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A COMPARISON OF THE PATTERNS OF LAMININ EXPRESSION IN FIBROADENOMA, FIBROCYSTIC DISEASES, PRE-INVASIVE AND INVASIVE DUCTAL BREAST CARCINOMA

WEI-QIANG ZHENG*, LAI MENG LOOI† AND PHAIK LENG CHEAH†

*Department of Pathology, Changhai Hospital, Second Military Medical University, Shanghai, China, and †Department of Pathology, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia

Summary

The basement membrane (BM), of which laminin is a major glycoprotein component, is an important barrier to tumour cells which must be breeched before metastatic spread can occur. We have compared the pattern of laminin expression in a range of benign and malignant breast lesions to better understand the process of tumour progression. A total of 162 cases of breast samples, comprising 18 fibroadenomas, 22 cases of fibrocystic disease, 96 cases of invasive ductal carcinoma and 26 carcinomas with intraductal components, were evaluated for laminin expression by a standard immunoperoxidase method on formalin-fixed, paraffin-embedded histological sections, using a commercial antibody against human laminin. The pattern of laminin expression was charted as follows: Type 1, >70% of BM complete/continuous; Type II, >70% of BM moderately disrupted; Type III, >70% of BM completely disrupted. The Type I pattern was observed in all cases of fibroadenoma and fibrocystic diseases, and in 77% of intraductal carcinoma components. Various patterns of BM disruption were observed in invasive ductal carcinoma. Severity of BM disruption correlated with histological grade of the carcinomas ($P < 0.001$). Small-sized tumours, those without lymphatic invasion and lymph node-negative tumours showed more complete patterns of laminin expression. The current study suggests that tumour cells with high histological grade possess an enhanced capacity to disrupt the basement membrane, an important step in the metastatic process. The detection of BM disruption by immunohistochemical staining for laminin is technically easy and may be usefully applied for the differentiation of *in situ* and microinvasive carcinoma.

Key words: Breast, carcinoma, laminin, immunohistochemistry.

Abbreviations: BM, basement membrane.

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INTRODUCTION

Basement membranes occur in connection with epithelium, endothelium, mesothelium, smooth and striated muscle cells, Schwann cells and fat cells. Basement membranes separate the epithelium from the mesenchymal tissues, and are composed mainly of a specialised generic type of collagen (type IV collagen) which resembles, in some respects, the procollagen forms of the interstitial collagens[1,2] and non-collagenous glycoproteins. Laminin, a non-collagenous glycoprotein component of basement membranes, has been identified.[3–5] It is a glycoprotein with a molecular weight of about 600 000 and it is also important in the cell adhesion process.

Metastasis is one of the most important reasons for deterioration of patients with breast cancer. The basement membranes are of particular interest in cancer invasion because they are regarded as a significant hindrance to the entry of cancer cells into the matrix and metastatic cells which attach to the basement membranes of endothelial cells in the spread of tumours via circulation.[6–8] In mammary carcinomas, the first step of tumoural invasion is characterised by the loss of basement components, particularly laminin.[9] Basement membranes have been extensively studied in neoplastic breast lesions.[10–12] Although basement membrane morphology in breast lesions has been the subject of a large number of studies, there have been relatively few detailed investigations of the patterns of basement membrane deposition in various benign and malignant breast lesions and the consistency of these patterns. It seemed important to examine the distribution of basement membrane laminin in non-malignant and malignant breast lesions in this study.

MATERIALS AND METHODS

Patients

A total of 162 breast samples of female patients, comprising 18 fibroadenomas, 22 cases of fibrocystic disease, 96 invasive ductal carcinomas and 26 lesions with intraductal carcinoma components, were retrieved from the archives of the Department of Pathology, University of Malaya. Formalin-fixed, paraffin-embedded histological sections of the lesions, stained with H&E, were reviewed by three pathologists for designation into the above diagnostic categories. Invasive ductal carcinoma were evaluated for histological grade according to the modified Bloom and Richardson method of Elston.[13]

Demographic and clinical data were obtained from histopathology request forms accompanying the samples. Information on the size of the breast carcinomas was extracted from the reports of the pathologists who performed the original pathological examinations on the lesions. Patients with invasive ductal carcinomas were divided into two age groups (<50 and ≥50 years) based on their presumed menopausal status.

Immunohistochemical staining

When more than one tissue block was available from a case, the one most representative of the lesion or, in the case of carcinoma, with the highest tumour content was selected for immunohistochemistry. Laminin

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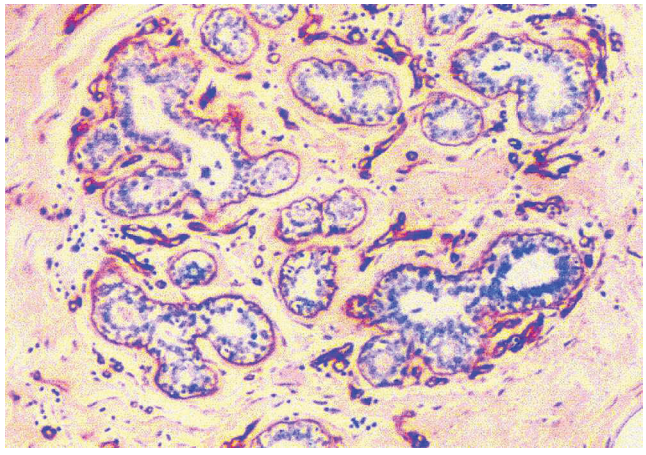

Fig. 1 Linear and continuous laminin staining of the epithelial basement membrane in a case of breast fibrocystic disease (laminin immunoperoxidase staining, original magnification, ×100).

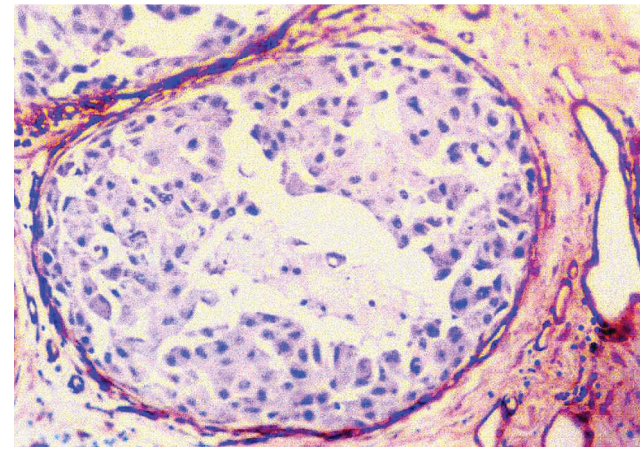

Fig. 2 Continuous linear staining of basement membrane around intraductal components of invasive ductal carcinoma (laminin immunoperoxidase staining, original magnification ×200).

expression was demonstrated by the standard avidin–biotin complex immunoperoxidase method on formalin-fixed, paraffin-embedded histological sections. Five-μm paraffin sections were melted overnight at 42°C, clear in xylene, and hydrated in a series of decreasing concentrations of ethanol (100, 80, 70%) and distilled water. The sections were then treated with 0.2% pepsin (Boehringer Mannheim, Germany) in 0.2 N HCl at 37°C for 30 min. The reaction was blocked by immersing the sections in distilled water for 5×3 min. After blocking of endogenous peroxidase activity with a 0.3% solution of hydrogen peroxide and methanol, slides were sequentially incubated with a dilution of 1:100 primary rabbit laminin antibody (Dako, USA) for 30 min, followed by incubation overnight at 4°C with biotinylated antirabbit immunoglobulin for 45 min, and avidin–biotin peroxidase complex for 40 min (Dako). Slides were washed three times in phosphate-buffered saline (PBS) for 5 min between the above steps. Freshly prepared 0.05 M Tris buffer, pH 7.6, containing 0.01% H_2O_2 3,3-diaminobenzidine (DAB) was used as chromogen in the application of hydrogen peroxide. The slides were then lightly counterstained with haematoxylin. Several dilutions of the antibody were tested to optimise the staining (and to avoid background staining) before the total series was processed. Positive controls consisted of sections from a breast carcinoma with known detectable laminin. Omission of the primary antibody incubation step from the procedure served as negative control.

Immunohistochemical evaluation

The pattern of laminin expression was charted as follows: Type I, the staining pattern of >70% of basement membranes in the range of one section was complete/continuous; Type II, >70% of BM moderately disrupted; Type III, >70% of BM completely disrupted.

Statistical analysis

The patterns of laminin positivity of the various breast lesions were compared against each other. The χ^2-test was used to evaluate significance of correlation between the various parameters under study.

RESULTS

Laminin immunostaining in fibroadenoma and fibrocystic disease showed a continuous line around lobules and ducts; the staining was located in basement membranes along the epithelial cell layers in contact with the stroma (Fig. 1). Also, regular linear staining was observed in basement membranes around blood vessels. No intracellular staining was observed.

In intraductal components of invasive ductal carcinoma, a continuous linear staining of basement membranes around the involved ducts was again present (Fig. 2). However, it appeared slightly irregular and thickened, as shown by darker staining, compared with that observed in the non-neoplastic breast. In addition, dystrophic basement membranes around ducts containing tumour cells were observed (6/26, 23%).

The staining pattern in invasive ductal carcinoma was quite heterogeneous, even within an individual carcinoma. The staining was discontinuous around the cancer cell nests infiltrating the stroma, with gaps and irregular thickening (Fig. 3). No staining was visible around tumour cell masses in some areas within an individual carcinoma. In some focal regions of well-differentiated carcinoma, partial basement membrane formation by the pseudo-duct structures was observed.

The correlation of immunostaining pattern and pathological features was evaluated. Various sized tumours did not produce a significant difference in laminin integrity ($P > 0.05$). Although the result of statistical analysis was not significant ($P > 0.05$), laminin absence appeared to be

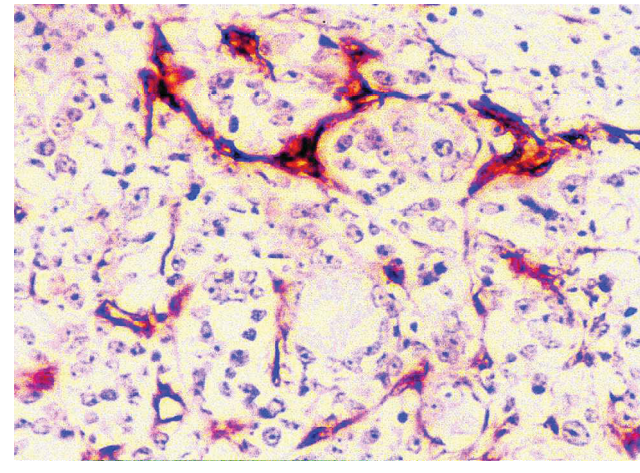

Fig. 3 Invasive breast carcinoma with discontinuous laminin staining around the malignant cells (laminin immunoperoxidase staining, original magnification, ×200).

TABLE 1 Correlation between laminin immunostaining and morphology in invasive ductal carcinoma of breast

	Number examined	Immunostaining pattern (%)			
		Type I	Type II	Type III	*P*
Histological grade					
I	14	9 (64.3)	4 (28.6)	1 (7.1)	
II	41	3 (7.3)	25 (60.9)	13 (31.7)	< 0.001
III	41	2 (4.9)	5 (12.2)	34 (82.9)	
Tumour size (diameter)					
< 3 cm	45	7 (15.6)	13 (28.9)	25 (55.5)	
3–6 cm	37	2 (5.4)	15 (40.5)	20 (54.1)	> 0.05
> 6 cm	14	5 (35.7)	6 (42.9)	3 (21.4)	
Total	96	14 (14.6)	34 (35.4)	48 (50.0)	

correlated with lymphatic invasion and lymph node metastasis because there was a tendency for cases with more lymphatic invasion and more lymph node metastases to show the higher-grade staining patterns. It was also evident that higher-grade patterns were likely to be present in cases with vascular invasion. Moreover, statistical significance was found between staining patterns and histological grade (Table 1); the continuous linear pattern was more frequently observed in low-grade carcinomas, whereas basement membrane disruption was more frequently observed in higher-grade carcinomas ($P < 0.001$).

DISCUSSION

The visualisation of basement membranes by laminin staining allowed us to study the organisation and role of basement membrane integrity in the progression of breast cancer in more detail.

In the current study, both malignant and non-malignant breast tissue were stained for laminin. It seems reasonable to conclude that laminin synthesis also occurs in carcinoma cells. The significance of this finding linked to the previous studies on the cell adhesion-promoting properties of laminin suggests that laminin could be important for the attachment and growth of tumour cells.[14,15] It has been reported[16–18] that malignant breast tumours only occasionally possess basement membranes. Pitelka *et al.*[19] reported that well-differentiated malignant mouse mammary tumour lines had intact basement membranes demonstrable by electron microscopy, whereas a poorly differentiated tumour line lacked them. Our study was in agreement with these previous investigations of basement membrane changes in breast carcinoma. As visualised by laminin staining, a progressive lack of basement membranes seemed to parallel an increasing degree of tumour dedifferentiation. We considered that this lack of basement membranes may be because a poorly differentiated tumour would partially or completely lose its ability to synthesise laminin and other components. In addition, the occurrence of basement membrane destruction by proteolytic enzymes released by stromal cells would be increased in a poorly differentiated carcinoma, thus the absence of a basement membrane barrier may facilitate tumour invasion and spread. This has been confirmed by the correlation found between the immunostaining patterns and histological grade or differentiated status, i.e., the more complete basement membrane was significantly associated with well-differentiated carcinomas, whereas basement membrane disruption more frequently occurred in poorly differentiated carcinomas. These findings suggest that tumours with a higher histological grade possess an enhanced disruption of the basement membrane, an important step in the metastatic process. The correlation of disintegration of the laminin-containing basement membranes of carcinomas with increasingly anaplastic appearance supported the notion that basement membranes may play a role in cancer progression.[20]

It was also noted that there were qualitative changes in laminin immunostaining of both invasive and intraductal carcinomas, even within an individual case. Most cancers originate from the malignant transformation of a single cell (monoclonal origin of tumours). Nevertheless, the inherent genetic instability of the malignant phenotype leads to the appearance of subpopulations with diverse biological characteristics and profound variations in their metastatic potential (tumour heterogeneity). The demonstration of tumour heterogeneity has led to the concept that at each step of the metastatic cascade, only the fittest cells survive. The heterogeneity of laminin distribution in the current study suggested such demonstration of subpopulations with diverse biological tumours. This heterogeneity of laminin immunostaining distribution indicated that both invasive and intraductal components of breast carcinomas were probably composed of heterogeneous subpopulations with variable abilities to produce laminin.

It is also implied that some intraductal carcinoma cells lose the ability to synthesise basement membranes, since dystrophic basement membranes around ducts composed of tumour cells were exhibited in some intraductal components. It is known that intraductal carcinomas are confined to the epithelium in which they arise. Microinvasion in a predominantly intraductal carcinoma consists of tumour penetration through the basement membrane of a duct involved by intraductal carcinoma. Distortion due to fibrosis, elastosis, and inflammation may make recognition of such penetration difficult, and it is also often difficult to determine whether small tumour glands represent invasion or merely intraductal carcinoma involving more distal units of the lobule. It appears that a proportion of histologically classified intraductal carcinomas of the breast has a disruption of the laminin layer which may be indicative of early invasion. Hence, laminin detection by immunohistochemistry may be a useful and important

marker to differentiate true '*in situ*' from 'microinvasion' breast carcinoma. Moreover, those intraductal components of carcinoma may be analogous to invasive carcinoma and it would be of interest to follow up whether they had obtained an invading propensity.

It has been reported[21] that profound changes occur in the distribution and quantity of the epithelial basement membrane during the transition from benign to invasive carcinoma. However, no matter how extensive the architectural disorganisation, these benign disorders are always characterised by a continuous basement membrane separating the epithelium from the stroma. In contrast, invasive ductal carcinoma consistently lack a complete extracellular basement membrane around the invading tumour cells in the stroma.

This study did not show an accurate correlation between laminin expression and lymphatic invasion and lymph node metastasis. Anyway, the possibility that the pattern of basement membranes in breast cancer might be of prognostic significance needs to be further explored. It will be important to evaluate in long-term clinical studies whether absence of laminin represents those subclones of the tumour with a special metastatic potential.

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Address for correspondence: Dr Wei-qiang Zheng, Room 406, No. 89, Lane 871, Xiang-Yin Road, Shanghai 200433, China. Email: zhengwqk@online.sh.cn

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