

Autoantibodies to Survivin in the Sera of Patients with Infiltrating Ductal Carcinoma of the Breast

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Summary

Autoantibodies to survivin have been reported in lung cancers and in gastrointestinal cancers. A few reports have also described a low prevalence of autoantibodies to survivin and at low titres in the sera of breast cancer patients with no implications for their clinical usefulness. This study was designed to re-examine the prevalence and the clinical correlations of autoantibodies to the tumour protein survivin, in the sera of patients with infiltrating ductal carcinoma of the breast using an ELISA assay. In spite of the low prevalence of autoantibodies to survivin (7%, n=57), their presence was associated with grade III tumours, with tumour sizes exceeding 10cm, with axillary lymph nodal involvement and with metastases. Moreover, all the autoantibody-positive cases were estrogen and progesterone receptors negative. Furthermore, all the autoantibody-positive cases expressed survivin with high scores.

Key Words: Autoantibodies, Survivin, Breast cancer

Introduction

Autoantibodies are antibodies that are reactive with self or autoantigens. They are commonly associated with autoimmune diseases. However, autoantibodies to a variety of cellular antigens have been associated with the development and progression of cancer¹. Hence during the past three decades, many reports have described the existence of autoantibodies to a variety of cancer-associated antigens. The main objective in such studies has been to employ these antibodies in investigating the role of their relevant autoantigens in the development and progression of cancer, in addition to their potential usefulness in diagnosis, prognosis and management of breast cancer patients. Examples of such autoantigens are the IgG Fab fragment², breast tumour-associated antigen³, murine mammary tumor virus⁴, T-cell antigens⁵. Furthermore, autoantibodies to proteins associated with malignant transformation of cells such as proto-oncogenes have been reported⁶, in addition to autoantibodies to cellular proteins involved

in the regulation of cell division and cell cycle such as P53, HER-2/neu and ras^{6,8}. Survivin is yet another cellular protein which functions in the regulation of cell division and acts in the prevention of apoptosis. Autoantibodies to survivin have been reported in lung cancers and in gastrointestinal cancers⁹⁻¹¹. In breast cancer, however, only few previous reports have described the presence of autoantibodies to survivin at low titres in the sera of breast cancer patients with no implications for their clinical usefulness^{10, 12, 13}. This study was designed to re-examine the prevalence and the clinical correlations of autoantibodies to the tumour protein, survivin in the sera of patients with infiltrating ductal carcinoma of the breast.

Materials and Methods

Antigens. Two peptides, peptide I with survivin amino sequence of MGAPTLPPAWQP (12 amino acids, molar mass 1245.48 g/mol, empirical formula C₅₉H₈₄N₁₄O₁₄S₁)

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and peptide II with survivin amino sequence of KEFEETAKKVRRAIEQLAAMD (21 amino acids, molar mass 2,354.76 g/mol and empirical formula $C_{106}H_{179}N_{31}O_{34}S_1$) were provided by Pepton Company, Korea. They were synthesized by the Fmoc solid method, with 95% purity and confirmed for the amino acid composition by mass spectrometry.

Sera. Blood samples from 57 female patients with infiltrating ductal carcinoma of the breast were obtained with informed consent from Universiti Sains Malaysia Hospital (HUSM), Kelantan, Kota Bharu General Hospital (HKB), Kelantan, and Kuala Terengganu General Hospital (HKT), Kuala Terengganu, Terengganu, Malaysia. The blood samples were collected as soon as diagnoses had been established and before any treatments were started. Healthy blood donors without any history of cancer were obtained from the Kelantanese Population for the control group and for the determination of a cutoff point for positivity in the ELISA assay. After clotting and centrifugation, sera were aliquoted and stored at -20°C.

ELISA. The indirect ELISA procedure used is a modification of a previously described method⁹. The two purified oligopeptides were diluted in 50mM bicarbonate buffer (pH 9.5) to a final concentration of 5 µg/ml each, as determined by the Bradford assay (BioRad) and were dispensed into 96-well plates (100 µl/well) and incubated overnight at 4 °C. After removal of the protein solution by flipping out, the wells were blocked for 1 h at 37°C with 100 µl/well of a 5% dried skimmed milk solution in phosphate-buffered saline (PBS). The plates were washed five times with phosphate-buffered saline-Tween (PBST). Serum samples diluted 1:100 in PBS were added at 100 µl well, incubated for 1 h. The plates were then flipped out and washed five times with PBST. Each well was then incubated for 1 h with 100 µl of 1:2000 dilution of goat antihuman IgG F(ab') labeled with horseradish peroxidase (DAKO), washed five times with PBST, and developed by adding 100 µl of a tetrabenzidine solution [1 mg of tetramethylbenzidine (Sigma-Aldrich) diluted in 10ml of 0.05 M citrate buffer (pH = 5.0) with 0.006% H₂O₂]. After a 5-min incubation in the dark, the reaction was stopped with 100 µl of 0.25 M H₂SO₄, and the absorbance was measured at 490 nm. All serum samples were run in duplicates and randomly distributed on the plates. Sera from cancer patients and sera from healthy donors were tested simultaneously.

Results

Sera from 57 women with ages ranging from 32 to 79 years (average age 46.75, SD ± 12.04 years) were recruited for this study. Blood was taken from each patient three days before surgery. The diagnoses were confirmed by histopathology. For the negative control group, sera from 44 apparently healthy homogenous Kelantanese donors, without any history of cancer were collected. Their ages ranged from 22 - 47 years (average age ± SD, 35.45 ± 7.89 years). They were selected to determine the cutoff point for positivity in the ELISA. The average absorbance at 490 nm ± SD obtained with the sera of blood donors was 0.045 ± 0.007 (n=44). The cutoff for positive antibody reactivity against survivin was taken as 0.059, which was defined as an absorbance greater than 2 SDs above the mean value of the controls (Figures 1 and 2).

The mean absorbance of the breast cancer group sera was 0.051 ± 0.007 (n=57) (Figure 2). Sera of 4 out of 57 of the breast cancer patients (7.0%) were reactive with the survivin oligopeptides in ELISA, whereas none of the control group sera from healthy blood donors recognized survivin. They were also strongly positive for survivin in the immunohistochemical analysis (unpublished data). Furthermore, all these four cases were Grade III (Bloom and Richardson's grading), with tumour sizes exceeding 10cm, all with metastases, and all with axillary nodal involvement, and estrogen and progesterone receptors negative.

Discussion

The autoantibody status of a concern patient may support a diagnosis, serve as predictors of disease progression, and or act as a guide to select the appropriate management. Survivin may have such a role and may be explored as a selected antigen for anti-tumor vaccination.

With clinical correlations, a negative survivin autoantibody status may be useful in excluding metastases, and a positive autoantibody status may confirm the grade or progression of a disease. This report confirms some previous reports on the prevalence of autoantibodies to survivin where relatively high cutoff points and low autoantibody titres were obtained, in addition to the variable prevalence rates of 6.3% (n=16) and 7.8% (n=64) and 23.9%^{10, 12, 13}. The variability in the prevalence of the survivin

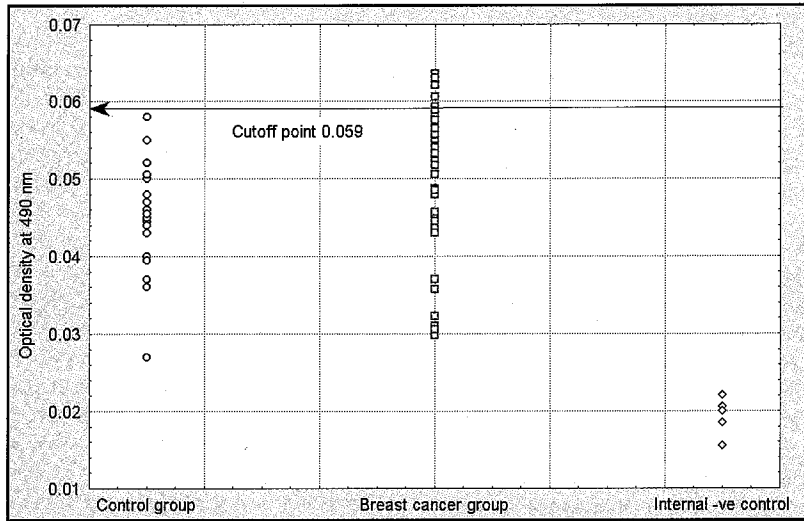


Fig. 1: The cutoff point of positivity for autoantibodies to survivin at 0.059 and the optical density distribution among the subject according to the groups and internal negative control in which sera of breast cancer were replaced by PBS.

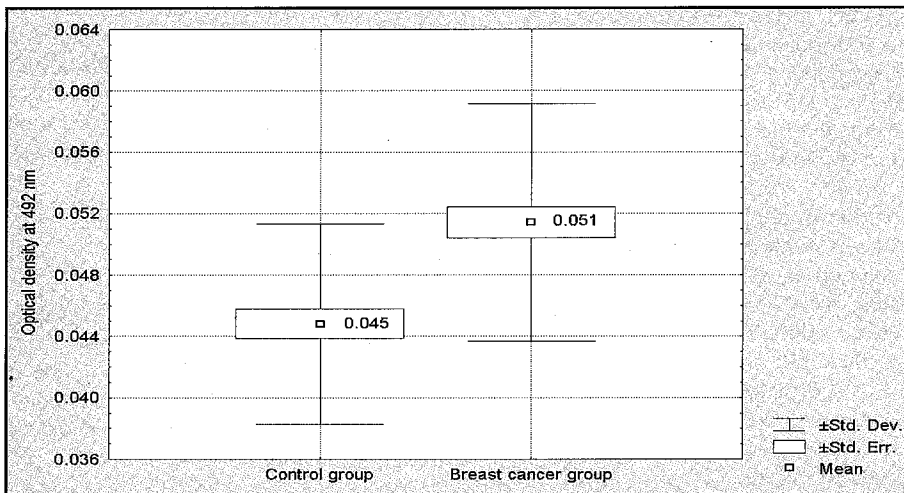


Fig. 2: The mean, standard deviation and standard error of the each group in the study.

autoantibody may be due to the nature of the disease, where cells in infiltrating ductal carcinoma of the breast do not have a great tendency to be desquamated off the tumour mass until the mass becomes big enough to increase the tumour cell load in the circulation. Cells of lung cancers and colorectal cancers may behave differently where they may have bigger tendencies to desquamate at earlier stages exposing their intrinsic

cellular antigens to the immune system. Hence, the prevalence of autantibodies to survivin in those reports ranged from 21.6% to 58.1%¹⁰⁻¹². It is generally believed that the bigger the size of a tumour, the more the probability of exposure of cellular antigens to the immune system, in which case, natural or chemically induced apoptosis or necrosis would be evident. The efficiency of the immune system may be another factor,

especially considering the immuno-compromised status of patients with advanced disease. Moreover, the immunosuppression may become more pronounced later due to the combined effect of chemotherapy and radiotherapy on the immune system, hiding any potential for autoantibody production.

Nevertheless, the low prevalence detected with breast cancer sera may be due to technical errors, especially considering the relatively high cutoff point and the low survivin-specific antibody titres in this report as well as in previous reports^{11, 12, 13}. This may prompt the development of a more sensitive assay system with a better magnification of the ELISA signal, such as using the avidin-biotin complex, or by the detection of survivin auto-reactive lymphocytes. Alternatively, dissection of the immune response in breast cancer patients by Epstein-virus transformation of lymphocytes of breast cancer patients may unveil minor or rare clones of survivin-reactive B-lymphocytes¹⁴, although such tests cannot be carried out in a routine clinical practice.

Ultimately, even with proven clinical significance, the data available may arouse an unnecessary confusion on their interpretation, unless higher values and lower cutoff points are achieved, in addition to consistent results obtained in trials utilizing larger numbers of patients to consolidate the autoantibody-metastasis correlations.

In addition, the findings raise the possibility that the anti-survivin immune response may be useful as a tool for investigating some aspects of the immune response to tumours, and may enhance the understanding of the mechanism (s) of breast cancer development and progression with special consideration of the correlation between the autoantibodies and the advanced disease status.

A clear example was the demonstration of autoantibodies to survivin in a patient one year after surgery for a biliary-tract cancer: the patient had no anti-survivin antibodies before or shortly after surgery. At the time reactivity was detected, a local recurrence of cancer was found¹⁰. Similarly, anti-p53 antibodies have been reported to be detectable several years before the clinical manifestation of lung cancer⁹. In the case of breast cancer patients in this study, only 7% of patients' sera were positive pre-operatively. No post-operative follow-up was performed. Hence, findings of such follow-up studies may yield significant information. Whether anti-survivin antibodies could also serve as early predictive markers in patients at high risk of developing cancer, remains an open question.

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