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Short Communication

Prognostic Value of Immunohistochemically Detected CD44 Isoforms CD44v5, CD44v6 and CD44v7-8 in Human Breast Cancer

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We investigated the expression of CD44 isoforms containing variant exons v5, v6 and v7-8 in 115 human breast cancer specimens by means of immunohistochemistry. CD44 isoforms CD44v5, CD44v6 and CD44v7-8 were detected in 56% (n = 64), 24% (n = 28) and 15% (n = 17), respectively. In 36 specimens of axillary lymph node metastasis, expression of CD44v5, CD44v6 and CD44v7-8 was found in 94% (n = 34), 92% (n = 33) and 89% (n = 32), respectively. Five year survival rates with or without CD44v5 and CD44v6 expression were 71% versus 86% (log-rank test, P = 0.02) and 62% versus 81% (log-rank test, P = 0.001), respectively. For disease-free survival, expression of CD44v5, CD44v6 and CD44v7-8 showed a prognostic impact (log-rank test, P = 0.004, P = 0.0001 and P = 0.0001, respectively). However, multivariate analysis revealed that all investigated CD44 isoforms failed to be independent predictors of the patient's outcome.

Key words: breast, neoplasm, adhesion molecules, prognosis

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INTRODUCTION

THE OVEREXPRESSION of CD44 isoforms has been shown to be associated with metastasis and poor prognosis in human malignancies such as cervical cancer, colorectal cancer, gastrointestinal lymphoma, non-Hodgkin's lymphoma, thyroid carcinoma, leukaemia and malignant brain tumours [1–7]. It has been speculated that tumour cells expressing CD44 isoforms could mimic lymphocytes thus exploiting homing receptors present on endothelial cells [8]. Another theory suggests that activated lymphocytes temporarily express CD44v6 [9]. Activated lymphocytes are known to reside for extended periods in the draining lymphoid tissue. Tumour cells expressing CD44v6 could thus enhance their chances of metastasis formation in the regional lymph nodes [8]. We examined the expression of the CD44 isoforms CD44v5, CD44v6 and CD44v7-8 in 115 breast cancer specimens and determined the prognostic value of these cell surface markers.

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MATERIALS AND METHODS

We investigated 115 paraffin-embedded tumour specimens of primary breast cancer and 36 specimens of axillary lymph node metastasis of a randomly selected patient sample. Histological staging was performed according to the current International Union Against Cancer (UICC) classification [10]. UICC stages pT1, pT2 and pT3 were present in 66, 46 and 3 cases, respectively. The median age at diagnosis was 54 years (range 23–81 years).

The median follow-up time was 78 months (range 11–172 months). During the observation period, 11 patients showed locoregional recurrence. 32 patients developed distant metastases. 7 patients had local recurrence and distant metastases. 38 patients died from the disease.

Immunohistochemistry

Immunohistochemical procedures were performed as described previously [3].

Statistics

The chi-square test was used where appropriate. Associations between the expression of different CD44 epitopes was

described by the Kendall Tau correlation coefficient. We calculated the survival probabilities by the product limit method of Kaplan and Meier. Differences between the groups were tested using the log-rank test. Cox proportional hazards regression model was used to assess the independence of effects of different prognostic factors. *P*-values <0.05 were considered statistically significant.

RESULTS

CD44 isoforms CD44v5, CD44v6 and CD44v7-8 were detected by means of immunohistochemistry in 56% ($n = 64$), 24% ($n = 28$) and 15% ($n = 17$) of the 115 tumour samples, respectively. In 36 specimens of axillary lymph node metastasis, expression of CD44v5, CD44v6 and CD44v7-8 was found in 94% ($n = 34$), 92% ($n = 33$) and 89% ($n = 32$), respectively.

We found homogenous staining in tumours that were considered positive for CD44 expression (Figure 1). The correlation coefficients between expression of CD44v5 and CD44v6, CD44v5 and CD44v7-8 and between the expression of CD44v6 and CD44v7-8 were 0.51, 0.22 and 0.33.

In the univariate analysis, the expression of CD44v5 and CD44v6 was correlated with a poor overall survival. Five year survival rates with or without CD44v5 expression were 71% versus 86% (log-rank test, $P = 0.02$). Five year survival rates with or without CD44v6 expression were 62% versus 81% (log-rank test, $P = 0.001$). The expression of CD44v7-8 could not predict patients' survival. For disease-free survival, expression of CD44v5, CD44v6 and CD44v7-8 showed a prognostic impact (log-rank test, $P = 0.004$, $P = 0.0001$ and $P = 0.0001$, respectively). In the axillary lymph node-negative patients ($n = 79$), the expression of CD44v6 was correlated with both a poor overall survival (log-rank test, $P = 0.04$) and a poor disease-free survival (log-rank test, $P = 0.03$).

We found a strong correlation between CD44v6 expression and histological grading (chi-square test, $P = 0.009$) and axillary lymph node metastasis (chi-square test, $P = 0.01$).

A multivariate analysis, correcting for the confounding variables axillary lymph node status, tumour size and histological grading was performed as shown in Table 1. Expression of none of the CD44 isoforms reached statistical significance.

Figure 1. Expression of CD44 isoforms containing variant exon v6 in a primary breast carcinoma specimen with staining of cell membranes. Stroma cells are negative. Magnification × 140; counterstain was haematoxylin.

Table 1. Multivariate analysis of prognostic factors for overall survival

Prognostic factors	*P*	Relative risk	95% confidence interval
Axillary nodal status (pN + versus pN−)	**0.001**	2.5	1.2–5.0
Tumour size (pT1 versus pT2+pT3)	**0.02**	2.4	1.1–5.2
Histological grading (G1 versus G2 versus G3)	**0.03**	1.8	1.0–3.1
CD44v5	0.06	2.1	0.95–4.5
CD44v6	0.09	1.8	0.90–3.7
CD44v7-8	0.7	1.2	0.51–2.7

DISCUSSION

In a previous study, Joensuu and colleagues investigated the expression of CD44 in 198 breast cancer patients [11]. They used the Hermes-3 monoclonal antibody which identifies the standard form of CD44 as well as isoforms of CD44 containing variant exons, and reported that the overexpression of CD44 was associated with aggressive histological features and poor outcome, but failed to be an independent prognostic factor in a multivariate analysis. In a recent study of 100 breast carcinomas, Kaufmann and associates showed that the expression of CD44v6 is an independent predictor of overall survival [12].

In the present study, the univariate analysis showed a strong correlation between CD44 isoform expression and outcome in the whole study group as well as in the subgroup of axillary lymph node-negative patients. However, multivariate regression revealed that CD44 isoform expression did not confer additional prognostic information over that obtained by established prognostic indicators.

In summary, our data support the thesis of Joensuu and colleagues, indicating that there is no independent prognostic value of CD44 isoform expression, presumably due to the correlation between CD44 isoform expression and other prognostic indicators such as lymph node status and histological grading. However, it cannot be ruled out that the differences reported so far are due to small sample sizes.

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