

Expression of 34 β E12 may be an independent predictor of survival in breast cancer

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Abstract

Objectives: To investigate the relationship between high-molecular-weight cytokeratin (34 β E12) and clinicopathological parameters (including HER-2, Ki67 and steroid receptors) in breast cancer to determine its usefulness as a prognostic marker.

Methods: In this retrospective study, the expression level 34 β E12 was assessed in surgically resected breast cancer specimens by immunohistochemical staining. Data were correlated with the patients' clinicopathological parameters.

Results: Of the 348 breast cancer tissue samples, 232 (67%) showed positive expression of 34 β E12. There were statistically significant differences between the positive and negative 34 β E12 expression groups in tumour size, lymph node involvement, oestrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor-2 (HER2) status. There were no differences between groups in age, tumour grade, or Ki67 status. In addition, patients who were positive for 34 β E12 had significantly extended overall survival. In multivariate analysis, the expression level of 34 β E12 was found to be a significant independent prognostic factor.

Conclusion: These results suggest that positive 34 β E12 expression is associated with a favourable outcome in breast cancer and so may be a useful prognostic factor. Further studies are required to confirm these results.

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Keywords

Breast cancer, HMW cytokeratins, 34 β E12, Prognosis, Oestrogen receptor, progesterone receptor, Human epidermal growth factor receptor-2, Ki67.

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Introduction

In China, breast cancer remains one of the most common cancers and the leading cause of death in women younger than 45 years.¹ Oestrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor-2 (HER2) status in primary tumours is an important indicator of breast cancer patients prognosis.^{2,3} Used in combination with clinicopathological factors, an assessment of receptor expression profile is an important tool in identifying patients that may benefit from certain therapies, for example hormonal therapy.⁴⁻⁶ However, further therapeutic stratification of breast cancer patients and more biomarkers are needed to improve patient outcome.

In human epithelial cells, cytokeratins are the main structural protein, and can be separated into two types, type I- acidic and type II- basic and neutral.⁷ The expression of cytokeratin proteins plays a critical role in regulated embryonic development and differentiation.^{8,9} The high molecular weight cytokeratin (HMWCK) antibody, clone 34 β E12, reacts with the basal cells and has been investigated as a prognostic marker in breast,^{10,11} renal cell,¹² urachal,¹³ prostate,¹⁴ and lung carcinomas.¹⁵ While inconclusive results have been found in one study of patients with triple negative breast cancer,¹⁰ another study in a similar group of patients showed that the expression of 34 β E12 was a good predictor of disease-free- and overall survival.¹¹ However, in a study of young women with

invasive breast cancer, researchers found that 34 β E12 immunopositivity and vimentin were correlated with adverse pathological parameters.¹⁶

The purpose of the present study was to investigate the relationship of the antikera-tin 34 β E12 with clinicopathological parameters, including the status of HER-2, Ki67 and steroid receptors, in breast cancer and determine its usefulness as a prognostic marker.

Patients and Methods

Patients

In this retrospective study, breast tissue samples were obtained from patients ≥ 18 years who were consecutively admitted to the Department of Breast and Thyroid Surgery, Shaoxing Hospital, Zhejiang University School of Medicine between December 2010 and November 2013 for surgical resection of breast cancer. Patients who had received pre-operation adjuvant treatment, or had radical mastectomy after neo-adjuvant chemotherapy, in addition to those with multifocal tumours and/or metastatic disease were excluded from the study.

The clinical characteristics of the patients were retrieved from medical records and patients were staged according to the 7th edition of the Union for International Cancer Control–American Joint Committee on Cancer (UICC-AJCC) staging manual. The clinicopathologic parameters included, age, tumour size,

tumour grade, lymph node metastasis, chemotherapy and expression levels of ER, PR, HER2, and Ki67. All patients provided written informed consent and the study was approved by the Ethics Committee of The Shaoxing People's Hospital.

Immunohistochemistry

The immunohistochemical analysis was performed by a single laboratory. Analysis of the expression of 34 β E12, ER, PR, HER-2, and Ki67 was performed on formalin-fixed, paraffin-embedded sections of surgical specimens. For this, breast cancer tissues were fixed with 10% formaldehyde, embedded in paraffin and cut at 4- μ m-thickness and mounted onto glass slides.¹⁷ The slides were deparaffinized in xylene and rehydrated in gradient ethanol solutions. Endogenous peroxidase was blocked with 0.3% hydrogen peroxide in methanol for 5 minutes. The slides were immersed in 10 mM citric buffer (pH 6.0) and heated for 15 minutes for antigen retrieval. Nonspecific binding was blocked by preincubation with 10% foetal calf serum in phosphate buffered saline (PBS) and 0.01% sodium azide and the slides were incubated in a humid chamber for one hour with antibody against 34 β E12 (Novocastra, Newcastle on Tyne, UK, 1:50). After washing three times in PBS, the slides were incubated with the EnVision-HRP complex (undiluted, DAKO) for 60 minutes. The slides were visualized with diaminobenzidine (DAKO Corp.) and then counterstained with haematoxylin & eosin (H&E). For substitute negative controls, the primary antibody was replaced with PBS. Positive control was breast cancer tissue known to exhibit high levels of 34 β E12.

The expression of the antibodies was assessed semi quantitatively by estimating the percentage of tumour cells with positive nuclei or cytoplasm staining on whole

tumour slides. Immunoreactivity was evaluated by determining the percentage of positive cells in each core and then taking the average of four cores. All slides were examined and scored independently by two experienced pathologists and expression levels were assessed according to procedures adopted in a previously published assay.¹⁷ Each slide was examined in its entirety under a light microscope, and initially a proportion score was assigned, which represented the estimated proportion of positive tumour cells (0, none; 1, 0%–10%; 2, 10%–50%; 3, 50%–100%). In addition, an intensity score was assigned which represented the average intensity of the positive tumour cells (0, none; 1, weak; 2, intermediate; and 3, strong). The proportion and intensity scores were then multiplied to obtain a total score, which ranged from 0–9, and 34 β E12, ER, PR, and HER-2 protein positive expression was defined as total score ≥ 2 . The evaluation criteria for Ki67 expression were interpreted based on the percentage of positive tumour cells (0, no staining; 1, <14% staining; 2, $\geq 14\%$ staining). A score of 0 was considered negative.

Follow-up

Post-operative follow-up of the patients had occurred at 3-monthly intervals for two years, and thereafter at 6-monthly intervals for a further three years. The follow-up consisted of physical examination, complete blood count and biochemistry. Computed tomography (CT) and position emission tomography (PET) scanning were performed if clinically indicated. The patients were followed-up until death or the date of the last follow-up of November 30, 2018.

Statistical analyses

All analyses were performed using Statistical Package for Social Sciences

(SPSS®) for Windows® release 15.0 (SPSS, Inc., Chicago, IL, USA) and a P -value <0.05 was considered to indicate statistical significance. Patients were separated into two groups depending on expression of 34 β E12 and the relationship between 34 β E12 expression and their clinicopathologic parameters were analysed using a 2-tailed χ^2 test. To assess potential risk factors for a poor prognosis, all clinicopathologic parameters were included in the univariate analysis and those with a P value of <0.05 were included in a Cox's proportional hazard model for multivariate analysis. Kaplan–Meier survival curves were generated for overall survival, and the log-rank test was used to test for differences between groups.

Results

Patients

Breast cancer tissues samples were obtained from 348 patients, 312 of whom had undergone modified radical mastectomy and 36 conservative surgery. No patients had been lost to follow-up. All patients were female and aged from 28 to 76 years (mean 51 years). Fifty-two patients (15%) died during the follow-up period (median 87 months [range: 11–91 months]). With regard to chemotherapy, 64 patients remained untreated, 172 had received adriamycin and 112 had received adriamycin and taxol (Table 1).

Expression of biomarkers in breast cancer tissues

In the 348 breast cancer tissue samples, positive expression of 34 β E12 was demonstrated in 232 (67%) patients and the staining was predominantly localized in the cytoplasm (Figure 1). Positive expression of ER protein was demonstrated in 238 (68%) patients, PR protein in 198 (57%)

patients, HER-2 protein in 81 (23%) patients and Ki67 in 270 (69%) patients. There were 22 (9%) deaths in the 34 β E12 positive group and 30 (26%) in the 34 β E12 negative group.

34 β E12 expression and clinicopathological characteristics

Patients were separated into two groups depending on expression of 34 β E12 (Table 1). There were statistically significant differences between the two 34 β E12 expression groups in tumour size ($P < 0.001$), lymph node involvement ($P < 0.001$), ER status ($P < 0.001$), PR status ($P < 0.001$) and HER-2 status ($P = 0.004$) (Table 1). An inspection of the data showed that patients with positive 34 β E12 status tended to have smaller tumours, less lymph node metastasis, fewer steroid receptors and HER-2 receptors than patients with negative 34 β E12 status. However, there were no differences between groups in age, tumour grade, Ki67 status or chemotherapy.

Univariate logistic regression analysis showed that predictors of a poor clinical prognosis were positive 34 β E12 status, positive ER status, lymph node metastasis and tumour size (Table 2). A multivariate logistic regression analysis was applied to the data to determine if they independently affected the clinical prognosis. Results showed that positive 34 β E12 status (OR, 0.28), positive ER status (OR, 0.26) and lymph node metastasis (OR, 1.17) were independent risk factors associated with a poor clinical prognosis for patients with breast cancer (Table 2).

Based on the expression level of 34 β E12, the mean overall survival times for positive and negative 34 β E12 groups were 86 and 81 months, respectively. The difference in survival between group was statistically significant ($P < 0.001$; Figure 2). These data

Table 1. Clinicopathologic parameters and their relation to 34 β E12 expression in breast cancer patients ($n = 348$).

	34 β E12 expression		Statistical significance
	Positive $n = 232$	Negative $n = 116$	
Age, years	50.4 \pm 8.9	51.1 \pm 10.9	ns
Tumour grade			ns
1	56 (24)	40 (35)	
2	88 (38)	40 (35)	
3	88 (38)	36 (31)	
Tumour size, cm			$P < 0.001$
≤ 2	164 (70)	56 (48)	
>2 and ≤ 5	60 (26)	48 (41)	
>5	8 (4)	12 (10)	
Lymph node metastasis			$P < 0.001$
0	184 (79)	80 (69)	
≥ 1 and <4	32 (14)	12 (10)	
≥ 4 and <9	4 (2)	12 (10)	
≥ 10	12 (5)	12 (10)	
Oestrogen receptor			$P < 0.001$
-ve	92 (40)	18 (16)	
+ve	140 (60)	98 (85)	
Progesterone receptor			$P < 0.001$
-ve	112 (48)	38 (33)	
+ve	120 (52)	78 (67)	
HER-2 status			$P = 0.004$
-ve	160 (69)	97 (84)	
+ve	72 (31)	19 (16)	
Ki67 status			ns
< 14	46 (20)	32 (28)	
≥ 14	186 (80)	84 (72)	
Chemotherapy			ns
Untreated	48 (21)	16 (14)	
Adriamycin	112 (48)	60 (52)	
Adriamycin and Taxol	72 (31)	40 (35)	

Values are shown as mean \pm SD or n (%).

Abbreviations: HER-2, human epidermal growth factor receptor 2; ns, not statistically significant.

suggest that 34 β E12 positive expression was correlated with a favourable prognosis.

Discussion

Although 34 β E12 expression has been investigated previously as a potential prognostic marker in several cancers including breast cancer, its role remains unclear.

In this present study the presence of the antikeratin was detected in the majority (67%) of breast cancer tissue samples and its presence was significantly correlated with a good prognosis. We found that positive 34 β E12 status was associated with small tumour size, less lymph node metastasis, fewer ER, PR and HER-2 receptors. Indeed, lymph node metastasis and size of

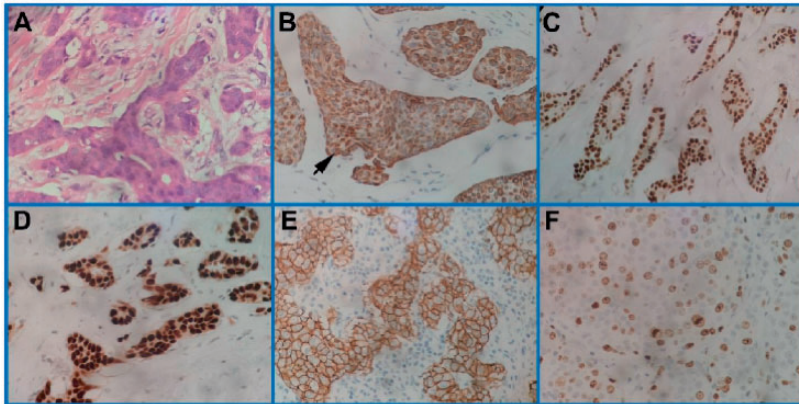


Figure 1. Examples of immunohistochemical analysis of breast cancer tissues. (a) Haematoxylin & Eosin (H&E) stained image of invasive breast cancer; (b) 34βE12 positive cytoplasmic staining; (c) Oestrogen receptor α (Er α) positive nuclear staining; (d) Progesterone receptor (PR) positive nuclear staining; (e) Human epidermal growth factor receptor-2 (HER-2) positive nuclear staining; (f) Ki67 positive nuclear staining.

Table 2. Univariate and multivariate analysis of predictors for a poor clinical response in breast cancer (n = 348).

Risk Factors	Univariate analysis	Multivariate analysis	
	Statistical significance	Hazard Ratio (95% CI)	Statistical significance
34βE12 (positive vs. negative)	$P < 0.001$	0.28 (0.14, 0.55)	$P < 0.001$
ER (positive vs. negative)	$P = 0.001$	0.26 (0.14, 0.49)	$P < 0.001$
Lymph node metastasis	$P < 0.001$	1.17 (1.13, 1.215)	$P < 0.001$
Tumour size	$P < 0.001$		ns
PR (positive vs. negative)	ns		ns
HER-2 (positive vs. negative)	ns		ns
Ki67 (≥ 14 vs. < 14)	ns		ns
Age (≥ 50 vs. < 50)	ns		ns
Tumour grade	ns		ns

Abbreviations: ER, oestrogen receptor; PR, progesterone receptor; HER-2, human epidermal growth factor receptor 2; ns, not statistically significant.

tumours are crucial parameters in predicting survival.¹⁸ Our findings are in agreement with a previous study in triple negative breast cancer that showed the expression of 34βE12 was associated with a good prognosis.¹¹ Therefore, these results suggest that 34βE12 may be a promising candidate as a prognostic biomarker in breast cancer.

It is unclear how changes in 34βE12 expression level affects survival in breast cancer patients. Some studies have suggested that a loss of 34βE12 expression could cause downregulation of cell-cell and cell-matrix contacts which play a major role in epithelial-mesenchymal transition (EMT), a process that is thought to increase migration and invasion of

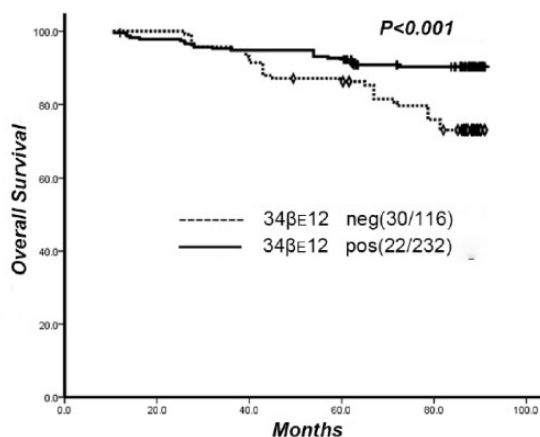


Figure 2. Kaplan-Meier survival curves showing survival time for 34βE12 positive (86 months) and 34βE12 negative (81 months) expression groups.

metastatic tumour cells.^{19,20} Moreover, HER-2 expression has been suggested to induce EMT in breast cancer through focal adhesion kinase signalling.^{21,22} Interestingly, in this present study, the incidence of HER-2 positive patients was low (23%) which may have impacted on the results from the 34βE12 positive breast cancer patients and be a factor in their favourable prognosis.

The study had some limitations. For example, it was a retrospective and uncontrolled and we did not explore differences in outcome between the 34βE12 subgroups. However, our findings suggest that evaluation of the 34βE12 status in patients with primary breast cancer may assist in selecting patients with a favourable prognosis and determine which patients will require aggressive adjuvant therapy. More controlled studies in large numbers of patients are required to confirm our findings.

Declaration of conflicting interest

The authors declare that there are no conflicts of interest.

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