ORIGINAL ARTICLE

Survivin Expression Correlates with Unfavourable Prognoses in Invasive Ductal Carcinoma of the Breast

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SUMMARY

Survivin is a 16.5-kDa intracellular protein also known as AP14 or BIRC5. It inhibits apoptosis and regulates cell division and belongs to the inhibitors of apoptosis (IAP) gene family. In the majority of neoplasms investigated for survivin expression, high levels of the IAP proteins were predictive of tumour progression, either in terms of disease-free survival or overall survival, thus providing significant prognostic information. Hence, the prognostic value of survivin expression in tumour masses of invasive ductal carcinoma has been investigated. It was found that negative and low expression of survivin correlated significantly with favourable outcomes. Conversely, high expression correlated with unfavourable outcomes. The five-year survival rate was higher among the cases with low and negative survivin expression, compared to those with higher survivin expression. However, this correlation was found to be insignificant statistically. Furthermore, a statistical model has been devised to explain the combined effects of survivin expression and its sub-cellular localisation, p-53 expression and lymph nodal involvement, on the outcomes of these patients.

KEY WORDS:
Survivin expression, Invasive ductal carcinoma, Prognosis

INTRODUCTION

Survivin is a 16.5-kDa intracellular protein also known as AP14 or BIRC5. It inhibits apoptosis and regulates cell division and belongs to the inhibitors of apoptosis (IAP) gene family [1,2]. In the majority of neoplasms investigated for survivin expression including breast, lung, colorectal, gastric, liver, bladder and kidney cancers, neuroblastomas, gliomas, soft tissue sarcomas and hematological malignancies, high levels of the IAP proteins were predictive of tumour progression, either in terms of disease-free survival or overall survival, thus providing significant prognostic information[2-4]. In addition to the potential of survivin in diagnosis, monitoring and detection of recurrence of cancer [2], it has been reported that survivin has a prognostic value in a number of cancer types, including adenocarcinoma of the lung[5], soft tissue sarcoma [6], squamous cell carcinoma of the cervix [7] and breast cancer [8, 9]. The sub-cellular localisation of survivin may somehow predict the aggressiveness of a tumour possibly due to the effective action of cytoplasmic survivin in blocking apoptosis where it is phosphorylated for binding to caspase-9 [10] as nuclear survivin may not assume this activity [8].

In this work, the prognostic value of survivin expression in tumour masses of invasive ductal carcinoma (IDC) has been investigated. It was found that negative and low expression of survivin correlated significantly with favourable outcomes. Conversely, high expression correlated with unfavourable outcomes. The five-year survival rate was higher among the cases with low and negative survivin expression, compared to those with higher survivin expression. However, this correlation was found to be insignificant statistically. Furthermore, a statistical model has been devised to explain the combined effects of survivin expression and its sub-cellular localisation, p-53 expression and lymph nodal involvement, on the outcomes of these patients.

MATERIALS AND METHODS

The tissue blocks, their histopathology reports with other clinical findings, and the disease outcomes of 170 patients with IDC of the breast were obtained from The Department of Pathology and from the Medical Records Unit of the Hospital of The University of Science of Malaysia (HUSM) from 1992 to 2000. The tissue expression of survivin was performed using an immunohistochemistry assay that utilized, as primary antibody, polyclonal rabbit anti-sera raised against oligopeptides of the survivin molecule[11]. The quantification of survivin expression was assessed according to intensity and percentage of cells expressing survivin [11-13]. Briefly, they were classified by a scoring system into negative (score <1), low, medium and high, calculated by multiplying the cellular intensity of survivin staining (0, 1, 2 or 3) by the percentage of cells expressing survivin, measured in five microscopic fields and expressed in five categories as follows: (a) 0 < 5% (b) 1 = 5-25% (c) 2 = 26-50% (d) 3 = 51-75% (e) 4 > 75%. The sub-cellular localisation of survivin was defined as predominantly nuclear, nuclear and cytoplasmic, predominantly cytoplasmic, or exclusively cytoplasmic. Similarly, the expression of p-53 was detected using an immunohistochemistry assay that utilized a mouse anti-human p-53 antibody (DO-7; Dako).

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Statistical analyses of data utilized the Statistical Package for Social Sciences (SPSS version 11.0 software package for Macintosh, SPSS Inc., Chicago, IL).

RESULTS

The correlation between the outcomes of patients with survivin expression.

The outcome of the study patients, being still alive or having deceased, showed a significant correlation with the survivin status (Pearson Chi-square = 43.509, Spearman Correlation = -0.506, p<0.001). Hence, 46.5% (n=79) of the patients who died expressed survivin, compared to 19.4% (n=33) with negative survivin expression. Among the patients who were still alive, only 5.9% (n=10) were survivin positive, compared to 28.2% (n=48) who were survivin negative (Figure 1). Furthermore, it was found that high survivin scores coincided with high death rates (49 out of 50 for high survivin scores, and 26 out of 29 for moderate survivin scores). Conversely, low survivin scores significantly coincided with a relatively lower death rate (4 out of 10) (Pearson Chi-square 28.142, Spearman Correlation= 0.433, p<0.001) (Figure 2).

Prognostic analysis in patients with IDC of the breast.

Using Kaplan-Meier curves, the percentage of survivors was plotted against the survival time (years) for both survivin positive and survivin negative cases. The overall 5-year survival among the survivin-positive cases was 21.5% (17 out of 79). The percentage of the 5-year survival was 33.3% among the survivin negative cases (11 out of 33). However, this difference was statistically not significant (p=0.4; log rank test) (Figure 3).

Influence of independent factors on the outcome variable (dependent factor).

Multiple regression was used in order to examine the influence of other factors on the outcome variable (alive or dead) such as age, survivin score, survivin expression and its' sub-cellular localisation, lymph node involvement and p53 expression. In this test, all independent factors were controlled as to see their influence on the outcome variable (Table I).

The F value was 30.106, which was significant with the value $R^2 = 0.565$, $p < 0.0001$, showing that this multiple regression model can explain 56.5% of the variation of the dependent outcome (alive or dead) among the subjects under study. This means that, once the age factor is controlled, survivin expression and its score, sub-cellular localisation, p53 expression, and the nodal involvement, can predict the outcome (alive or dead) significantly (Table I). Based on the results, the multiple regression model obtained is:

Outcome (alive or dead) = 1.1946 - 1.5155 (survivin expression) + 0.2797 (survivin sub-cellular localisation) + 0.4269 (survivin score) + 0.1379 (p53 expression) + 0.1029 (lymph nodal involvement)

DISCUSSION

The assessment of prognosis is important in patients with malignancies because their results serve to separate large heterogeneous populations into smaller populations with more concisely predictable outcomes [14, 15]. In this report, survivin expression, especially the cytoplasmic, is being shown to have a significant correlation with the disease outcome. This has been explained by the finding that cytoplasmic survivin is less efficient in preventing apoptosis than nuclear survivin [13, 16]. The scoring system of survivin expression was found to be useful as a prognostic indicator since high survivin scores coincided with elevated death rates and low scores coincided with high survival rates. The prognostic value of this system may prove to be more accurate than that of the general expression of survivin. It is notable that the majority of patients presented with high survivin scores. This has been investigated previously, and it was found that the high survivin expression correlated strongly with higher histopathological grades [17]. Furthermore, many cases in Malaysia present with high pathological grades at first diagnosis [17, 18].

It the present study, it was found that survivin expression significantly correlated with the outcome in invasive ductal carcinoma of the breast. The high expression of survivin was detected mostly in patients who died within relatively short periods of time. On the contrary, patients who survived the disease had lower survivin expression. These findings are in agreement with previous studies which reported that the overall survival of survivin-positive patients was significantly less than that of individuals whose tumors were negative for survivin expression[5]. Other studies have implicated survivin as an independent prognostic indicator [19] and that it may predict response to therapy [20]. Overall, the expression of survivin in tumour cells may be an indicator, and possibly a factor of poor prognosis [6] and high aggressiveness of tumour cells [7]. In addition, it is thought that survivin may be a promoter of cell transformation [21].

The relationship between prognosis and the sub-cellular expression of survivin has witnessed some controversies; these may be due to the different techniques used, various antibody populations utilized, different scoring systems adopted, and various sample sizes used, as well as different interpretations. There are some findings suggesting that the sub-cellular localisation of survivin may somehow predict the aggressiveness of a tumour [10]. This may be due to the effective action of cytoplasmic survivin in blocking apoptosis where it is phosphorylated for binding to processed caspase-[9 22]. Nuclear survivin may not assume this activity.

Table I: Multiple regression "Forward Stepwise" to see the influence of other factors on the outcome: alive or dead.

	Dependent variable: Outcome (alive or dead) R= 0.7519, R^2=0.5654, F=30.106		
Independent variables (n=170)	**Regression coefficients (β)**	**t value**	**p value**
Constant	1.1946	6.3123	p<0.0001
Survivin expression	-1.5155	-12.6023	p<0.0001
Survivin sub-cellular localisation	0.2797	8.3610	p<0.0001
Survivin score	0.4269	7.8042	p<0.0001
p53 expression	0.1379	2.3578	p<0.0001
Nodal involvement	0.1029**	2.0267	p<0.05

** β is significant at p< 0.05

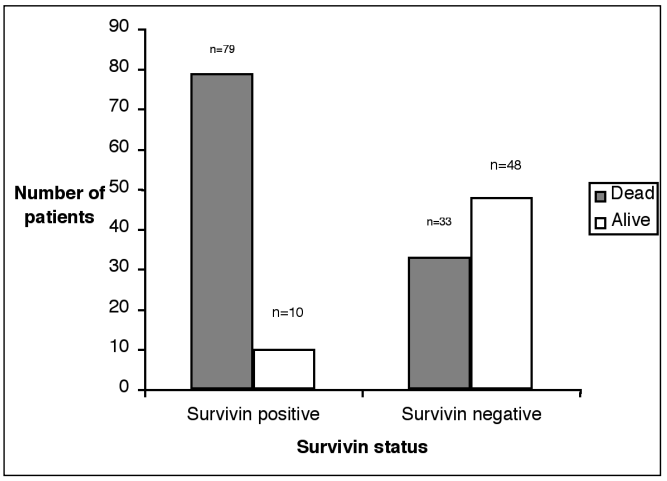

Fig.1: The survivin status among the dead and alive patients from 1992 to 2000 until December 2004 (Pearson Chi-square= 43.509, p< 0.001).

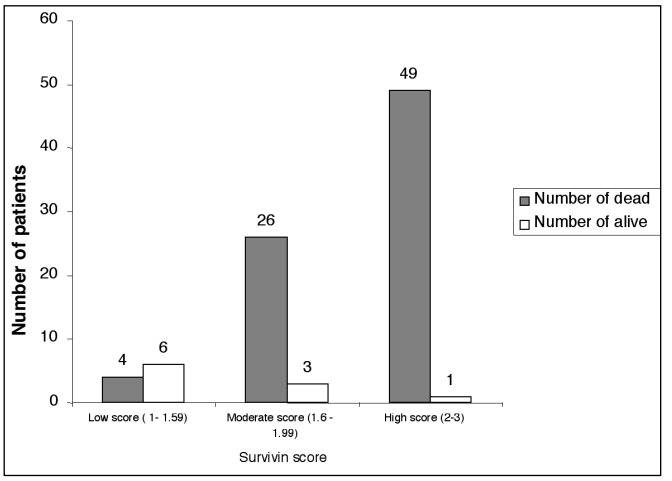

Fig.2: The survivin score classified according to the outcome of patients with invasive ductal carcinoma of the breast having positive survivin staining (Pearson Chi-square 28.142, Spearman Correlation= 0.433, p<0.001).

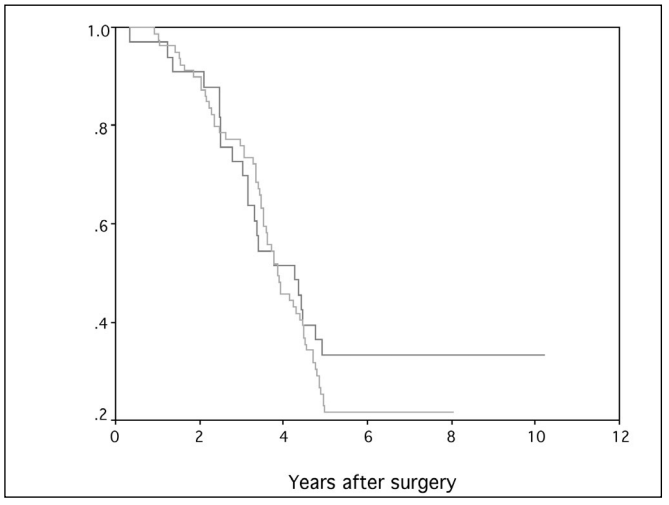

Fig.3: Kaplan-Meier curves for overall 5-year survival rates of invasive ductal carcinoma of the breast patients, categorized according to survivin expression. No significant difference was found between the groups (p=0.4; log-rank test)

Previous results obtained demonstrated that both nuclear and cytoplasmic staining was higher in the presence of lymph node involvement compared to no lymph node involvement. In positive lymph node involvement, nuclear staining was only 5.8%, cytoplasmic staining only 17.8% whereas both nuclear and cytoplasmic staining was predominant with 21.5% expression [17]. 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 [7]. Hence, positive expression of survivin may be related to the aggressiveness of tumour cells. Hence, survivin expression may serve as a marker for prognosis. In addition, its intra-cellular location may aid in the diagnosis. Furthermore, its prognostic implications coupled with the knowledge of its biologic functions suggest that survivin may serve as therapeutic target.

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