Intermediate Filament Proteins in Spindle Cell Carcinoma of the Larynx and Tongue


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In an analysis of intermediate filament protein expression of spindle cell carcinoma, a variant of squamous cell carcinoma, occurring in larynx and tongue, vimentin positivity was found in sarcomatoid areas in 12 of 13 patients. Scattered expression of keratin was observed in sarcomatoid areas in tumours of 10 patients. However, large parts of sarcomatoid areas of such tumours were negative for keratin. Overlying dysplastic epithelium and squamous cell carcinoma components were positive for keratin. In a number of cases there was a strong indication of co-expression of keratin and vimentin in parts of cells that, on histological grounds, belong to sarcomatoid areas or to cells in the interface between carcinoma and spindle cell area. The use of keratin and vimentin type intermediate filament antibodies could be of great help for the correct classification of these tumours. Key words: intermediate filaments, sarcomatoid squamous cell carcinoma, upper respiratory tract.

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Spindle cell carcinoma of larynx and tongue is a rare, mostly polyloid tumour. The age at presentation is the same as that for patients with the more common squamous cell carcinoma.

In the literature, spindle cell carcinoma is known under various synonyms, viz. carcinosarcoma, pseudosarcoma, sarcomatoid squamous cell carcinoma and polyloid carcinoma.

It is now generally believed that spindle cell carcinoma is a variant of squamous cell carcinoma. Both squamous and spindle cell tumour compartments have malignant properties and are capable of metastasizing. It is sometimes hard to find histological or ultrastructural evidence of epithelial properties in sarcomatoid areas. Especially in metastases, a correct diagnosis is necessary for a successful search for a primary tumour. In reported cases with ultrastructural examination, some authors (1, 2, 3, 4) noted squamous cell properties, while others (5, 6) detected only fibroblastic cells with active secretion in spindle cell compartments.

Immunohistochemical techniques for the detection of the intermediate filament proteins (IFP), keratin and vimentin, could in principle distinguish between epithelial and mesenchymal properties within such tumours. Two case-studies have examined the expression of keratin and vimentin in spindle cell carcinoma. In one report (7) keratin was found positive in both carcinomatous and sarcomatoid parts of the tumour, while in the other report (8), keratin was positive in the carcinomatous areas and vimentin in the sarcomatoid part, with some cells in the interface between the two tumour types showing co-expression of both types of IFP. Recently, in a series of 25 cases (4) keratin expression was present in 8 of 18 biphasic and 4 of 7 ulcerated monophasic spindle-cell tumours. Most spindle cells in all tumours showed vimentin expression. Three biphasic tumours, examined with the alkaline phosphatase double labelling technique, contained only a small number of spindle cells with co-expression of keratin and vimentin. Another very recent report (9) on 21
mucosal spindle-cell tumours showed keratin positivity in 62% of cases. Co-expression of keratin and vimentin was present in 59% of cases.

In this study we examined 13 cases of spindle cell carcinoma of larynx and tongue, giving special attention to the (co-)expression of keratin and vimentin.

MATERIALS AND METHODS

Tissues

The tissue specimens in this study were formalin-fixed paraffin-embedded samples from 13 patients diagnosed between 1960 and 1985 as having a carcinosarcoma. A survey of the clinicopathological data of the cases is given in Table I.

Table I. Clinical data of 13 patients with spindle cell carcinoma of the upper respiratory tract

<table>
<thead>
<tr>
<th>Patient</th>
<th>Symptom</th>
<th>Site</th>
<th>Appearance</th>
<th>TNM class</th>
<th>First histology*</th>
<th>Treatment</th>
<th>Clinical course (eventual second histology)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/M/61</td>
<td>Hoarseness</td>
<td>TVC</td>
<td>Pedunc.</td>
<td>T3N0MO</td>
<td>SPCA</td>
<td>25 Gy, TLE</td>
<td>NED 13 yr.</td>
</tr>
<tr>
<td>2/M/55</td>
<td>Hoarseness</td>
<td>FVC</td>
<td>Polypoid</td>
<td>T2N0MO</td>
<td>SPCA</td>
<td>40 Gy, TLE</td>
<td>Stoma-recurrence, 2 years later excision (SPCA), Died of septic shock</td>
</tr>
<tr>
<td>3/M/45</td>
<td>Hoarseness</td>
<td>FVC</td>
<td>Polypoid</td>
<td>T2N0MO</td>
<td>SPCA</td>
<td>60 Gy, TLE</td>
<td>Residual disease (SPCA) NED 3 yr., died of alcohol-abuse NED 3 yr.</td>
</tr>
<tr>
<td>4/M/70</td>
<td>Hoarseness</td>
<td>TVC</td>
<td>Diffuse</td>
<td>T1N0MO</td>
<td>SPCA</td>
<td>60 Gy</td>
<td>Recurrence (SPCA), TLE died after 3 yr. of pulm. metast. (no hist.) More subia due to suflocation. Autopsy SPCA in cervical lymphnodes</td>
</tr>
<tr>
<td>5/M/64</td>
<td>Hoarseness</td>
<td>TVC</td>
<td>Polypoid</td>
<td>T2N0MO</td>
<td>SPCA</td>
<td>60 Gy</td>
<td></td>
</tr>
<tr>
<td>6/M/68</td>
<td>Hoarseness</td>
<td>TVC</td>
<td>Pedunc.</td>
<td>T4N1MO</td>
<td>SPCA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7/M/61</td>
<td>Dysphagia, otalgia</td>
<td>Tongue</td>
<td>Exophytic</td>
<td>T3N1MO</td>
<td>SPCA</td>
<td>Composite resection</td>
<td></td>
</tr>
<tr>
<td>8/M/55</td>
<td>Hoarseness</td>
<td>TVC</td>
<td>Pedunc.</td>
<td>T1N0MO</td>
<td>SPCA</td>
<td>63 Gy</td>
<td>NED 1 yr., later bronchus ca. T1N0MO, thoracotomy NED 8 yrs later</td>
</tr>
<tr>
<td>9/M/68</td>
<td>Hoarseness</td>
<td>TVC</td>
<td>Polypoid</td>
<td>T2N0MO</td>
<td>SPCA</td>
<td>62 Gy</td>
<td>NED 4 yrs later</td>
</tr>
<tr>
<td>10/M/62</td>
<td>Hoarseness</td>
<td>TVC</td>
<td>Polypoid</td>
<td>T1N0MO</td>
<td>SPCA</td>
<td>60 Gy</td>
<td>NED 4 yrs later</td>
</tr>
<tr>
<td>11/M/59</td>
<td>Hoarseness</td>
<td>TVC</td>
<td>Diffuse</td>
<td>T1N0MO</td>
<td>SQCA</td>
<td>60 Gy</td>
<td>Recurrence 4 yrs TLE (SPCA) NED 2 yrs later</td>
</tr>
<tr>
<td>12/M/56</td>
<td>Hoarseness</td>
<td>TVC</td>
<td>Polypoid</td>
<td>T1N0MO</td>
<td>SQCA</td>
<td>60 Gy</td>
<td>Recurrence 2 yrs TLE (SPCA) NED 3 yrs later</td>
</tr>
<tr>
<td>13/F/42</td>
<td>Hoarseness</td>
<td>TVC</td>
<td>Diffuse</td>
<td>T1N0MO</td>
<td>SPCA</td>
<td>Radiation</td>
<td>Lost for follow-up</td>
</tr>
</tbody>
</table>

TVC: True vocal cord; SPCA: Spindle cell carcinoma; NED: No evidence of disease; SIN PI. Sinus piriformis; FVC: False vocal cord; SQCA: Squamous cell carcinoma; EPIG: Epiglottis; TLE: Total laryngectomy.

* Histology at first presentation.

* Histology after recurrence of the tumour.
A primary diagnosis in all cases was made by routine light microscopy, using haematoxylin and eosin (H&E) stained tissue sections or (when appropriate) supplemented with additional histochemical staining procedures, while in 2 cases (cases 5 and 7) electron microscopic examination had been performed.

Antibodies

The following primary antibody preparations were used in this study:
1. An affinity purified polyclonal rabbit antiserum directed against human skin keratins. For preparation and specificity see (10)
2. An affinity purified polyclonal rabbit antiserum raised against vimentin, isolated from calf lens cells by preparative gel electrophoresis. Preparation and specificity testing have been described elsewhere (10).

Table II. Histological and immunohistochemical data

<table>
<thead>
<tr>
<th>Patient/sex/age</th>
<th>Histology</th>
<th>Immunology</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1/M/61</td>
<td>Mostly SQCA only in polyp spindle cell area before and after irradiation</td>
<td>K+ V−</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2/M/55</td>
<td>Biopsy SQCA after radiotherapy in TLE 90% spindle cells only dysplastic overlying cells with incipient invasion</td>
<td>K+ V−</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3/M/45</td>
<td>In biopsy and TLE 80% SQCA 20% spindle cells before and after radiation</td>
<td>K+ V− spK+ V+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4/M/70</td>
<td>Mostly spindle cells with overlying dysplastic cells with incipient invasion</td>
<td>K+ V−</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5/M/64</td>
<td>Balanced composition of SQCA and spindle cells with overlying dysplastic epithelium focal myxoid areas (before and after irradiation) in EM only SQCA-properties</td>
<td>K+ V−</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6/M/68</td>
<td>Only spindle cells with overlying ulceration. Autopsy: spindle cell areas and SQCA in neck lymph node. META:</td>
<td>K+ V−</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7/M/61</td>
<td>SQCA with fluent transition to spindle cell area. In EM only squamous cell properties. In lymph node meta neck only SQCA</td>
<td>K+ V−</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8/M/55</td>
<td>Dysplastic epithelium with invasive SQCA and spindle cell areas</td>
<td>K+ V− spK+ V+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9/M/68</td>
<td>Overlying dysplastic epithelium with incipient invasion. Moreover epithelialoid and spindle cell type SPCA</td>
<td>K+ V−</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10/M/62</td>
<td>Dysplastic epithelium with incipient invasion. Elsewhere myxoid pattern with stellate, spindle and epithelioid areas</td>
<td>K+ V−</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11/M/59</td>
<td>Biopsy: SQCA: radiotherapy 4 yrs later TLE. Balanced composition of SQCA and spindle cell areas with overlying dysplasia</td>
<td>K+ V−</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12/M/56</td>
<td>Multiple biopsies before and after irradiation: spindle cells without clear-cut SQCA: overlying ulceration and ulceration</td>
<td>K+ V−</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13/F/42</td>
<td>Spindle cells with overlying dysplastic epithelium</td>
<td>K+ V−</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

K: keratin; spK: sporadic keratin; V: vimentin; EM: electron microscopy.
* NT: not tested; no material left of spindle cell area.
Fig. 1. Immunohistochemical demonstration of keratin (a, c, e) and vimentin (b, d, f) in paraffin sections of spindle cell carcinoma of upper respiratory tract (×350). (a, b) Transitional zone between carcinoma in situ and/or squamous cell carcinoma and spindle cell carcinoma areas (arrow: solitary positive cell for vimentin in interface). (c, d) Spindle cell carcinoma area. (e, f) Myxoid areas of spindle cell carcinoma.
The immunoperoxidase (PAP) technique was used for the detection of the antigens and has been described before (10).

RESULTS
The male/female ratio of cases presented was 12:1 and the age range was 45–70 years. Complaints at first presentation were hoarseness (11/12 cases), pain (1/12 cases), stridor (1/12 cases) and dysphagia (1/12 cases). In 10 cases, tumours had a polyoid or pedunculated appearance.

In a subset of 5 patients whose tumour recurred or persisted after a full course of radiotherapy, the initially diagnosed squamous cell carcinoma had changed into a spindle cell carcinoma on two occasions. In the 3 other cases a spindle cell carcinoma was already present in the first biopsy (see Table I).

Histology
Microscopically (see Table II), an overlying carcinoma in situ, with or without limited invasion, was observed in 9 cases.

In cases of squamous cell carcinoma, the tumour was of a classic type with visible cell bridges. Mostly, this part of the tumour was found superficial to sarcomatoid areas, while the proportion of tumour-area containing classic squamous cell- and spindle cell carcinoma components varied. A clear preponderance of sarcomatoid areas was noted in 10 of 16 specimens (from 13 patients with spindle-cell carcinoma). In three specimens, classic squamous cell carcinoma was the most abundant component, while in three other specimens a more balanced ratio between the two components existed.

In a number of cases a gradual transition between both types of tumour differentiation was seen. Sarcomatoid compartments consisted mostly of cell-rich areas of spindle-shaped
cells with only a few thin reticulin fibres in between. Very often these cells had relatively abundant faintly stained eosinophilic cytoplasm and clearly visible cell-borders, sometimes giving cells a more epithelioid appearance. In other areas a dissociated growth pattern was observed with widely spread solitary cells. In 2 cases (nos. 5 and 10) a myxoid pattern was seen with abundant alcian-blue-positive material. In one of these 2 cases (no. 5) a myxoid pattern was only seen in the laryngectomy-specimen obtained after radiotherapy. In case 12 a cell-rich tumour was found, with large polygonal tumour cells showing a medullary growth-pattern. In the basal cell layers of overlying dysplastic mucosa, morphologically identical polygonal cells were also found. Also in these areas, a so-called ‘dropping-off’ phenomenon was seen, meaning that a gradual transition of tumour cells from dysplastic mucosa or in situ carcinoma to invasive parts was observed. On the basis of this phenomenon we decided that this tumour represented spindle cell carcinoma. In case 6, a metastasis containing spindle cell component was detected in a cervical lymph node.

Electron microscopy
In cases 5 and 7 in which electronmicroscopic examination was performed, cells present in the sarcomatoid areas showed desmosomes and tonofilaments.

Immunohistochemistry
All specimens were examined for the presence of keratin and vimentin (see Table II). In all cases, microscopically classic squamous cell carcinoma components as well as overlying dysplastic mucosa and in situ carcinoma-areas were clearly positive for keratin and negative for vimentin (Fig. 1a, b). In transitional zones between squamous cell and spindle cell areas, some of the cells were found positive for keratin and most cells were vimentin-positive (see arrow, Fig. 1b). As far as we can conclude from the immunoperoxidase-stained paraffin sections, co-expression of keratin and vimentin seems to occur in a number of these cells.

In the sarcomatoid areas (12 cases) the cells were predominantly vimentin-positive and keratin-negative. In 8 cases, a few solitary keratin-positive cells were seen in sarcomatoid areas (Fig. 1c, d).

In the myxoid regions, all tumour cells were positive for both keratin and vimentin (thus with obvious co-expression; Fig. 1e, f).

DISCUSSION
We believe, like most other authors, that spindle cell carcinoma of the upper respiratory tract is a variant of squamous cell carcinoma. Some investigators (6), however, could not find epithelial properties in sarcomatoid areas and concluded that these were benign stromal reactions.

Prognosis of this tumour type seems to be significantly better than for classic squamous cell carcinoma. This may be due to the relatively superficially infiltrative growth pattern of these often exophytic polypoid tumours (11).

Our clinical data suggest that the spindle cell component of laryngeal carcinoma is not radiation-induced (1). Eleven patients showed a sarcomatoid pattern before radiotherapy. Only 2 patients developed a spindle cell carcinoma after radiotherapy for a recurrent squamous cell carcinoma. Radiotherapy therefore does not seem to be a major pathogenetic factor. Histological diagnosis of spindle cell carcinomas of the upper respiratory tract may be difficult, especially in cases with presentation of pure spindle cell tumour, as a primary cancer, or as a metastasis. This tumour type can often not be readily distinguished from a sarcoma. Therefore we wondered if immunohistochemical techniques using anti-
bodies to keratin and vimentin could be of help. Keratins have been detected mainly in
epithelial tissues and their tumours, while vimentin is present in normal mesenchymal
tissues and their neoplasms, but may be co-expressed next to other intermediate filament
proteins (12).

Specificity of keratin and vimentin antibodies is best illustrated in cases of histological
dysplastic mucous and/or squamous cell carcinoma. Epithelial components were keratin-
positive and vimentin-negative, while stroma components were vimentin-positive and
keratin-negative.

In spindle cell areas as well as in the interface between carcinoma and sarcomatoid
areas, most tumour cells were vimentin-positive, with occasional cells (also) positive for
keratin. In contrast, cells in myxoid areas showed a strong co-expression of keratin and
vimentin.

Double expression of keratin and vimentin has been shown to occur in other epithelial
tumours such as tumours from thyroid (13) and salivary gland (14), in mesotheliomas (15),
in renal cell carcinomas (12) and in nephroblastomas (16). Also, epithelial tumour cells
present in serosal body cavities may show temporary vimentin expression next to keratin
(17).

Some authors (18) believe that this co-expression is due to dedifferentiation, with
regression to embryonal type of IFP.

Experiments with cultured cells (19) and cells shed into body cavities (20) show that it
may also be a consequence of adaptation of cells to environmental influences or to be
related to reduced cell-to-cell contact. This latter possibility may be applicable to myxoid
tumour areas.

Strikingly, however, compact and cell-rich spindle cell areas show almost complete loss
of keratin expression and show high concentrations of vimentin. In this component of the
tumour a relation between morphological characteristics and IFP expression seems to
exist. Apparently, the spindle cell shape of these cells is correlated to the presence of
vimentin and absence (in most cells) of keratin. Such a connection has also been demon-
strated for spindle cell mesotheliomas (15).

Preliminary results with a limited number of urothelial cell carcinoma, showing pseudo-
sarcomatoid growth patterns, also indicate that in these tumours the spindle cells acquire a
vimentin-type cytoskeleton, in addition to the keratin cytoskeleton.

From a practical point of view, application of keratin and vimentin antibodies in spindle
cell tumours could be of help in those cases with presentation of a primary sarcomatoid
neoplasm in the upper respiratory tract without recognizable squamous cell carcinoma or
in situ carcinoma component. Moreover, such antibodies may contribute to the diagnosis
of spindle cell carcinoma metastases. However, one should keep in mind that spindle cell
carcinomas, such as those described above, may possibly give rise to metastases which
are completely devoid of keratin-positive cells. The metastatic tumour described above
contained, however, a keratin-positive squamous cell carcinoma besides spindle cell
areas. Since reports in the literature have described pure spindle cell type carcinoma
metastases (21), future studies will have to elucidate the IFP protein expression pattern of
such neoplasms.

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REFERENCES
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