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Immunohistochemical evaluation with Ki-67: An application to salivary gland tumours

M. MURAKAMI, M.D.,* I. OHTANI, M.D.,* H. HOJO, M.D.,+ H. WAKASA, M.D.+ (Fukushima, Japan)

Abstract

Using Ki-67, a monoclonal antibody, the proliferating capacity of 15 salivary gland tumours, including nine pleomorphic adenomas, four adenoid cystic carcinomas, one mucoepidermoid carcinoma and one acinic cell carcinoma was determined immunohistochemically, using normal salivary gland tissue as a control. The frequency of Ki-67 positive cells was 4.7 per cent in the normal salivary gland and one per cent in pleomorphic adenomas, whereas the average frequency in malignant tumours was 18.3 per cent. Among adenoid cystic carcinomas, the frequency was related to the morphological type; the solid sub-type had the highest frequency of Ki-67-positive cells. As this sub-type is recognized as the most aggressive of these tumours, this technique has the potential of providing an early indication of the clinical behaviour of a tumour.

Introduction

The morphological classification of salivary gland tumours is based on histological patterns. However, the morphology may correlate poorly with clinical behaviour and prognosis and tumours of similar histological type may exhibit quite different clinical courses. A prediction of tumor behaviour may be difficult, although appropriate therapeutic decisions depend on this information.

Ki-67 (Gerdes *et al.*, 1983; 1984) is a monoclonal antibody which reacts with proliferating cells in human neoplastic tissue, except for those in the G0 period of the cell cycle. Ki-67 has been used for estimating the presence of malignancy in brain tumours, lymphomas and other tumours; the close correlation between the frequency of Ki-67-positive cells and the level of malignancy is well established (Barnard *et al.*, 1987; Weiss *et al.*, 1987; Grogan *et al.*, 1988; Hall *et al.*, 1988; Lorz and Meyer-Breiting, 1988; Shibata *et al.*, 1988).

We have found no reports evaluating the utility of Ki-67 in salivary gland tumours. Using normal salivary gland tissue as a control, the frequency of Ki-67-positive cells was determined in benign and malignant salivary gland tumours to explore correlations between the frequency of Ki-67-positive cells, the malignancy of the tumour and its clinical behaviour.

Material and method

Tissue specimens

Tissue samples were obtained from five normal salivary gland and 15 salivary gland tumors, including nine

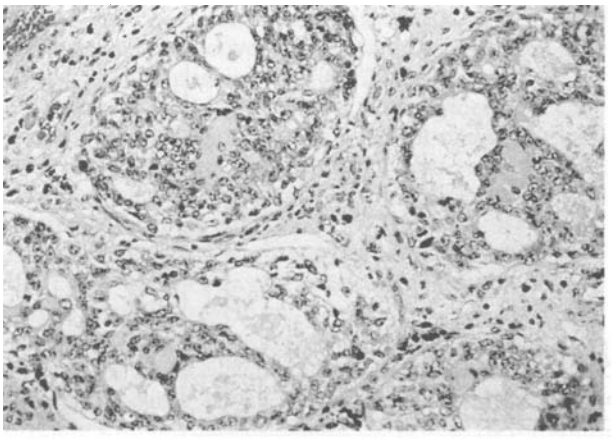

FIG. 1a
Adenoid cystic carcinoma (cribriform lesion: H–E staining).

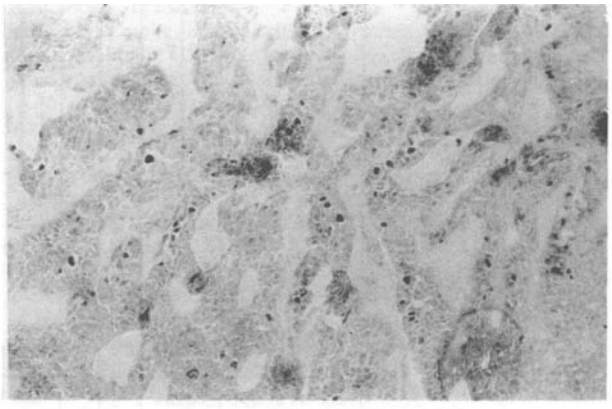

FIG. 1b
Adenoid cystic carcinoma (cribriform lesion: Ki-67) A number of nuclei of tumor cells are strongly stained.

From the department of Otolaryngology* and the Department of Pathology+ Fukushima Medical College, Fukushima, Japan.
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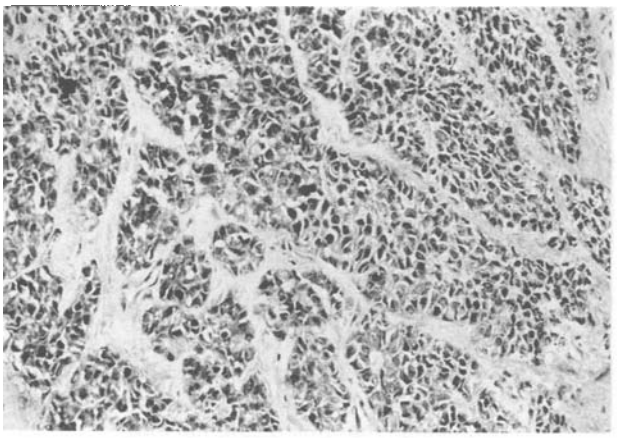

FIG. 2a
Adenoid cystic carcinoma (solid lesion: H–E staining).

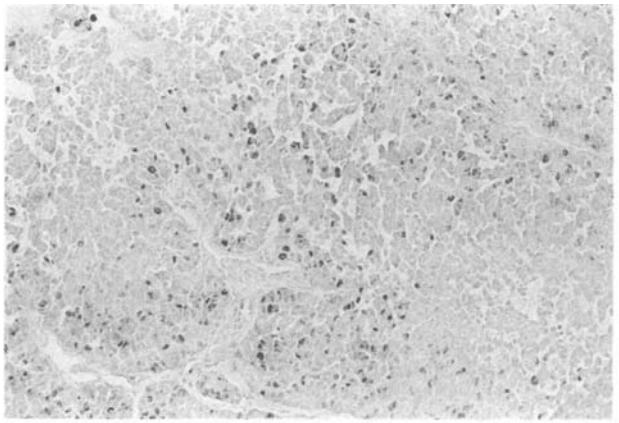

FIG. 2b
Adenoid cystic carcinoma (solid lesion: Ki-67) Numerous nuclei of tumour cells are positive for Ki-67.

pleomorphic adenomas, four adenoid cystic carcinomas, one mucoepidermoid carcinoma and one acinic cell carcinoma.

Antibody

Ki-67, a mouse monoclonal antibody which reacts with human nuclear antigen in proliferating cells, was purchased from DAKOpatts.

Immunostaining method

Fresh tissue from normal salivary glands and salivary gland tumours were rapidly fixed with periodate-lysine-formaldehyde (PLP) fixative for eight hours, embedded in OCT compound (Ames) and frozen at −80°C. Frozen tissue was cut into 4–5 μm slices with a cryostat and stained using a 4-step peroxidase-anti peroxidase (PAP) staining technique (Sternberger *et al.* 1970).

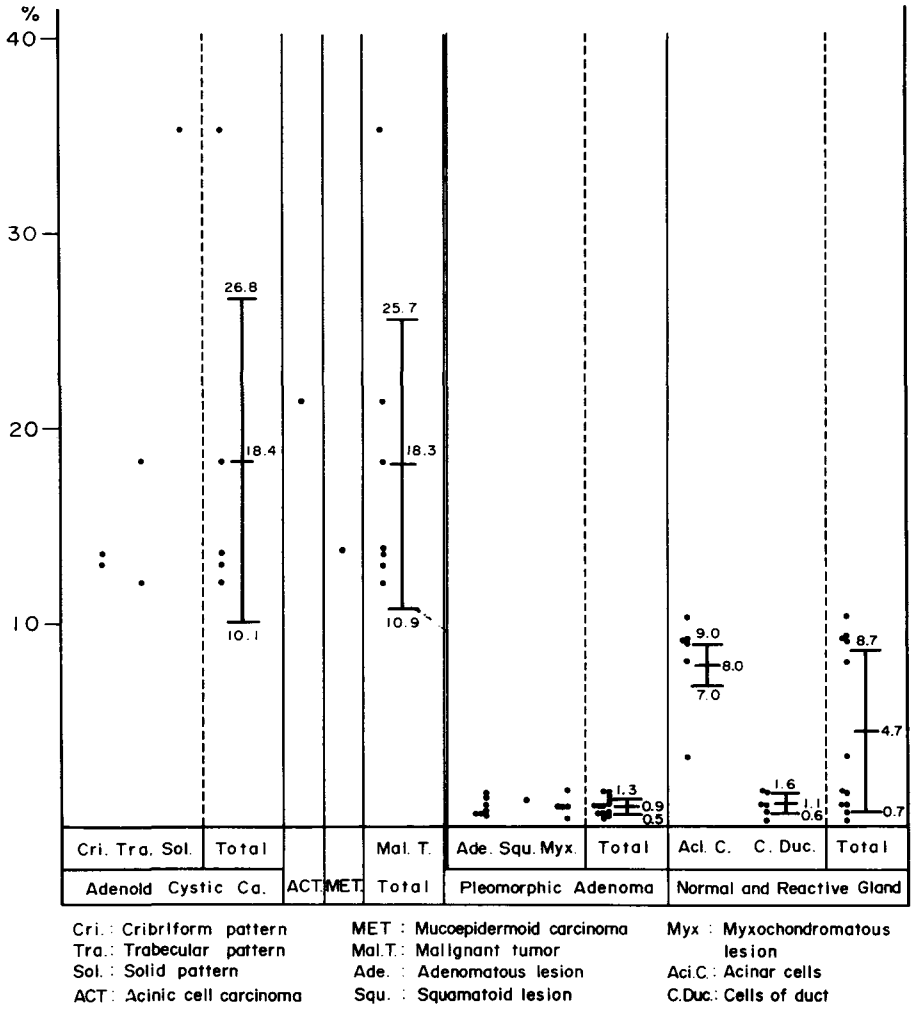

FIG.3
Frequency Ki-67-positive cells in salivary glands tumours and in normal salivary gland.

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To determined frequency of Ki-67-positive cells in these tissues, the number of Ki-67-positive cells found in samples consisting of 1,000–4,000 cells from various areas of the tumour were counted and expressed as a percentage. In the normal salivary gland, the frequencies of Ki-67-positive cells among acinar cells and ductal cells (including myoepithelial cells) were calculated separately. The pleomorphic adenoma specimens were subdivided into adenomatous lesions with solid area, myxochondromatous lesions and squamous lesion; the adenoid cystic carcinoma specimens were subdivided into cribriform, tubular/trabecular and solid lesions. The frequency of Ki-67-positive cells was calculated separately for each subdivision, and overall for each tumour type.

Immunoelectron-microscopic method

Immunostaining and electron microscopy was performed on the adenoid cystic carcinoma specimens using a modification of Tsunoda's technique. The frozen sections were treated with the PAP method detailed above, fixed with in cold 1.25 per cent glutaraldehyde in 0.1M cacodylate buffer (pH 7.4), and post-fixed in one per cent buffered osmium tetroxide for 30 mins prior to the diaminobenzidine reaction. Following dehydration in a graded series of alcohols, they were embedded in Epon 812. Ultrathin sections without counterstain were observed under a JEM-1000CX electron microscope.

Results

In the normal salivary gland, Ki-67-positive cells were observed in the nuclei of acinar and ductal cells with a frequency of eight and one per cent, respectively. In the pleomorphic adenomas, Ki-67-positive cells were distributed focally, and accounted for one per cent of cells, almost equal to the frequency observed in ductal cells in the normal salivary gland.

There was a significantly higher frequency of Ki-67-positive cells in malignant salivary gland tumours. Among the adenoid cystic carcinoma specimens, the frequency varied among the sub-types and was 13.6, 15.3 and 34.7 per cent in cribriform, tubular/trabecular, and solid tumours respectively (Figs. 1a,b & 2a,b). The frequency of Ki-67-positive cells was 21.5 per cent in acinic cell carcinoma and 14 per cent in mucoepidermoid carcinoma. The average frequency for all malignant salivary gland tumours was 18.3 per cent, significantly higher than that in normal salivary gland tissue ($P<0.01$) and in pleomorphic adenomas ($P<0.05$).

Immuno-electron microscopic observation of the adenoid cystic carcinoma specimens revealed that Ki-67-positive material appeared to be distributed within the nucleus. Ki-67-positive tumour cells usually had a large euchromatic nucleus with a distinct nucleoli or diffusely in the heterochromatin, however, these patterns did not correlate with the morphological features of the tumour cells.

Discussion

The behaviour and aggressiveness of a malignancy may have an important impact on the choice of therapy. Ki-67, a monoclonal antibody against an antigen closely related to nucleic acid synthesis, detects proliferating cells in all cell cycle phases except G0. Ki-67 has been used to establish malignancy and tumour grade in brain tumours, lymphomas and other neoplasms. However, there have been no reports of its use in salivary gland tumours. Our results demonstrated that the frequency of Ki-67-positive cells was significantly higher in malignant salivary gland tumours compared to normal tissue ($P<0.01$) or benign tumours ($P<0.05$) of the salivary gland. Ki-67 appears to be useful in distinguishing the proliferating capacity of different sub-types of adenoid cystic carcinoma; the solid sub-type had the highest frequency of Ki-67-positive cells. These findings correlate with the clinical behaviour as the solid sub-type of adenoid cystic carcinoma usually displays more malignant behaviour than the other sub-types. Thus, Ki-67 may be used to indicate the relative malignancy of a tumour's clinical behaviour and could be used to direct treatment planning.

Conclusion

Ki-67 may be useful for the evaluation of salivary gland tumours. The frequency of Ki-67-positive cells within a tumour specimen differentiates benign from malignant tumours, and may be related to the relative malignancy of the tumour's clinical behaviour. Morphology alone does not always offer an accurate prediction of subsequent clinical behaviour. Further study may demonstrate the utility of this monoclonal antibody in predicting clinical behaviour and directing treatment planning of patients with malignant tumours of the salivary gland.

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Address for correspondence:
Dr M. Murakami, M.D.,
Matsukichyo 1–23,
Fukushima City,
Fukushima 960,
Japan.

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