Laminin and fibronectin in adenoid cystic carcinoma

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SUMMARY The distribution of fibronectin and laminin was examined by immunohistochemistry in 11 adenoid cystic breast carcinomas, six adenoid cystic carcinomas of mouth and salivary gland, and six cribriform ductal breast carcinomas. Both proteins were present lining cystic lumina and around tumour islands in all the adenoid cystic breast carcinomas and in five of six salivary gland tumours. Abundant laminin and fibronectin were dispersed among adenoid cystic tumour cells arranged in sheets. One adenoid cystic carcinoma from buccal mucosa showed a transition from a cribriform tumour positive for both fibronectin and laminin to a cribriform tumour negative for fibronectin and laminin to undifferentiated carcinoma. Fibronectin and laminin seemed to disappear simultaneously from tumour cell surfaces. Another adenoid cystic carcinoma from buccal mucosa was negative for fibronectin and laminin from the time of initial biopsy. This was the only tumour that gave rise to disseminated metastases, resulting in the death of the patient within two years of surgery. In cribriform invasive ductal breast carcinomas the linings of cystic lumina were always negative for fibronectin and laminin. Varying quantities were present at the tumour boundaries. We suggest that staining for fibronectin and laminin may be a valuable aid to the diagnosis of adenoid cystic carcinomas and that the absence of these proteins may have important prognostic implications.

Adenoid cystic carcinomas are tumours which arise most often in the mouth and salivary glands, but which may also occur in other sites—namely, the tracheobronchial tree, nasopharynx, maxillary sinus, uterine cervix, skin and breast.^{1–5} They are composed of two cell types: those resembling myoepithelial cells and duct lining cells. Myoepithelial like cells are predominant. In adenoid cystic carcinomas with the classical cribriform pattern, groups of cells are separated to form false cysts, which on ultrastructural examination are seen to be lined by basal lamina.^{6–8} True ductal lumina are also present. In some adenoid cystic carcinomas false cysts are less conspicuous, and there is a sheet like pattern of growth. Adenoid cystic carcinomas of the mouth and salivary glands are usually slow growing if infiltrative but may display more overtly aggressive behaviour and give rise to disseminated metastasis.² In some cases this is preceded by transformation to carcinomas with a more anaplastic morphology. Local recurrences are common. Adenoid cystic carcinomas of the breast are generally associated with a better prognosis.^{3,9,10} It is therefore important to distinguish them from the more common invasive ductal carcinomas,

some of which display a cribriform pattern.¹¹ This distinction may present a problem.³

Fibronectin and laminin are both non-collagenous glycoproteins associated with basal lamina.^{12,13} Fibronectin is also found in interstitial connective tissue and in extracellular body fluids.¹⁴ Ultrastructural immunohistochemistry has localised laminin to both the lamina rara and lamina densa of basement membranes^{15,16}; the precise localisation of fibronectin is more controversial.^{14,17–19} A previous study showed the presence of fibronectin lining the cystic spaces of an adenoid cystic carcinoma of the submandibular salivary gland.²⁰ In this study we compared the distribution of fibronectin and laminin in a series of adenoid cystic carcinomas from breast with their distribution in cribriform ductal carcinomas of breast and in adenoid cystic carcinomas of the mouth and salivary gland. The aim was to determine whether staining for fibronectin and laminin may be of value in diagnosing these tumours and whether the presence of these proteins has any prognostic implications.

Material and methods

Tissue was examined from eleven adenoid cystic

Table 1 Adenoid cystic carcinomas of mouth and salivary glands

Case No	Age and sex at diagnosis	Site	Time since diagnosis	Outcome
1	50 F	Parotid gland	12 years	Alive and well; four local recurrences
2	64 F	Submandibular gland	5 years	Alive and well; no local recurrence
3	61 F	Buccal mucosa	3 months	Alive and well; good initial recovery
4	77 F	Parotid gland	3 years	Alive and well; one local recurrence
5	61 M	Buccal mucosa	5 years	Died when transition to undifferentiated carcinoma occurred with extensive local recurrence 5 years after diagnosis
6	57 F	Buccal mucosa	18 months	Died 18 months after diagnosis with lymph node, lung, and bone metastases

breast carcinomas, six adenoid cystic carcinomas of the mouth and salivary gland, and six cribriform invasive ductal breast carcinomas. All of the tissue used in the study was paraffin embedded. Most was fixed in formalin, but in a few instances when patients had been referred from elsewhere the mode of fixation was unknown. Sections were stained for fibronectin and laminin by the indirect immunoperoxidase method following protease digestion: details of this method have been described previously.^{21 22} Rabbit antibody to human fibronectin and peroxidase conjugated anti-rabbit IgG were obtained from Dakopatts, Mercia Brocades Ltd. Rabbit antilaminin antibody was obtained from Bethesda Research Laboratories. All antisera were diluted 1/50 in phosphate buffered saline, pH 7.3. Effectiveness of the technique was judged by the staining of basal lamina in blood vessels and around normal breast acini. These structures acted as an internal positive control. Negative controls were incubated with non-immune rabbit IgG or with phosphate buffered saline in place of the primary antiserum.

Results

ADENOID CYSTIC CARCINOMA OF THE MOUTH AND SALIVARY GLANDS

Table 1 summarises the clinical details of these cases. Four of the six tumours (cases 1–4) showed strong positive staining for fibronectin and laminin along cell boundaries both within and at the periphery of tumour islands. The immunostaining was seen lining pseudocysts, along the margins of epithelial cell cords, and abundantly between those tumour cells which were arranged in sheets (Fig. 1). This staining pattern was present in local recurrences as well as in the initial tumour. Only one case (2) showed focal cytoplasmic staining for laminin (Fig. 2). No intracellular fibronectin was shown.

In case 5 there was a transition from typical adenoid cystic carcinoma to a poorly differentiated car-

cinoma with a focally squamoid appearance. In one part of the tumour fibronectin and laminin were present lining pseudocystic lumina and peripherally around individual islands of carcinoma cells. Adjacent tumour cells showed retention of the classical cribriform pattern but loss of fibronectin and laminin (Fig. 3). Staining consecutive serial sections for both these proteins suggested that they were lost simultaneously, initially from the pseudocystic spaces and subsequently from the tumour boundaries. The cribriform tumour negative for fibronectin and laminin merged into a poorly differentiated carcinoma in which laminin staining was discontinuous and irregular at the margins, or was completely absent. Fibronectin showed a similar distribution but was found in increased amounts in the tumour stroma. Case 6 was a carcinoma of cribriform appearance in which cystic lumina were negative for both fibronectin and laminin from the time of initial biopsy. Scanty and fragmented laminin was evident at some tumour boundaries but most were negative. Parts of the tumour were less well differentiated and these showed increased stromal fibronectin.

ADENOID CYSTIC CARCINOMAS OF BREAST

Table 2 summarises the clinical details of these cases. Fibronectin and laminin were seen lining pseudocystic spaces within all these tumours (Fig. 4). Where tumour cells were arranged in sheets fibronectin and laminin were dispersed irregularly among them and sometimes outlined small pseudocysts that were not otherwise readily detectable. Only one case showed focal intracytoplasmic staining for laminin. Both proteins were present around the periphery of tumour cell islands. In a few cases laminin staining appeared weak and patchy, but as this applied to normal structures as well as to the tumour it was attributed to staining artefact. These findings may have been the result of the method of fixation rather than attributable to a genuine difference in distribution of laminin.

Fig. 1a Adenoid cystic carcinoma of parotid gland (case 4) showing abundant laminin (black) among tumour cells arranged in sheets. (Immunoperoxidase haematoxylin.) $\times 250$.

Fig. 1b Adenoid cystic carcinoma of parotid gland (case 4) showing laminin lining small cysts within tumour. (Immunoperoxidase haematoxylin.) $\times 400$.

CRIBRIFORM DUCTAL CARCINOMAS OF BREAST

Table 3 gives the clinical details of these cases. Both fibronectin and laminin outlined the basement membranes of ducts containing intraductal carcinoma, but neither was seen lining cystic spaces within tumour masses (Figs. 5 and 6). Basement membrane staining

Fig. 2 Adenoid cystic carcinoma of submandibular gland (case 2) showing laminin lining cysts and focally within tumour cell cytoplasm. (Immunoperoxidase haematoxylin.) $\times 400$.

around the in situ component of the carcinomas was usually continuous, but a single section of one tumour showed a duct with focal discontinuity of basement membrane laminin (Fig. 6). Laminin staining around the invasive carcinoma was discontinuous or absent. None was shown in the cytoplasm of the tumour cells.

In contrast to its distribution in normal breast tissue and adenoid cystic carcinoma, fibronectin in invasive ductal carcinoma differed substantially from that of laminin. It was focally abundant in the stroma of tumour and was also present in irregular condensations of fibres around infiltrating islands of carcinoma. The linings of cysts in cribriform carcinomas were always negative, although diffuse staining for fibronectin was sometimes evident in the central areas of necrosis. One carcinoma showed weak focal cytoplasmic positivity for fibronectin.

All sections incubated with non-immune rabbit IgG or with phosphate buffered saline in place of the primary antiserum were negative. In sections staining positively for fibronectin there was a strong reaction in mast cell granules, as noted previously.²³

Discussion

This study indicates that fibronectin and laminin staining may be a valuable aid in the diagnosis of adenoid cystic carcinoma. The technique readily distinguishes between the pseudocysts lined with basal lamina of an adenoid cystic tumour and the cysts negative for fibronectin and laminin present in other carcinomas with a cribriform pattern. Its advantage

Fig. 3 Adenoid cystic carcinoma of buccal mucosa stained for laminin showing transition from tumour positive for laminin (a) to cribriform tumour negative for laminin (b) to undifferentiated carcinoma (c). (Immunoperoxidase haematoxylin.) (a) $\times 400$; (b) $\times 400$; (c) $\times 360$.

Table 2 Adenoid cystic carcinomas of breast

Case No	Age and sex at diagnosis	Site	Time since diagnosis	Outcome
7	54 F	Breast	5½ years	Local intermittent breast pain; no recurrence or metastasis
8	F	Breast	Not known	
9	58 F	Breast	9 years	No known recurrence
10	47 F	Breast	4 years	Local recurrence at 4 years; no metastasis
11	74 F	Breast	5 years	No recurrence or metastasis
12	55 F	Breast	18 years	Local chest wall recurrence at 15 years; no metastasis
13	F	Breast		No information
14	66 F	Breast		No local recurrence
15	60 F	Breast	3 years	
16	F	Breast	5 years	Bilateral mastectomy
17	67 F	Breast	2 years	No known recurrence

Fig. 4 Adenoid cystic carcinoma of breast showing laminin lining both sides of epithelial cords. (Immunoperoxidase haematoxylin.) $\times 400$.

Fig. 5 Cribriform carcinoma of breast showing fibronectin associated with interstitial collagen fibres but not lining lumina within tumour. (Immunoperoxidase haematoxylin.) $\times 400$.

over electron microscopy is that a greater volume of tissue can be examined and it is less time consuming. In many instances adenoid cystic carcinomas have a sufficiently distinctive appearance on routine staining to make special techniques for their diagnosis superfluous. Contrary to the view, however, that their diagnosis is always easy,²⁴ they may cause problems in some circumstances. In particular, cribriform ductal breast carcinomas may be misdiagnosed as adenoid cystic carcinomas.^{3,25} Another appearance that may cause difficulty is the arrangement of adenoid cystic tumour cells in sheets as opposed to the classical cribriform pattern.² The presence of abundant laminin between these cells may indicate

which diagnosis to make. Some areas within salivary gland pleomorphic adenomas or monomorphic adenomas²⁶ may occasionally resemble adenoid cystic carcinoma.² As pleomorphic adenomas may also possess abundant basal lamina²⁰ and cystic areas within them may be lined by basal lamina (d'Ardenne *et al*, unpublished observations) staining for fibronectin and laminin is not of value in this particular differential diagnosis.

The most important factor limiting the diagnostic usefulness of fibronectin and laminin staining is the effectiveness of the immunohistochemical technique. Excellent reproducible results are usually achieved with paraffin embedded tissue that has been fixed for

Table 3 Non-adenoid cystic carcinoma of breast

Case No	Age and sex at diagnosis	Site	Time since diagnosis	Outcome	Diagnosis
18	64 F	Breast	5 years	No recurrence or metastasis	Atypical lobular proliferation
19	55 F	Breast	3 years	No recurrence or metastasis	Invasive cribriform carcinoma
20	52 F	Breast	1 year	No recurrence or metastasis	Invasive cribriform carcinoma
21	51 F	Breast	3 months	No recurrence or metastasis	Invasive cribriform carcinoma
22*	46 F	Breast			Invasive cribriform carcinoma "Pseudo ACC"
23	43 F	Breast	2 months	No recurrence or metastasis	Invasive cribriform carcinoma
24	66 F	Breast	1 month	No recurrence or metastasis	Invasive cribriform carcinoma

*Previously published by Harris.³⁶

Fig. 6 Intraduct carcinoma of breast showing discontinuous laminin staining in basement membrane of duct. (Immunoperoxidase haematoxylin.) $\times 80$.

up to 48 hours in formalin, but variations in fixation may affect the antigenicity of these matrical proteins.²¹ Trypsin is unsatisfactory for "unmasking" laminin and fibronectin antigens in formalin fixed tissue, and pepsin or pronase should be used.²¹ Fortunately, almost all tissue sections incorporate inbuilt positive controls in the form of blood vessels and other normal structures possessing basal lamina. It is therefore easy to determine if the result is genuinely negative, or negative due to inadequate staining.

Loss of fibronectin and laminin by an adenoid cystic carcinoma may occur during transformation to a more aggressive malignancy, as shown in this series (case 5). Case 6 was the only tumour of apparent adenoid cystic morphology (although the diagnosis may be open to question) that was negative for fibronectin and laminin from the time of initial biopsy. This was also the only case in which the patient died with disseminated metastases within two years of surgery. Such findings suggest that the presence or absence of fibronectin and laminin may have important prognostic implications. A larger series with longer follow up would be required to verify this hypothesis. Nevertheless, adenoid cystic breast carcinomas have a better prognosis than those found in salivary glands,^{3,9} and it is noteworthy that none was negative for fibronectin and laminin in our series. Prognosis may be related to site for several reasons, including the ease of complete excision. Both the cases resulting in

death in the present series arose in buccal mucosa where adequate excision is sometimes difficult.

Interestingly, the case, in which there was a morphological transition in the tumour from positivity for fibronectin and laminin to absence of fibronectin and laminin staining of consecutive serial sections, suggested that these two proteins were lost simultaneously from the tumour cell margins. A similar phenomenon was observed in a leiomyosarcoma from the ileum.²³ These findings resemble the simultaneous loss of fibronectin, laminin, and heparan sulphate proteoglycan from the surfaces of cultured rat kidney cells after viral transformation.²⁷ They suggest that there is a close relation between the expression of fibronectin and laminin. The parallel micro-anatomical distribution of these proteins points to fibronectin as a genuine constituent of basal lamina. It has been clearly shown that it is not exclusively associated with interstitial collagen, fibronectin being found in sites where interstitial collagen is absent.^{13 20 23} The differing distribution of fibronectin and laminin in invasive ductal carcinoma of the breast may nevertheless be explained by increased production of fibronectin, together with interstitial collagen, while basal lamina material is progressively lost. Differing interpretations of the relation of fibronectin around invasive carcinoma to stroma or tumour cells may account for discrepancies in the reported distribution of fibronectin in breast cancer.^{14 20 28} Stampfer *et al* reported the presence of fibronectin around the entire external surface of infiltrating breast carcinoma cells, with the exception of an undifferentiated carcinoma from which it was absent.²⁹ Labat-Robert *et al* described loss of fibronectin from carcinoma cell membranes and partial loss in dysplasia.³⁰ Similar discrepant observations have been recorded on cells cultured from breast cancer in man.^{29 31 32}

The distribution of laminin in ductal breast carcinomas noted in this study accords with other reports, although no intracytoplasmic laminin was shown.³³⁻³⁵ Discontinuous basement membrane laminin around intraductal breast carcinoma has been observed previously,^{34 35} and this was seen in one case in the present investigation. The importance of this finding with regard to tumour invasion remains to be determined. Laminin was only seen intracellularly in two of the sixteen adenoid cystic carcinomas studied, one from the breast and one from a submandibular gland. In both cases cytoplasmic staining was focal only and possibly related to excessive production of basal lamina.

We conclude that immunostaining for fibronectin and laminin is worthwhile to help make the distinction between genuine adenoid cystic carcinomas and morphologically similar tumours.

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