Our case must be distinguished from malignant mixed müllerian tumour which occurs in the same age group and has carcinomatous and sarcomatous components. However, these tumours are generally solid, lack the characteristic squamous epithelial lined cyst and show different mucin expression. Immature malignant teratoma occurs in a younger age group and shows a variety of lines of mesenchymal differentiation, notably towards neural elements, which were absent in this case.

Germ cell tumours of all types derive from neoplastic totipotent stem cells whose progeny retain the ability to differentiate towards tissues of all three embryonic somatic layers and the transitional extraembryonal structures. The original pattern of the better differentiated members of this group suggests that local cellular influences are important in determining the final phenotype expressed by individual cells. The histological behaviour and histological appearances of these tumours are determined by the degree to which this embryogenetic environmental responsiveness is retained. In contrast, our case resembles carcinomas and sarcomas at other sites in that there was an essentially random mixture of stromal and epithelial elements. This reflects a probabilistic commitment of individual cells and clones to one or other expressed phenotype without regard to organogenetic influences. Our case implies glandular malignant transformation within the previously benign, fully differentiated, glandular component of a mature cystic teratoma, although no premalignant changes were identified despite thorough sampling.

Adenocarcinomas are the second most common malignancies arising within dermoid cysts and may display gastrointestinal phenotypes, but lack the malignant stroma as in our case. Sarcomas alone or in combination with squamous carcinoma have been described arising in a mature cystic teratoma. To the best of our knowledge, no case of sarcoma arising in association with adenocarcinoma has been described before.

The prognosis of patients with malignant transformation in teratoma is very poor, with most women dying within one year. Occasional cases with prolonged survival have been reported. Poor prognostic factors include tumour dissemination, cyst wall invasion, ascites, spontaneous or accidental rupture, adhesions, and tumour type other than squamous carcinoma. Treatment is surgical, with pelvic clearance in most cases. Chemotherapy may be indicated according to the tumour type.


Tenascin in human papillomavirus associated lesions of the uterine cervix

R Pöllänen, Y Soini, S Vuopala, E Läärä, V-P Lehto

Abstract
The immunohistochemical expression of tenascin was studied in 80 morphologically diagnosed condylomas and cervical intraepithelial neoplasia (CIN) lesions. The results were compared with the human papillomavirus (HPV) DNA subtype, which was determined by HPV dot blot and in situ hybridisation. Tenascin mRNA synthesis was also determined in 10 selected cases by in situ hybridisation. No statistically significant association was found between tenascin expression and the degree of dysplasia or the HPV subtype. There was, however, a strong correlation between the extent of tenascin immunoreactivity and the degree of inflammation. Synthesis of tenascin mRNA was detected in basal keratinocytes and in fibroblasts by in situ hybridisation. The lack of association between the grade of CIN and tenascin expression precludes its use as a marker of premalignancy in CIN.

Keywords: tenascin, extracellular matrix proteins, human papillomavirus infection, HPV DNA, inflammation.

Human papillomavirus (HPV) is the aetiological agent of various genital lesions and there is
a significant correlation between infection with specific HPV subtypes and the nature of the lesion. Thus, HPV types 6 and 11 are most commonly found in association with benign condylomata whereas HPV types 16, 18 and 31 are found in high grade dysplasia and cervical cancer.

Tenascin is an extracellular matrix protein that plays a role in tissue interactions during fetal development and in malignant transformation. It is involved in critical functions such as epithelial-mesenchymal interactions, organogenesis, somatic growth regulation, epithelial renewal, cell adhesion, morphogenesis, and cell migration.

To determine whether tenascin expression is correlated with the degree of cervical dysplasia and the HPV subtype, we investigated its expression in 80 condylomata and cervical intraepithelial neoplasia (CIN) lesions and correlated the results with the HPV subtypes present in these lesions.

Methods

Samples for the study were obtained at colposcopy from 80 patients. Nine patients with normal histology or with cervicitis with no indication of condyloma or dysplasia were also included. Biopsy specimens for routine histological evaluation were fixed in 10% formalin, processed routinely, and embedded in paraffin wax using standard techniques. Histological diagnosis was made using generally accepted criteria. The extent of the inflammatory reaction was scored as follows: none (-); weak (+); moderate (++); and strong (++++).

HPV DNA was detected by dot blot hybridisation using the Virapap HPV DNA screening kit, the Viratype HPV DNA typing kit (Digene, Gaithersburg, Maryland, USA) and the Affiprobe kit (Orion, Helsinki, Finland) according to the manufacturers’ instructions. HPV DNA was detected by the Virapap HPV DNA screening kit, using a mixture of 32P-labelled RNA probes detecting HPV types 6, 11, 16, 18, 31, 33, and 35. Specimens displaying a positive hybridisation signal were typed by using the Viratype kit or the Affiprobe kit with probes delineating three (6/11, 16/18 and 31/33/35) or four viral groups (6/11, 16/18, 31/33, and 31), respectively.

In situ DNA hybridisation was used in cases with negative scores on dot blot hybridisation. For that purpose, the Biohit in situ HPV DNA typing test kit (Biohit, Helsinki, Finland), containing biotin labelled probes for HPV types 6, 11, 16, 18, 31, 33, and 35, was used as described by the manufacturer. Positive controls included cervical samples positive for each of the HPV subtypes tested, and HPV 16 containing CaSki cells provided with the kit.

For immunohistochemistry, the sections were incubated with a monoclonal tenascin mouse antibody (143CB7), and then with the biotinylated rabbit anti-mouse antibody and the avidin-biotin peroxidase complex (Dako, Glostrup, Denmark). Diaminobenzidine was used as the chromogen. Negative control consisted of substituting the primary antibody with phosphate buffered saline and normal mouse serum. The maximal width of the anti-tenascin reactive zone was measured using an ocular micrometer.

### Table 1: Distribution of HPV positive and negative patients and tenasin immunoreactivity in the cervical lesions

<table>
<thead>
<tr>
<th>Condition</th>
<th>HPV subtype</th>
<th>Low risk (n = 10)</th>
<th>High risk (n = 24)</th>
<th>Tenasin (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Condyloma</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CIN</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>grade I</td>
<td>32</td>
<td>5</td>
<td>9</td>
<td>100.0 ± 92.5</td>
</tr>
<tr>
<td>grade II</td>
<td>14</td>
<td>2</td>
<td>8</td>
<td>122.5 ± 170.0</td>
</tr>
<tr>
<td>grade III</td>
<td>12</td>
<td>1</td>
<td>3</td>
<td>167.0 ± 113.0</td>
</tr>
</tbody>
</table>

| HPV 6 or 11; HPV 16, 18, 31, 33, or 35. Tenasin immunoreactivity was scored as the maximal width of the anti-tenasin reactive zone, measured using an ocular micrometer. |

### Results

In all 80 cases HPV infection was diagnosed on the basis of characteristic morphological features. HPV DNA was detected by in situ or dot blot hybridisation in 32 (40%) patients. The low risk HPV subtypes (6 or 11) were detected in 10 patients and the high risk subtypes (16, 18, 31, 33, and 35) in 24 (table 1). The extent of the tenasin positive zone in relation to the morphology of the lesion, the presence or absence of HPV DNA and the degree of inflammation is shown in tables 1 and 2. There was no statistically significant difference in the extent of tenasin immunoreactivity between the different CIN lesions. Patients with no or weak inflammation showed significantly less tenasin immunoreactivity (91.5 ± 114.0 μm) than those with strong inflammation (185.5 ± 175.5 μm) (p = 0.002, Fisher’s exact test). There was no significant difference in tenasin immunoreactivity between patients with benign (71.0 ± 93.0 μm) and malignant (88.5 ± 84.5 μm) HPV subtypes (p = 0.84, Fisher’s exact test). According to a three-way analysis of variance, inflammation had a statistically significant (p = 0.03) effect on the accumulation of tenasin but not on the degree of dysplasia (p = 0.25) or the HPV subtype (p = 0.44).

In the 10 cases studied by in situ hybridisation for tenasin mRNA, labelling was ob-
Table 2. Extent of the tenascin positive zone in relation to the degree of dysplasia, the presence of HPV and the degree of inflammation.

<table>
<thead>
<tr>
<th>Tenascin immunoreactivity (n; pm)*</th>
<th>1-25</th>
<th>26-100</th>
<th>101-250</th>
<th>&gt; 250</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dysplasia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CIN 0</td>
<td>11</td>
<td>5</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>CIN I</td>
<td>12</td>
<td>8</td>
<td>11</td>
<td>1</td>
</tr>
<tr>
<td>CIN II</td>
<td>5</td>
<td>4</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>CIN III</td>
<td>1</td>
<td>5</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>HPV subtype</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>low</td>
<td>15</td>
<td>11</td>
<td>14</td>
<td>6</td>
</tr>
<tr>
<td>high</td>
<td>6</td>
<td>1</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>CIN</td>
<td>7</td>
<td>12</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Inflammation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>none</td>
<td>16</td>
<td>8</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>weak</td>
<td>8</td>
<td>11</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>moderate</td>
<td>4</td>
<td>2</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>strong</td>
<td>0</td>
<td>2</td>
<td>3</td>
<td>1</td>
</tr>
</tbody>
</table>

*n = number of patients.

Tenascin in human papillomavirus associated lesions of the uterine cervix.

R Pöllänen, Y Soini, S Vuopala, E Läärä and V P Lehto

doi: 10.1136/jcp.49.6.521

Updated information and services can be found at:
http://jcp.bmj.com/content/49/6/521

These include:
Email alerting service
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/