Immunohistochemical localization of basement membrane type VII collagen and laminin in neoplasms of the head and neck


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The distribution pattern of the basement membrane components type VII collagen and laminin, was studied immunohistochemically in normal human head and neck tissues and in a series of benign and malignant tumours from the same site. Using monoclonal antibodies, a basement membrane containing type VII collagen and laminin could be demonstrated beneath the epithelial cell layer in 16 normal head and neck tissues from different localizations. Unlike type VII collagen, laminin was also abundantly present around blood vessels and muscle fibres.

With respect to 42 squamous cell carcinomas studied, type VII collagen and laminin were present in basement membranes surrounding small and large tumour fields, independent of the tumour grade. Type VII collagen was demonstrated in the cytoplasm of tumour cells in 36% of the cases, while the antibody to laminin displayed a basement membrane staining pattern mainly. Both antibodies showed a staining gradient in more than half of the cases, with strong staining in the centre of the tumour and weakening of the staining towards the tumour periphery.

In a series of 22 salivary gland tumours consisting of 19 pleomorphic adenomas and three adenoid cystic carcinomas, the distribution pattern of type VII collagen and laminin was very heterogeneous. Laminin was present in 17 and type VII collagen in 10 of 19 cases of pleomorphic adenoma, mostly scattered throughout the tumour fields. In the tumours positive for type VII collagen areas with little or no positivity were also found. A correlation between type VII collagen positivity and the presence of basal cell keratin 14 positivity was noticed in the majority of cases. The three adenoid cystic carcinomas studied displayed random positivity with the laminin antibody, whilst the antibody to type VII collagen showed little positivity in these cases.

Keywords: basement membrane, type VII collagen, laminin, head neoplasms, neck neoplasms

Introduction

Basement membranes are complex extracellular matrix structures, involved in a number of biological processes and acting as a scaffold for maintaining structure and function of organisms. They form an important structural barrier influencing the passage of macromolecules and cells. The constituents of basement membranes are numerous and not yet all known, while considerable heterogeneity in composition between different basement membranes also exists. The main basement membrane components include laminin, type IV collagen, proteoglycans, entactin/nidogen and fibronectin. Another basement membrane component, type VII collagen, has been demonstrated as the major
protein constituent of the anchoring fibrils, connecting basement membranes with the underlying connective tissue.

Basement membranes form a natural barrier in the process of tumour invasion. Extensive studies have been performed on the function of basement membranes in neoplasms, predominantly using antibodies to laminin and type IV collagen. Several authors have reported a partial or complete loss of basement membrane during the process of tumour invasion in several different tissues. A more or less continuous basement membrane, on the other hand, was observed in invasive squamous cell carcinomas of the head and neck and in infiltrating squamous carcinomas of epidermal origin. The basement membrane component type VII collagen was shown to be more restricted in its appearance than laminin and type IV collagen. In normal tissues, type VII collagen is found in basement membranes lining combined and stratified epithelia, such as breast, prostate, larynx, oesophagus, trachea and epidermis. In previous reports, we have demonstrated that the presence of type VII collagen in malignant tumours seems to be related to the differentiation of the tumours, rather than to their origin. Type VII collagen was predominantly found in squamous cell carcinomas of, for instance, lung, head and neck, cervix, vulva and vagina.

The presence of type VII collagen has been shown to be related to the presence of basal/myoepithelial cells, as demonstrated in normal tissues and in situ and invasive carcinomas of the breast. In a previous study on squamous cell carcinomas of the lung, we reported correlation between type VII collagen and a basal cell phenotype of the tumour, as shown by the presence of keratin 14 and keratin 17 positive tumour cells. In the present study we have investigated the localization of type VII collagen in normal, benign and malignant tissues of the head and neck region, and compared its distribution to that of laminin. In addition, an antibody directed to keratin 14 was included as a basal cell marker in the case of salivary gland tissues.

### Materials and methods

Fresh normal (n = 16), benign (19) and malignant (45) human tissues from the head and neck region (Table 1),

<table>
<thead>
<tr>
<th>Tissues (no. of cases)</th>
<th>Staining pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal squamous and columnar epithelium (n = 16)</td>
<td>Type VII collagen</td>
</tr>
<tr>
<td>floor of mouth</td>
<td>+</td>
</tr>
<tr>
<td>tongue</td>
<td>+</td>
</tr>
<tr>
<td>palatum durum</td>
<td>+</td>
</tr>
<tr>
<td>palatum molle</td>
<td>+</td>
</tr>
<tr>
<td>nasopharynx</td>
<td>+</td>
</tr>
<tr>
<td>pharynx</td>
<td>+</td>
</tr>
<tr>
<td>sinus piriformis</td>
<td>+</td>
</tr>
<tr>
<td>post-cricoid region</td>
<td>+</td>
</tr>
<tr>
<td>supralarynx: epiglottis</td>
<td>+</td>
</tr>
<tr>
<td>plica vestibularis</td>
<td>+</td>
</tr>
<tr>
<td>larynx: plica vocalis</td>
<td>+</td>
</tr>
<tr>
<td>subglottis</td>
<td>+</td>
</tr>
<tr>
<td>trachea</td>
<td>+</td>
</tr>
<tr>
<td>Salivary gland combined epithelum (n = 2)</td>
<td>BM +: C+(6)</td>
</tr>
<tr>
<td>Squamous carcinomas of the oral cavity (n = 23)</td>
<td>BM +: C+(9)</td>
</tr>
<tr>
<td>Squamous laryngeal carcinomas (n = 19)</td>
<td>H+: - (9)</td>
</tr>
<tr>
<td>Pleomorphic adenomas (n = 19)</td>
<td></td>
</tr>
<tr>
<td>Adenoid cystic carcinomas (n = 3)</td>
<td></td>
</tr>
</tbody>
</table>

BM + = basement membrane positivity; C+ = cytoplasmic positivity; () = number of cases; H+ = heterogeneous positivity of basement membrane staining; = no positivity.

Table 1. Immunostaining patterns of head and neck tissues with antibodies to type VII collagen and laminin.
obtained after surgery or during autopsy, were immediately frozen and stored in liquid nitrogen. Cryostat sections (3–5 μm thick) were air-dried for 24 h at room temperature and, if necessary, stored at −80°C until use. For immunohistochemistry the sections were fixed for 10 min in acetone at room temperature, and incubated with the primary antibodies for 60 min, also at room temperature. Monoclonal antibody LH7.2, reacting with type VII collagen, was used as culture supernatant diluted 1:10 in phosphate-buffered saline (PBS, pH 7.4). Monoclonal antibody 4E10, reacting with laminin, was used as ascites fluid diluted 1:1000 in PBS. Monoclonal antibodies RCK107 and IL002, both reacting with keratin 14, were used as undiluted culture supernatant or diluted 1:10, respectively. Negative control sections were obtained by replacing the primary antibodies with PBS, while appropriate positive control sections were also run.

After repeated washings with PBS for 20 min, sections were incubated for 30 min with peroxidase-conjugated rabbit-anti-mouse IgG (Dako Patts, Glostrup, Denmark; diluted 1:80–160 in PBS). After a second series of washing steps with PBS the peroxidase activity was detected with 3,3′-diaminobenzidine tetrahydrochloride (DAB; Sigma Chemical Co., St. Louis, MO). Sections were counterstained with Harris’ haematoxylin, dehydrated, cleared in xylene and finally embedded in permount (Fisher Scientific Co., Fair Lawn, NJ).

Results

NORMAL HEAD AND NECK TISSUES

The normal head and neck squamous and columnar epithelia and the salivary gland combined epithelium studied displayed a positive basement membrane reaction with the antibodies to type VII collagen and laminin (Table 1, Figure 1). However, the staining reaction with the laminin antibody was often very weak, while the intensity of the reaction with the antibody to type VII collagen was very strong and discrete. In a minority of tissues a slight granular staining of blood vessels, lying in the close vicinity of the basement membrane, for type VII collagen could be noticed.

SQUAMOUS TUMOURS OF HEAD AND NECK

Type VII collagen and laminin were present in all 42 carcinomas of the head and neck region (Table 1). Areas with dysplasia showed a continuous basement membrane staining with both antibodies (Figure 2a,b). In 15 of 42 cases a large number of tumour cells showed a strong cytoplasmic staining reaction using the type VII collagen antibody. With the antibody to laminin, on the other hand, little or no significant cytoplasmic reactivity could be seen, since this antigen seemed restricted to the basement membrane (Figure 2c,d). In some cases the cytoplasmic type VII collagen reactivity was restricted to...
the basal cells lining tumour nests (Figure 2e), while cytoplasmic reactivity in combination with basement membrane positivity could also be found. In about half of the tumours (18 of 42) a granular staining reaction could be observed focally in the stroma, possibly representing cross-sections of type VII collagen fibrils.

In 18 of 25 cases a staining gradient with the type VII collagen antibody was noted, resulting in decrease of staining intensity towards the peripheral part of the tumour (Figure 2f). In the remaining 17 cases only the centre of the tumours were visible, and no tumour peripheries were present. This phenomenon was less obvious with the laminin antibody, which showed a more consistent staining pattern.

The group of squamous carcinomas of the head and neck consisted of poorly-, moderately- and well-differentiated tumours. No correlation between staining pattern and degree of differentiation was noticed. Staining of these squamous cell carcinomas with the antibody to keratin 14 showed diffuse positivity of virtually all tumour cells, in all cases.

SALIVARY GLAND TUMOURS

The group of salivary gland tumours consisted of 19 pleomorphic adenomas and three adenoid cystic carcinomas (Table 1). The pleomorphic adenomas displayed a heterogeneous staining pattern with the antibodies to basement membrane components. Laminin was found in 17 cases while two cases were completely negative. Type VII collagen was absent in nine of 19 cases, and the remaining 10 displayed a heterogeneous reaction pattern. Within a given tumour, areas with abundant type VII collagen positivity were found next to areas with little or no staining reaction. In the adenomas, type VII collagen positivity was observed, related to more differentiated areas, i.e. areas with tubular structures. In the majority of cases a correlation between the presence of
Figure 3. Two sections of the same case of pleomorphic adenoma stained a with the antibody to type VII collagen and b the antibody to keratin 14 (RCK107).

Type VII collagen has been shown to correlate with the presence of both constituents in invasive and metastatic squamous carcinomas. From these studies it was concluded that loss of basement membrane integrity is not generally associated with invasive behaviour of squamous cell carcinomas. Since our earlier study demonstrated that the expression of type VII collagen is more related to the type of tumour differentiation than is the case for laminin, we have compared the immunoreactivity of a monoclonal antibody to type VII collagen to the distribution pattern of laminin, in a series of benign and malignant tumours of the head and neck region.

With respect to the squamous cell carcinomas of the head and neck, it was remarkable to observe a cytoplasmic reaction of the type VII collagen antibody in the majority of the tumour cells in 36% of the cases. Whilst staining of the laminin antibody was mostly found in the basement membrane with virtually no cytoplasmic reactivity. Visser et al. and Suki et al. noted the presence of intracytoplasmic laminin and type IV collagen in squamous head and neck tumours, although their reports show cytoplasmic staining of only some neoplastic cells and focal intracellular laminin staining. The abundant intracellular type VII collagen staining pattern described here may indicate a failure of tumour cells to deposit this component extracellularly, resulting in its accumulation within the cytoplasm. The fact, however, that tumour fields containing such cells also exhibit a basement membrane type VII collagen distribution pattern, indicates an increased production of this basement membrane protein.

Our study on lung tumours revealed the presence of type VII collagen in moderately well differentiated squamous carcinomas, while it was decreased in poorly differentiated tumours. No such relationship with tumour progression could be found in the series of head and neck squamous cell carcinomas. In addition no relation between type VII collagen reactivity and the tissue of origin could be demonstrated. The fact that type VII collagen is expressed independent of tumour grade in head and neck squamous cell carcinomas and that the staining reaction is often intracytoplasmic may be useful as diagnostic criteria, for example in case of a differential diagnosis where a metastasis has to be identified as being derived from lung or a head and neck squamous cell carcinoma.

The observation that type VII collagen expression is gradually lost when comparing the centre of a squamous cell carcinoma with its periphery must be interpreted with great care. It is not possible to completely exclude the diminishing of the staining reaction to be the result of epitope masking or epitope destruction. The absence of type VII collagen at the periphery of the tumour may
possibly be related to a higher proliferative capacity of the tumour cells in this area, and may be related with the ability of the tumour cells to invade the surrounding stromal tissues in these areas. Alternately, the tumour cells in this area may not have had the opportunity to produce a basement membrane. The granular type VII collagen expression pattern, found in nearly half of the cases, might indicate the first steps in assembly of the anchoring fibrils. It cannot be excluded, however, that cross-sectional profiles of type VII collagen fibrils were seen.

With respect to salivary gland tumours, Caselitz et al. reported the heterogeneous expression of basement membrane components laminin and type IV collagen in a series of pleomorphic adenomas. These authors reported the presence of basement membrane in those areas where myoepithelial cells predominated. Our results, combining both markers for basal/myoepithelial cells and basement membrane constituents, confirm these findings. In a pattern similar to that in carcinomas of the breast and lung, type VII collagen expression was also strongly related to keratin 14 positive cells, as well as in pleomorphic adenomas of the head and neck. This may point to a specific synthesis of this membrane constituent by basal or myoepithelial cells, both normal and malignant. The virtual absence of type VII collagen in the three adenoid cystic carcinomas studied may be an indication of the malignant behaviour of these tumours, while type VII collagen positivity may be a diagnostic feature in pleomorphic adenoma.

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