Immunoreactivity of α smooth muscle actin in salivary gland tumours: a comparison with S100 protein

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Abstract
Antibodies against α smooth muscle actin (ASMA) and S100 protein were applied to paraffin wax embedded sections from 40 salivary gland tumours and seven normal salivary glands. The results indicate that ASMA is useful in the diagnosis of epithelial-myoepithelial carcinoma, but is otherwise only of limited use in diagnostic practice. An unexpected finding was the failure of ASMA to react with myoepitheliomas.

Results (table)
S100 positivity was indicated by uniform cytoplasmic and nuclear staining. ASMA positivity was indicated by uniform or patchy cytoplasmic positivity; a filamentous pattern was not seen.

In normal salivary glands ASMA was shown in the myoepithelial cell population around the acini and terminal and small ducts, but not in the epithelial lining cells. S100 was also strongly positive for the myoepithelial elements but was also weakly positive for the epithelial cells. No reaction with either marker was seen around large (striated) ducts as they lack easily recognisable myoepithelial cells.

Benign tumours
In all three Warthin’s tumours (adenolymphoma) staining was generally absent with both markers, although there was some epithelial reaction to S100. Blood vessels within the lymphoid tissue showed strong ASMA positivity.

In the 10 pleomorphic adenomas the inner duct lining cells were negative for ASMA, but focal weak positivity was noted in some tubules in an apparent myoepithelial cell layer. Only occasional cells in the matrix expressed ASMA. In contrast, most cells within the myxoid/chondroid stroma reacted positively with S100, as did some of the epithelial cells. Myoepithelial cells around some ducts expressed S100 strongly. Three additional pleomorphic adenomas were particularly rich in myoepithelial cells and these were strongly positive for S100, but two showed only focal weak ASMA positivity, a third case being completely negative.

Myoepitheliomas are believed to be composed totally of myoepithelial cells. One predominantly spindle tumour and one lesion composed of plasmacytoid cells were examined. ASMA was negative in both cases, even when repeated at a concentration of 1 in 5000. S100 was strongly positive in all areas.

Malignant tumours
In the four epithelial-myoepithelial carcinomas ASMA was strongly positive in the outer cell layer but was completely negative for the inner layer. S100 reacted strongly with the outer...
Immunoreactivity of salivary gland tumours

Immunoreactivity of 40 salivary gland tumours and seven normal salivary glands to antibodies against a smooth muscle actin (ASMA) and S100 protein

<table>
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<tr>
<th>ASMA</th>
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<td>-</td>
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Normal salivary glands:
- Epithelium: 7
- Duct: 7
- Acini: 7
- Myoepithelial cells: 7
  - Acini: 7
  - Ducts within acini: 7
  - Terminal ducts: 7
- Large ducts: 7
- Blood vessels: 7
- Nerve: 7

Epithelial/myoepithelial carcinomas:
- Inner cells: 4
  - 3f
  - 1f
- Surrounding cells: 4
- Acinic cell carcinoma: 2
  - 1
  - 1
- Myoepithelioma: 2
  - 2
- Warthin’s tumour: 3
  - 1
  - 2f
- Salivary duct carcinoma: 4
- Polymorphous low grade adenocarcinoma: 1
  - 3f
  - 2f
  - 1f
  - 4f
- Adenoid cystic carcinoma: 1
  - 2f
  - 1
  - 2f
  - 1
- Monomorphous clear cell carcinoma: 2
  - 2
  - Pleomorphic adenoma:
    - Classic: 2
      - f6
      - 1
      - 1
    - Myoepithelial rich: 1
      - f2
      - 1
      - 3
      - 3

f indicates focal immunoreactivity.

Immunohistochemical reactions varied across the salivary glands, with the inner layer showing focal positivity but patchy weak crossreactivity with the inner cells. This was true in all three main histological patterns (classic, sclerotic, and clear cell predominant).

In six polymorphous, low grade adenocarcinomas focal positivity varied with S100 which was strong in places. Five displayed some staining with ASMA, but this was much less pronounced than the S100 in three cases consisting of just isolated cells. Two tumours showed small foci resembling epithelial-myoeptihelial carcinoma, and both ASMA and S100 highlighted the outer cell layer. Two out of three adenoid cystic carcinomas showed focal weak ASMA immunoreactivity. All three tumours showed foci of variable positivity with S100 but only in one case was this considered a strong reaction.

Discussion

Myoepithelial cell identification by actin immunohistochemistry depends on the isoform of actin identified. The α smooth muscle isoform recognised by ASMA is the only isoform specific to myoepithelial cells in the salivary glands (non-specific anti-actin antisera and anti-sera to β and γ isoforms react

**Figure 1** Epithelial-myoeptihelial carcinoma: immunoperoxidase reaction to ASMA showing strong outer layer reactivity and absence of inner layer cross reactivity.

**Figure 2** Myoepithelioma: immunoperoxidase reaction to ASMA clearly showing vascular reactivity (arrowed) but tumour negativity.
with epithelial cytoplasmic actin). We have demonstrated ASMA positive tumour cells in pleomorphic adenomas, adenoid cystic carcinomas, and epithelial myoepithelial carcinomas. These findings support a myoepithelial component to these tumours, previously suggested by S100 immunohistochemistry and ultrastructural studies.4-10

The consistently negative ASMA immunoreactivity of the myoepitheliomas (all S100 positive) was unexpected. Ultrastructural studies have shown that the cells exhibit both desmosomes and myofilaments. It should be noted, however, that cells thought to be of myoepithelial origin in pleomorphic adenomas, especially myoepithelial rich pleomorphic adenomas, also show only weak focal positivity for ASMA. It is therefore possible to postulate that myoepithelial elements within these tumours (pleomorphic adenomas and myoepitheliomas) lose their ASMA expression at some stage in tumour growth.

In practice, the main use of ASMA seems to be in the diagnosis of epithelial-myoepithelial carcinoma. The tumour comprises ducts lined by epithelial cells with a surrounding mantle of myoepithelial cells. The latter were highlighted by ASMA far better than by S100, which often crossreacted with the epithelial cells. An important diagnostic problem is differentiating polymorphous low grade adenocarcinoma from adenoid cystic carcinoma. Focal or weak ASMA positivity was seen in both tumour types, and thus cannot help differentiate them.

In summary the results of this study show that ASMA has a place in the investigation of salivary neoplasms, particularly in highlighting myoepithelial cells. It has advantages over S100 but these are relatively limited.