin, it was found that a considerable portion of the results displayed positive survivin staining for both nuclear and cytoplasmic tests. (14.4% on the left, 12.0% on the right). However, there was no statistically significant correlation between tumour side and subcellular localisation of survivin. In addition, there was a significant correlation between the subcellular localisation of survivin and lymph node involvement

Table II. The distribution of subcellular localisation of survivin among the survivin positive invasive ductal carcinoma of the breast according to the tumour size range.

Tumour size (cm)	Subcellular survivin expression (n = 210)			Statistical analyses*
	Nuclear staining only No. (%)	Cytoplasmic staining only No. (%)	Both nuclear and cytoplasmic staining No. (%)	
< 1	0 (0)	0 (0)	1 (0.3)	df = 12 LR = 21.787 p = 0.040 CV = 0.135
1–2	1 (0.3)	4 (1.0)	5 (1.3)	
> 2–5	17 (4.5)	14 (3.7)	11 (2.9)	
> 5–10	19 (5.0)	30 (7.9)	34 (8.9)	
> 10	26 (6.8)	44 (11.5)	54 (14.1)	

*Chi-square test (χ^2) was used for analysis by using likelihood ratio (LR); $p < 0.05$ is significant, df = degree of freedom; CV: Cramer's V test

Table III. The distribution of subcellular localisation of survivin among the survivin positive invasive ductal carcinoma of the breast according to the tumour grade.

Tumour grade	Subcellular survivin expression (n = 210)			p-value
	Nuclear staining only No. (%)	Cytoplasmic staining only No. (%)	Both nuclear and cytoplasmic staining No. (%)	
Grade I	10 (2.6)	10 (2.6)	10 (2.6)	NS 0.690
Grade II	29 (7.6)	34 (8.9)	45 (11.8)	
Grade III	24 (6.3)	48 (12.6)	50 (13.1)	

Table IV. The distribution of subcellular localisation of survivin among the survivin positive invasive ductal carcinoma of the breast according to the lymph node status.

Lymph node involvement	Subcellular survivin expression (n = 210)			Statistical analyses*
	Nuclear staining only No. (%)	Cytoplasmic staining only No. (%)	Both nuclear and cytoplasmic staining No. (%)	
Yes	22 (5.8)	68 (17.8)	82 (21.5)	χ^2 test = 40.039 p = 0.000 df = 3 CV = 0.324
No	41 (10.7)	24 (6.3)	23 (6.0)	

χ^2 : Pearson chi-square test; df: degree of freedom; CV: Cramer's V test

(Pearson chi-square test = 40.039; Cramer's V = 0.324; $p < 0.001$). The lymph node involvement coincided mostly with both nuclear and cytoplasmic staining (21.5%) compared to nuclear staining only (5.8%) and cytoplasmic staining only (17.8%). The involvement of lymph nodes was dependent on the subcellular localisation of survivin (Table IV).

However, there was no significant correlation between the subcellular localisation of survivin and the ER status ($p > 0.05$), though it was demonstrated that both nuclear and cytoplasmic staining was predominant regardless of the ER status, compared to nuclear staining only and cytoplasmic staining only. Similarly, the PR status did not correlate significantly with the subcellular localisation of survivin, since positive PR status coincided with 13.5% of cases with nuclear staining only, 19.3% of cases with cytoplasmic staining only, and with 18.9% of cases with both nuclear and cytoplasmic staining. Hence, survivin tended to be expressed more in cases with negative PR

status compared to those with positive PR status, although that relation was not statistically significant ($p > 0.05$).

DISCUSSION

Ethnic Malays from the predominant ethnic group (96%) in the states of Kelantan and Terengganu.⁽³⁹⁾ This may be the reason for the uneven ethnic distribution of the disease, with Malays forming the majority of patients with invasive ductal carcinoma of the breast (83.5%). No other conclusions could be drawn from the data obtained. Among the patients studied, grade III was the most common diagnosis (47.1%), followed by grade II (41.1%) and grade I (11.8%). These results were also directly proportional to findings on large tumour size (> 10 cm) and elevated levels of lymph node metastases. As the ratio of tumour position (left to right) was roughly equal (187:184), there was no significant findings to suggest a predisposition towards malignancy in a particular breast. Similar findings have been reported previously in the state

of Kelantan⁽³⁹⁾ as well as nationwide in Malaysia,^(41,42) where breast cancer would be at advanced stages on first diagnosis. This may be attributable to social and cultural factors. The age group of 40–49 years was found to have the highest incidence of breast cancer, a finding that corroborates with previous findings in Malaysia.^(39,41) This may be attributable to the fact that this age group represents the relatively highest population among the age-risk groups.

The aim of this study was to investigate the expression of survivin in invasive ductal carcinoma of the breast using IHC. Survivin expression was detected in 68.1% (260/382) of invasive ductal carcinoma of the breast patients studied. In previous reports, the survivin detection range was 60%–72.3%.^(5,6,10,32) Survivin was not detected in adjacent normal tissues (n = 30). This may point to a significant role for survivin detection, in making a diagnosis, or at least in the confirmation of malignancy. Furthermore, the subcellular distribution of survivin is predominant in both nuclear and cytoplasmic sites simultaneously (27.5%), whereas it was less detected in cytoplasmic staining alone (24.1%), and nuclear staining alone (16.5%). Previous reports have highlighted the significance of intracellular locations of survivin in relation to prognosis.^(5,6) Nevertheless, high survivin expression has been reported to correlate with poor prognosis⁽⁴³⁾ and has been used as an indicator to predict poor response to endocrine therapy, but a good response to chemotherapy in advanced breast cancer.⁽⁴⁴⁾ Similar findings have been reported in bladder mucosa, transitional cell carcinoma⁽⁴⁵⁾ and in gastric cancer.⁽⁴⁶⁾ Using monoclonal and polyclonal antibodies, different subcellular pools of survivin have been detected. A nuclear pool that segregates with nucleoplasmic proteins was identified, and a separate, and predominantly cytosolic pool, was associated with interphase microtubules, centrosomes, spindle poles, and mitotic spindle microtubules at the metaphase and anaphase. These two types of survivin are immunochemically distinct, independently modulated during cell cycle progression, and only cytosolic survivin associates with p34^{cdc2}. Phosphorylation of survivin by p34^{cdc2} – cyclin B has been identified as a requisite for apoptosis inhibition.^(47,48) The postulated explanation for these findings was that separate post-translational modifications could differently affect epitope accessibility of nuclear versus cytosolic microtubule-bound survivin in vivo. Hence, when nuclear survivin cannot associate with p34, an essential step in apoptosis, apoptosis may eventually be induced. This may explain why different patterns of survivin localisation are seen in different tumour types and associated with different prognoses. Moreover, it was reported that survivin-3B may act as an anti-apoptotic factor in breast cancer, where the expression of the variants of survivin varies differentially

with tumour progression and treatment.⁽⁴⁹⁾

It was also found that subcellular survivin expression was significantly correlated with lymph node involvement (p < 0.001). Hence nuclear expression had a minimum correlation with lymph nodal involvement, in contrast to cases that included cytoplasmic expression, solely or in combination with nuclear expression; these showed a stronger correlation with lymph nodal involvement. These findings logically suggest that patients with lymph node metastasis are likely to have more aggressive tumours compared to patients with no lymph node metastasis,⁽⁵⁰⁾ and positive expression of survivin may relate to highly aggressive tumour cells. Such information may be useful for oncologists in making decisions on the choice of treatment, especially when aggressive tumours would probably require relatively aggressive treatment.

ACKNOWLEDGEMENT

This work was supported by a Universiti Sains Malaysia short-term grant.

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