Primary cutaneous adenoid cystic carcinoma: case report, immunohistochemistry, and review of the literature

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Accepted for publication 16 September 1987

SUMMARY

We report a case of primary cutaneous adenoid cystic carcinoma in a 75-year-old man.

Electron microscopy revealed similar features to those previously described in adenoid cystic carcinomas of salivary gland origin: wide intercellular spaces, pseudocysts containing replicated basement membrane-like material and true lumina lined by cytoplasmic membranes with numerous microvilli. Immunohistochemistry using antibodies to several cytoskeletal proteins (keratins and actin) indicated the presence of two epithelial tumour cell populations, one with the phenotype of myoepithelial cells, lining the pseudocysts and the periphery of the tumour fields, and another with the phenotype of acinar cells of the secretory coil of sweat glands. In addition, the tumour showed immunoreactivity for epithelial membrane antigen, but not carinoembryonic antigen. A review of the literature on other cases of primary cutaneous adenoid cystic carcinoma showed that this tumour generally affects older patients with a female to male ratio of 4:1. The most common tumour site appears to be the scalp (40%), and the recurrence rate is 50%. Surgical treatment with extensive resection margins is recommended.

Adenoid cystic carcinomas (ACC) most commonly originate in the major and minor salivary glands. Other, rare, primary locations may be the breast,1 the major bronchi,2 the uterine cervix,3 the external auditory canal,4 and the skin.5-16 Cutaneous localization of ACC may also result from direct extension of ACC of the minor salivary glands situated in the paranasal sinuses,17 and rarely a distant cutaneous metastasis of a primary salivary gland ACC occurs.18 Primary cutaneous ACCs are generally considered as eccrine sweat gland carcinoma.19 Electron microscopic and immunofluorescence studies have implicated myoepithelial cells as the histogenetic precursors of ACC of the salivary glands and uterine cervix.20,21

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The expression of a distinct set of keratins and other cytoskeletal proteins (e.g. actin) by tumour cells may permit a more detailed characterization of the various epithelial cells within a given tumour. We present here histology, electron microscopy and immunohistochemistry of a case of primary cutaneous ACC, together with a review of the literature.

CASE REPORT

A 75-year-old Caucasian man presented with a tender, mobile tumour, 2 cm in diameter, on his chest. The nodule was firm, reddish in colour, with an irregular lobular surface and had slowly increased in size over several years. The lesion was surgically excised and histopathology revealed an adenoid cystic carcinoma. The resection margins were not free of tumour. Nine months later a firm tumour 2 cm in diameter recurred in the scarred area. The lesion was removed surgically and the histopathological diagnosis of adenoid cystic carcinoma was again established. No pathology of the salivary glands or the external auditory canals was found.

METHODS

Tissue

All tissue obtained at the first excision was fixed in 10% phosphate buffered formalin and processed routinely for microscopic examination. Part of the recurrent tumour was also processed for electron microscopy and a few slices of tumourous tissue were snap frozen in chilled isopentane and stored in liquid nitrogen.

For electron microscopy, tissue samples obtained immediately after surgical removal of the tumour were cut into small cubes, fixed in a solution of glutaraldehyde-formaldehyde, postfixed in phosphate buffered osmium-tetroxide, dehydrated in a graded series of ethanol, and embedded in Epon resin. Semithin 1 μm sections stained with toluidin-blue permitted the

### Table 1. Antibodies used in the study

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Antigen recognized</th>
<th>Antibody type</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>RCK 105</td>
<td>keratin 7</td>
<td>mouse monoclonal</td>
<td>Dr F. Ramackers$^{13}$</td>
</tr>
<tr>
<td>RKSE 60</td>
<td>keratin 10</td>
<td>mouse monoclonal</td>
<td>Organon Technika, Holland$^{24}$</td>
</tr>
<tr>
<td>RGE 53</td>
<td>keratin 18</td>
<td>mouse monoclonal</td>
<td>Organon Technika, Holland$^{23}$</td>
</tr>
<tr>
<td>ER-TR5</td>
<td>keratin (undefined)</td>
<td>rat monoclonal</td>
<td>Dr E. van Vliet$^{26}$</td>
</tr>
<tr>
<td>pKer</td>
<td>several keratins</td>
<td>rabbit polyclonal</td>
<td>Organon Technika, Holland$^{37}$</td>
</tr>
<tr>
<td>pVim</td>
<td>vimentin</td>
<td>rabbit polyclonal</td>
<td>Sambio, Holland$^{28}$</td>
</tr>
<tr>
<td>RAc1</td>
<td>actin</td>
<td>mouse monoclonal</td>
<td>Dr F. Ramackers</td>
</tr>
<tr>
<td>12E9</td>
<td>epithelial membrane antigen</td>
<td>mouse monoclonal</td>
<td>DAKO, Denmark$^{29}$</td>
</tr>
<tr>
<td>PARLAM-4</td>
<td>carcinomaembryonic antigen</td>
<td>mouse monoclonal</td>
<td>Organon Technika, Holland</td>
</tr>
</tbody>
</table>
Primary cutaneous adenoid cystic carcinoma

selection of cribriform pseudocyst containing areas for thin sectioning. Ultrathin sections were stained with uranyl acetate and examined in a Zeiss EM 902 electron microscope.

Antibodies
The antibodies used in the study are listed in Table 1. The monoclonal antibodies directed against epithelial membrane antigen (EMA), carcinoembryonic antigen (CEA), and the polyclonal antibodies directed against vimentin and keratin were applied to deparaffinized tissue sections. For demonstration of keratin and vimentin, deparaffinized tissue sections were pretreated with 1% pronase (Sigma) for 25 min at 37°C. The monoclonal antibodies specific for actin (RAct), keratins 7, 10 and 18 (RCK 105, RKSE 60 and RGE 53, respectively) and the rat monoclonal antibody ER-TR5 were only applied to frozen sections, since no immunostaining on formalin-fixed or paraffin-embedded sections has been achieved.23 ER-TR5 is a rat monoclonal antibody specific for a restricted number of mouse and human epithelia. Immunoelectron microscopy has shown that it specifically stains intermediate filaments of epithelial cells.26 The immunoperoxidase technique on paraffin-embedded and frozen tissues was performed using the indirect conjugated peroxidase method as described elsewhere.26 In order to visualize the binding of the IgM monoclonal anti-actin antibody, an FITC-conjugated goat anti-mouse IgM(Fc) was used.

Figure 1. Cribriform and tubular tumour masses of ACC localized in the reticular dermis and extending into the subcutis. No contiguity with the epidermis. (Haematoxylin and eosin stain; original × 125).
RESULTS

Histopathology and electron microscopy
The primary and recurrent tumours were composed of tubular and cribriform epithelial cell masses distributed throughout the reticular dermis and extending into the subcutis. The papillary dermis was not involved. No connection with the epidermis or hair follicles was observed (Fig. 1). The tumour cells had a basaloïd appearance, and contained uniform round to oval somewhat vesicular nuclei with an occasional rather conspicuous solitary nucleolus. The cytoplasm was sparse and several mitoses were present. Palisading of tumour cells was not evident. Most of the lumina of the tubular and cribriform masses were empty, but some contained homogeneous material that reacted with periodic acid-Schiff, periodic acid-Schiff-diastase, and alcain blue (pH 2.5) stains. The latter staining reaction was abolished by pre-treatment with hyaluronidase. In the primary and the recurrent tumour perineural invasion was found. Some tumour masses were surrounded by alcian blue positive material.

Electron microscopy of the tumour masses revealed wide intercellular spaces, while the cytoplasmic processes of the tumour cells were joined by desmosomes. The larger cystic spaces in the cribriform masses contained filamentous material (Fig. 2). True lumina were identified by the presence of junctional complexes between the surrounding cells and by the presence of numerous microvilli protruding into the lumen. The lumina contained flocculated material, most probably inspissated mucin. Two types of tumour cells could be distinguished: an electron-dense cell type with deeply indented nuclei, lining the pseudocysts (Fig. 3) and a less electron-dense cell type with round nuclei (Fig. 4). Both cell types contained numerous mitochondria and ribosomes. In some cells secretory vacuoles and Golgi membranes were observed.

Figure 2. Electron micrograph of ACC. The pseudocysts contain filamentous material (asterisk). (Original × 3000).
Figure 3. Electron micrograph of a myoepithelial cell. Note the elongated appearance of the cell, the electron-dense cytoplasm and the indented nucleus. (Original × 4400).

Figure 4. Electron micrograph of acinar cells showing abundant cytoplasm and numerous villous cytoplasmic projections (arrow). (Original × 4400).
Immunohistochemistry

The paraffin-embedded tumour material was immunoreactive for EMA and keratin, but no staining of tumour cells was found with antibodies to CEA and vimentin. EMA-positive material was found predominantly in the microcystic spaces of the tubular and cribriform tumour fields as well as on the surfaces of the cells lining the lumina of the tubular tumour strands. Faint cytoplasmic staining for EMA was present in a proportion of the cells at the periphery of the tumour fields. The larger pseudocystic spaces within the cribriform masses were not stained for EMA (Fig. 5). The polyclonal anti-keratin antiserum stained virtually all tumour cells, but a more intense staining was observed in the tumour cells lining the pseudocysts within the cribriform masses and in cells at the periphery of tubular and cribriform tumour masses. The monoclonal antibodies specific for keratins 7 and 18 gave intense staining of all epithelial tumour cells, except those lining the pseudocysts of the cribriform tumour fields and those at the periphery of the tubular and cribriform tumour fields (Fig. 6). No reactivity of tumour cells was observed with the monoclonal antibody specific for keratin 10. The rat monoclonal antibody ER-TR5 revealed a picture complementary to that observed with the antibodies against keratins 7 and 18. Thus, epithelial cells lining pseudocysts and those situated at the periphery of the tubular and cribriform tumour fields were moderately stained by ER-TR5. Occasional intensely ER-TR5 positive cells were, however, also present within the tumour fields.

Immunofluorescence studies on the expression of actin revealed an intense fluorescence along the circumference of the tumour fields and at the margins of the pseudocysts of the cribriform tumour masses (Fig. 7). A faint cytoplasmic staining was observed in the other tumour cells. Also occasional strongly actin positive tumour cells were present within the tumour fields.
Figure 6. Cribriform masses of ACC stained for keratin 18 with monoclonal antibody RGE 53. The inner cells of the tumor show cytoplasmic staining. Cells lining the pseudocysts and at the periphery of the tumor masses are not stained. Nuclei counterstained with haematoxylin. (Original × 375).

Figure 7. Cribriform masses of ACC stained for actin. Intense immunofluorescence staining of the outer rim of the tumour masses and along the pseudocysts can be seen. The remaining tumour cells are faintly stained. (Original × 250).
DISCUSSION

The cutaneous tumour described here fully meets the histopathological criteria for the diagnosis of an ACC. The ultrastructural features, including the presence of basement membrane-like material within the so-called pseudocysts, further confirm the diagnosis of ACC. The differential diagnosis of cutaneous ACC must include adenoid type basal cell carcinoma. Adenoid type basal cell carcinoma may also show a cribriform growth pattern and areas of cystic degeneration. The presence of palisading in solid tumour masses and contiguity of the tumour with overlying epidermis or hair follicles are the main histopathological criteria by which adenoid type basal cell carcinoma can be distinguished from ACC. In addition, from the results of Wick and Swanson and the present findings it is apparent that immunohistochemistry can contribute to the separation of adenoid type basal cell carcinoma and cutaneous ACC. Cutaneous ACCs express EMA, in contrast to adenoid type basal cell carcinomas which do not. However, the presence of CEA in cutaneous ACC reported by Wick and Swanson was not confirmed in the present study, using a monoclonal anti-CEA antibody. Probably this discrepancy is due to a difference in epitope specificity of the monoclonal antibodies used.

Our results on frozen sections indicate that cutaneous ACCs consist of two populations of epithelial cells. One population is made up of keratin 7-positive, keratin 18-positive cells, which express small amounts of actin. This population of tumour cells has a well circumscribed localization on the inside of the tumour fields and is surrounded by the population of epithelial tumour cells not expressing keratin 7 or 18, but high levels of actin. In addition, this population of cells possesses intermediate filaments recognized by ER-TR5. No immunoreactivity of any of the tumour cells was found with antibodies to keratin 10 or vimentin.

The apparent lack of detectable expression of vimentin in the present case of cutaneous ACC contrasts with the previously reported vimentin-positivity of six ACC of salivary gland origin. The absence of vimentin in the myoepithelial cells of sweat glands and the reported presence of this intermediate filament protein in triangular duct cells of the normal salivary glands is in keeping with the contrasting observations in ACCs of salivary gland and sweat gland origin.

Keratins 7 and 18 are found to occur normally in glandular epithelia, but not in squamous epithelia such as the epidermis. Keratin 18 is expressed by nearly all glandular epithelia, while keratin 7 has a more restricted pattern of expression. In the skin, keratins 7 and 18 are solely present in the acinar epithelial cells of the secretary coil of sweat glands. Ductal epithelium and myoepithelial cells of sweat glands do not express keratins 7 or 18, nor do sebaceous glands or hair follicles. In the skin, keratins 7 and 18 must thus be considered highly specific for sweat gland acinar epithelial cells. In contrast, expression of keratin 10 is entirely restricted to epidermal epithelial cells. Therefore, we consider the presence of keratin 7 and 18 and the absence of keratin 10 in cutaneous ACC as a strong argument for the sweat gland origin of this tumour type.

The polyclonal antikeratin antisera was used in the present study stains all epithelial cells of the epidermal appendages, including the myoepithelial cells of the sweat glands (data not shown). Similarly, this antisera was reactive with all epithelial tumour cells of cutaneous ACC. Moreover, a higher intensity of immunostaining was observed in the population of epithelial cells at the periphery of the tumour fields and those lining the pseudocysts in the cribriform areas. In the skin, ER-TR5 exclusively stains the basal cells of the epidermis and the myoepithelial cells of the sweat glands (data not shown). The presence of ER-TR5 in the population of epithelial cells lining the tumour fields and the pseudocysts is therefore highly suggestive of a myoepithelial origin. The intense staining of this cell population by our anti-
actin monoclonal antibody further substantiates this suggestion. Previous studies on salivary gland ACC and uterine cervix ACC also indirectly indicated the presence of myoepithelial cells in these tumours. The accumulation of basement membrane material in the pseudocysts of ACC may thus be the result of a stimulatory effect of neoplastic transformation of the myoepithelial cells which normally produce basement membranes. The data from the present study did not enable us to establish whether the histogenetic precursor cell of ACC resides in the myoepithelial or the acinar cell population, or, more probably, in a precursor common to both these cell types.

Cutaneous ACC is an uncommon tumour of the skin, which was first described as a separate entity in 1975 by Boggi and Sanderson and Batten. The review by Cooper et al. included eight cases of cutaneous ACC. Since then five more cases have been reported in the literature. A summary of published cases is given in Table 2. To our knowledge, the case reported by Sanderson and Batten, represents the only case of cutaneous ACC with a distant metastasis. All other cases of cutaneous ACC have been characterized by a prolonged indolent disease course, without distant metastases. ACCs in locations other than skin and breast seem to have a more aggressive disease course. The recurrence rate of cutaneous ACC is 50% (Table 2). Recurrence seems not to be related to the presence of perineural invasion or to tumour size. The data show a female predominance of 4:1, and 40% of the tumours appear to originate in the scalp. Since irradiation of the tumour does not prevent recurrence, surgical treatment with extensive resection margins is recommended.

### Table 2. Reported cases of cutaneous adenoid cystic carcinoma

<table>
<thead>
<tr>
<th>Reference</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Tumour location</th>
<th>Tumour size (cm)</th>
<th>Perineural invasion</th>
<th>Recurrence</th>
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<tr>
<td>Stout and Cooley</td>
<td>52</td>
<td>F</td>
<td>abdomen</td>
<td>1</td>
<td>+</td>
<td>-(82 months)*</td>
</tr>
<tr>
<td>Miller</td>
<td>66</td>
<td>F</td>
<td>arm</td>
<td>0.5</td>
<td>not reported</td>
<td>-(19 months)</td>
</tr>
<tr>
<td>Freeman and Winkelmann</td>
<td>34</td>
<td>F</td>
<td>scalp</td>
<td>3</td>
<td>not reported</td>
<td>+(40 years)</td>
</tr>
<tr>
<td>Boggi</td>
<td>66</td>
<td>F</td>
<td>scalp</td>
<td>8</td>
<td>+</td>
<td>not reported</td>
</tr>
<tr>
<td>Sanderson and Batten</td>
<td>46</td>
<td>F</td>
<td>scalp</td>
<td>5</td>
<td>+</td>
<td>+(11 years) (including pulmonary metastasis)</td>
</tr>
<tr>
<td>Headington et al.</td>
<td>60</td>
<td>F</td>
<td>scalp</td>
<td>not reported</td>
<td>+</td>
<td>+(21 months)</td>
</tr>
<tr>
<td>Headington et al.</td>
<td>47</td>
<td>M</td>
<td>axilla</td>
<td>2.5</td>
<td>+</td>
<td>+(18 months)</td>
</tr>
<tr>
<td>Dissanyake and Salm</td>
<td>47</td>
<td>M</td>
<td>scalp</td>
<td>3</td>
<td>not reported</td>
<td>-(30 months)</td>
</tr>
<tr>
<td>Cooper, Adelson and Holhaus</td>
<td>61</td>
<td>F</td>
<td>arm</td>
<td>8</td>
<td>+</td>
<td>+(30 months)</td>
</tr>
<tr>
<td>Boggi</td>
<td>59</td>
<td>F</td>
<td>suprapubic</td>
<td>7</td>
<td>+</td>
<td>+(5 years)</td>
</tr>
<tr>
<td>Beck</td>
<td>41</td>
<td>F</td>
<td>back</td>
<td>not reported</td>
<td>not reported</td>
<td>+(35 years)</td>
</tr>
<tr>
<td>Beck</td>
<td>40</td>
<td>M</td>
<td>hand</td>
<td>not reported</td>
<td>not reported</td>
<td>-(9 months)</td>
</tr>
<tr>
<td>Wick and Swanson</td>
<td>76</td>
<td>F</td>
<td>scalp</td>
<td>2</td>
<td>-</td>
<td>-(10 years)</td>
</tr>
<tr>
<td>Lang, Metcalf and Maize</td>
<td>63</td>
<td>F</td>
<td>back</td>
<td>not reported</td>
<td>-</td>
<td>+(5 years)</td>
</tr>
<tr>
<td>Present report</td>
<td>75</td>
<td>M</td>
<td>chest</td>
<td>3</td>
<td>not reported</td>
<td>+(9 months)</td>
</tr>
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* Period of follow-up.
ACKNOWLEDGMENTS

The authors thank Miss M. Hanegraaff for typing the manuscript and Miss P. Delfos for the photography.

REFERENCES

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