

CD44 expression and axillary lymph node metastasis in infiltrating ductal carcinoma of the breast

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Abstract

Metastasising ability connotes one of the most important life-threatening properties of malignant neoplasms. Recent studies indicate that CD44 proteins, multifunctional cell adhesion molecules which contribute to “homing” of lymphocytes to lymph nodes as well as cell-cell and cell-matrix interactions, are potential markers of tumour progression. However, whether CD44 expression by human tumours contribute to increased metastatic risk remains controversial. In an attempt to clarify its role in breast cancer, we have investigated the correlation between CD44 expression by breast carcinoma and the presence of axillary lymph node metastases. CD44 expression was detected using a standard immunoperoxidase method on formalin-fixed, paraffin-embedded, primary infiltrating ductal breast carcinoma tissues taken from 60 female patients who underwent mastectomy with axillary node clearance. Tumours were graded according to the modified Bloom and Richardson criteria. 62% of patients had histologically-proven lymph node metastasis. 40% of primary cancers exhibited cytoplasmic membrane immunopositivity for CD44. 46% of primary tumours which have metastasised to axillary lymph nodes were CD44 positive whereas 30% of tumours which have not metastasised expressed CD44. CD44 positivity was expressed by 20% of grade 1, 31% grade 2 and 58% grade 3 tumours. Our results suggest that CD44 may have a role in the progression of breast cancer and emphasise the need to investigate its interaction with other mechanisms of cancer advancement.

Key words: CD44, breast cancer, metastasis, tumour progression.

INTRODUCTION

Neoplastic diseases constitute a major cause of morbidity and mortality in most populations in the world, including developing countries such as Malaysia. In general, it is recognized that the main factors which influence the prognosis of patients with malignancies are tumour size, histological type and grade, and clinical stage. Metastasising ability connotes one of the most important life-threatening properties of malignant neoplasms. Recent studies indicate that CD44 proteins, multifunctional cell adhesion molecules which contribute to “homing” of lymphocytes to lymph nodes as well as cell-cell and cell-matrix interactions, are potential markers of tumour progression.^{1,2} However, whether CD44 expression by human tumours contribute to increased metastatic risk remains controversial.³⁻⁶ In an attempt to clarify its role in breast cancer, we have investigated the correlation between CD44 expression by breast carcinoma and the presence of axillary lymph node metastases.

CD44 expression was also compared against histological grade, another important prognostic indicator.⁷

MATERIALS AND METHODS

Consecutive female patients who underwent mastectomy with axillary node clearance for primary infiltrating ductal breast carcinoma at the University of Malaya Medical Centre, Kuala Lumpur over a two-year period were included in this retrospective study. Formalin-fixed, paraffin-embedded tissues taken from the primary tumours were processed for histopathological examination. All sections made were reviewed for confirmation of the diagnosis, histological typing, and grading according to the modified Bloom and Richardson criteria.⁷ All axillary lymph nodes were examined histologically for the presence of metastasis. Only infiltrating ductal breast carcinomas were included in this study. CD44 expression by the tumour cells was detected by a standard immunoperoxidase

method using a commercial mouse anti-human monoclonal antibody (Dako) against standard isoform CD44. A tumour was considered to be CD44 positive if more than 10% of tumour cells expressed membrane immunopositivity against the antibody (Figure 1).

RESULTS

Sixty infiltrating ductal carcinoma of breast treated by mastectomy and axillary lymph node clearance were included in this study. The patients' ages ranged from 27 years to 83 years. 10 (17%) tumours were categorised histologically as grade 1, while 26 (43%) were grade 2 and 24 (40%) were grade 3. 37 (62%) patients had histologically-proven lymph node

metastasis. 24 (40%) primary cancers exhibited cytoplasmic membrane immunopositivity for CD44.

Table 1 compares CD44 immunopositivity against histological grade and the presence of axillary lymph node metastasis. There was a progressive increase in percentage of CD44 positive tumours according to histological grade, with a positivity rate of 58% in grade 3 tumours. Although a larger proportion (46%) of primary tumours which had metastasised to axillary lymph nodes were CD44 positive compared with tumours which had not metastasised, this difference did not reach statistical significance (p=0.285).

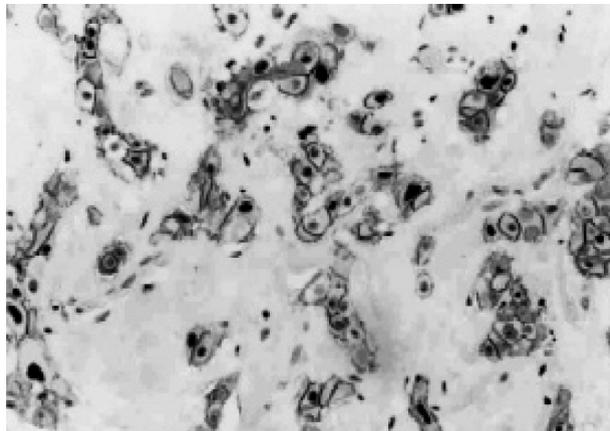


FIG. 1: Infiltrating ductal carcinoma of breast showing membrane immunopositivity for CD44.

TABLE 1: Correlation between CD44 immunopositivity, histological grade and axillary lymph node metastasis in 60 infiltrating ductal carcinoma of breast

| | <i>CD44 Positive No. (%)</i> | <i>CD 44 Negative No. (%)</i> | <i>Total No.</i> |
|----------------------|----------------------------------|-----------------------------------|------------------|
| <i>Grade 1</i> | 2 (20.0) | 8 (80.0) | 10 |
| <i>Grade 2</i> | 8 (30.8) | 18 (69.2) | 26 |
| <i>Grade 3</i> | 14 (58.3) | 10 (41.7) | 24 |
| <i>Node positive</i> | 17 (45.9) | 20 (54.1) | 37 |
| <i>Node negative</i> | 7 (30.4) | 16 (69.6) | 23 |
| | 24 (40.0) | 36 (60.0) | 60 |

DISCUSSION

Our results indicate an increasing trend in CD44 expression with increasing histological grade of breast cancer suggesting that CD44 may be linked to tumour dedifferentiation and increasing aggressiveness. CD44 was also expressed in a higher proportion of tumours which had metastasized by the time of surgery compared to those which had not. Thus, although this finding did not reach statistical significance, the possibility that CD44 may play a role in the cancer progression cannot be dispelled. It can also be speculated whether node-negative, CD44-positive tumours face imminent metastasis but this had not yet occurred at the time of surgery or was not detectable by conventional histopathology.

CD44 is a widely expressed, multi-structural, multifunctional, cell adhesion molecule encoded by 20 exons, at least 10 of which are subjected to alternative splicing, resulting in the generation of a large amount of CD44 variants.² These isoforms modulate the many reported roles of CD44 in cell biology and pathology, including activation and homing of lymphocytes, T-cell activation, binding to hyaluronic acid, cell-cell and cell-extracellular matrix interaction.^{1,2,8} Based on these, CD44 appears promising as a modulator of wound healing, angiogenesis and the cancer metastatic process.^{8,9} *In vitro* studies suggest that CD44 may play an important role in the mechanisms whereby tumours migrate and invade into tissues.^{9,10} However, the complexity of the molecule and its many spliced variants as well as subsequent modification by glycosylation and proteases has caused great difficulty in elucidating its role in cancer progression, and probably explain the many conflicting findings that abound in the scientific literature.¹¹⁻¹⁷ Our study, being based on the immunohistochemically detectable expression of unspliced CD44 standard, does not determine the expression patterns of the various isoforms and thus may not reflect the true status of CD44 expression by the tumours studied. Nevertheless, in spite of its limitations, a trend suggestive of a positive relationship between CD44 expression and tumour aggressiveness (grade) and metastasis has been observed. We emphasise the need to further investigate the interaction of CD44 and its variants with other cellular mechanisms which may contribute to cancer advancement.

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REFERENCES

1. Marhaba R, Zoller M. CD44 in cancer progression: Adhesion, migration and growth regulation. *J Mol Histol* 2004; 35: 211-231.
2. Naor D, Sionov RV, Ish-Shalom D. CD44: structure, function, and association with the malignant process. *Adv Cancer Res* 1997; 71: 241-319.
3. Lopez JI, Camenisch TD, Stevens MV, Sands BJ, McDonald J, Schroeder JA. CD44 attenuates metastatic invasion during breast cancer progression. *Cancer Res* 2005; 65: 6755-63.
4. Götte M, Yip GW. Heparanase, hyaluronan, and CD44 in cancers: a breast carcinoma perspective. *Cancer Res* 2006; 66: 10233-7.
5. Watanabe O, Kinoshita J, Shimizu T, Imamura H, Hirano A, Okabe T, Aiba M, Ogawa K. Expression of a CD44 variant and VEGF-C and the implications for lymphatic metastasis and long-term prognosis of human breast cancer. *J Exp Clin Cancer Res* 2005; 24: 75-82.
6. Draffin JE, McFarlane S, Hill A, Johnston PG, Waugh DJ. CD44 potentiates the adherence of metastatic prostate and breast cancer cells to bone marrow endothelial cells. *Cancer Res* 2004; 64: 5702-11.
7. Elston CW, Ellis IO. Pathological prognostic factors in breast cancer. I. The value of histological grade in breast cancer: experience from a large study with long-term follow-up. *Histopathology* 1991; 19: 403-410.
8. Borland G, Ross JA, Guy K. Forms and functions of CD44. *Immunology* 1998; 93: 139-48.
9. Jothy S. CD44 and its partners in metastasis. *Clin Exp Metastasis* 2003; 20: 195-201.
10. Bourguignon LY, Singleton PA, Diedrich F, Stern R, Gilad E. CD44 interaction with Na⁺-H⁺ exchanger (NHE1) creates acidic microenvironments leading to hyaluronidase-2 and cathepsin B activation and breast tumor cell invasion. *J Biol Chem* 2004; 279: 26991-7007.
11. Ichikawa W. Positive relationship between expression of CD44 and hepatic metastasis in colorectal cancer. *Pathobiology* 1994; 62: 172-9.
12. Liu YJ, Yan PS, Li J, Jia JF. Expression and significance of CD44s, CD44v6, and nm23 mRNA in human cancer. *World J Gastro* 2005; 11: 6601-6.
13. Ma W, Deng Y, Zhou L. The prognostic value of adhesion molecule CD44v6 in women with primary breast carcinoma: a clinicopathologic study. *Clin Oncol* 2005; 17:258-63.
14. Abraham BK, Fritz P, McClellan M, Hauptvogel P, Athellogou M, Brauch H. Prevalence of CD44+/CD24-/low cells in breast cancer may not be associated with clinical outcome but may favor distant metastasis. *Clin Cancer Res* 2005; 11: 1154-9.

15. Berner HS, Suo Z, Risberg B, Villman K, Karlsson MG, Nesland JM. Clinicopathological associations of CD44 mRNA and protein expression in primary breast carcinomas. *Histopathol* 2003; 42: 546-54.
16. Wobus M, Rangwala R, Sheyn I, Hennigan R, Coila B, Lower EE, Yassin RS, Sherman LS. CD44 associates with EGFR and erbB2 in metastasizing mammary carcinoma cells. *Appl Immunohistochem Mol Morphol* 2002; 10: 34-9.
17. Günthert U, Stauder R, Mayer B, Terpe HJ, Finke L, Friedrichs K. Are CD44 variant isoforms involved in human tumour progression? *Cancer Surv* 1995; 24: 19-42.