Int. J. Oral Maxillofac. Surg. 2006; 35: 733–739
doi:10.1016/j.ijom.2006.01.012, available online at http://www.sciencedirect.com

International Journal of
Oral & Maxillofacial Surgery

Research Paper
Head and Neck Oncology

The role of Schwann cell differentiation in perineural invasion of adenoid cystic and mucoepidermoid carcinoma of the salivary glands

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X.-L. Luo, M.-Y. Sun, C.-T. Lu, Z.-H. Zhou: The role of Schwann cell differentiation in perineural invasion of adenoid cystic and mucoepidermoid carcinoma of the salivary glands. Int. J. Oral Maxillofac. Surg. 2006; 35: 733–739.

Abstract. Twenty cases of adenoid cystic carcinoma (ACC) and 18 cases of mucoepidermoid carcinoma (MEC), were examined for expression of the Schwann cell markers S100 protein and glial fibrillary acidic protein (GFAP) by immunohistochemical staining. The relationship between expression of S100 and GFAP and the occurrence of perineural invasion was assessed. Ultrastructural localization of S100 and GFAP was examined by immunoelectron microscopy, and the co-expression of S100 and muscle actin by double fluorescence immunostain.

Perineural invasion was found in 11 ACCs (55%) and 0 MECs (0%). S100 and GFAP were expressed in most of the ACCs but none of the MECs; the difference in the rate of perineural invasion and expression of S100 and GFAP was significant between ACC and MEC ($P < 0.001$). There was a correlation between the expression of S100 and GFAP and perineural invasion in salivary malignancy ($P < 0.001$). The ultrastructures of S100- and GFAP-positive cells were consistent with the characteristics of myoepithelial cells. Double fluorescence immunostain also showed that S100 and muscle actin were expressed in the same type of ACC cells.

These results indicate that Schwann cell differentiation correlates with perineural invasion in salivary malignancy, and occurs in modified myoepithelial cells of ACC.

Key words: Schwann cell differentiation; perineural invasion; adenoid cystic carcinoma; mucoepidermoid carcinoma; myoepithelial cell.

Accepted for publication 17 January 2006
Available online 2 March 2006

Adenoid cystic carcinoma (ACC) and mucoepidermoid carcinoma (MEC), both predominantly originating from the major and minor salivary glands, are the commonest malignant neoplasms of the salivary glands, the former accounting for 21.9%[16] of all salivary malignancies and the latter for about 35%[11]. ACC cells have a known propensity for perineural invasion. They are regularly found to encircle nerve sheaths and invade neural tissue directly[3]. This leads to a high incidence of local recurrence and causes great difficulty in the curative resection of ACC[1]. Although there have been numerous studies reporting

0901-5027/080733 + 07 $30.00/0

the mechanisms of invasion and metastasis of ACC, tissue-specific invasion of ACC cells remains poorly understood.

Desmoplastic melanoma[13] also has a striking propensity for perineural invasion. IWAMOTO et al.[5,6] found a close correlation between expression of the Schwann cell marker p75NTR and the desmoplastic phenotype, which supports the hypothesis that a propensity for perineural invasion reflects Schwann cell differentiation of desmoplastic melanomas.

TOTH et al.[17] evaluated the Schwann cell-staining properties of ACC and polymorphous low-grade adenocarcinoma (PLAA) using antibodies to S100 protein, glial fibrillary acidic protein (GFAP), neuron-specific enolase, and using a solochrome stain. They found that most showed positive Schwann cell-staining properties, suggesting that Schwann cell differentiation may occur within these tumours. No direct evidence has been reported supporting a correlation between Schwann cell differentiation and perineural invasion of salivary malignancies. Also, there are two types of cells in ACC: myoepithelial cells and canula endothelial cells[1]. Although modified myoepithelial cells have been shown to possess characteristics of a variety of cell types, they have not been found to have neuronal differentiation. So whether Schwann cell differentiation occurs in the modified myoepithelial cells of ACC needs to be identified.

In this study, we examined the expression of the Schwann cell markers S100 and GFAP in cases of ACC and MEC, and assessed the correlation of these proteins with perineural invasion. In order to identify whether Schwann cell differentiation occurs in modified myoepithelial cells of ACC, we examined the ultrastructural localization of S100 and GFAP expression using immunoelectron microscopy and co-expression of S100 and muscle actin (MA) using double immunofluorescence histochemistry.

Materials and methods

Subjects

The study comprised 20 ACC patients (12 males and 8 females; mean age 46.5 years, range 21–63 years) and 18 MEC patients (10 males and 8 females; mean age 44.8 years, range 19–71 years) treated at the Department of Oral and Maxillofacial Surgery, Stomatological College, the Fourth Military Medical University from January 2001 to June 2002. These salivary malignancies were all pathologically diagnosed by preoperative biopsy. All tissue samples were obtained during operation. Informed consent was obtained from all subjects before obtaining the tissue samples.

Tissue preparation

All tissue samples from the 38 malignancies were obtained during surgery, and each tissue sample was divided into two parts. For immunohistochemical labelling and haematoxylin and eosin (H&E) staining, one part was fixed immediately in 10% formalin for 24 h and embedded in paraffin according to routine methods. Then 5-μm thick serial sections were prepared with a microtome. For immunoelectron microscopy, the other part was immediately fixed in an ice-cold mixture of 4% paraformaldehyde and 0.05% glutaraldehyde (pH 7.4) for 2 h. Serial sections of 50-μm thickness were prepared with a vibratome (VT 1000S, Leica, Heidelberger, Nussloch, Germany).

Identification of perineural invasion

Slides were prepared from each sample using 5-μm H&E-stained serial sections, and reviewed by two independent oral pathologists blind to clinical findings. The presence or absence of microscopic perineural invasion was recorded.

Immunohistochemical staining

Rabbit anti-human S100 and mouse anti-human GFAP were used as primary antibodies, and biotinylated IgG was used as secondary antibody. They were reacted with streptavidin–biotin–peroxidase complex. The primary antibodies and Labelled Streptavidin Biotin (LSAB) Kit were purchased commercially (Dako, Denmark). For negative controls, the primary antibodies were replaced by normal serum.

Immunohistochemical staining was conducted according to standard protocols using the LSAB technique by an experienced cytopathology laboratory technician. Briefly, paraffined sections were dewaxed and rehydrated routinely. The sections were incubated with 0.01% trypsin for 15 min in a humid chamber at 37 °C, and then in 3% hydrogen peroxide solution for 15 min. They were then incubated with horse serum (1:20) for 30 min to suppress non-specific binding, followed by incubation with the primary antibodies against S100 (1:80) or GFAP (1:50) for 2 h in a humid chamber at 37 °C. After washing in phosphate-buffered saline (PBS), the sections were incubated with biotinylated IgG, and subsequently with streptavidin–biotin–peroxidase complex for 30 min at 37 °C in a humid chamber. Finally, a substrate reagent (diaminobenzidine tetrahydrochloride) was added to all sections and the sections were mounted. For negative control sections, the primary antibodies were replaced by PBS.

The results of immunohistochemical staining were evaluated by two independent pathologists blind to clinical findings.

Immunoelectron microscopy

The specimens were obtained during operations and fixed immediately in an ice-cold mixture of 4% paraformaldehyde and 0.05% glutaraldehyde (pH 7.4) for 2 h. Each specimen was divided into 18–20 50-μm sections, each to include a small part of the tumour. The sections were placed in PBS containing 25% sucrose and 10% glycerol for 1 h for cryoprotection. After freeze–thaw treatment, they were immersed in PBS containing 5% bovine serum albumin and 5% normal goat serum for 4 h to block non-specific immunoreactivity. All immunohistochemical procedures were done at room temperature.

The sections were then incubated overnight in primary antibody of rabbit anti-human S100, diluted to 1:80, or mouse anti-human GFAP, diluted to 1:50 in PBS containing 1% bovine serum albumin and 1% normal goat serum. They were then washed in PBS and incubated overnight in secondary antibody, goat anti-rabbit/mouse IgG conjugated to 1.4 nm gold particles at a 1:100 dilution (Nanoprobes, Stony Brook, NY, USA). The sections were rinsed and post-fixed in 2% glutaraldehyde in PBS for 45 min. Silver enhancement was performed in the dark with an HQ Silver Kit (Nanoprobes) for visualization of S100/GFAP immunoreactivity. Before and after the silver-enhancement step, the sections were rinsed several times with de-ionized water.

Immunolabelled sections were fixed with 0.5% osmium tetroxide in 0.1 M phosphate buffer for 1 h, dehydrated in graded ethanol series and then in propylene oxide, and finally flat-embedded in Epon 812. After polymerization, the sections were examined under a light microscope. There was no S100/GFAP immunoreactivity in the sections of MEC. Most ACCs showed strong intensity staining for S100/GFAP. Three to four sections showing S100/GFAP immunoreactivity were selected from each ACC specimen, trimmed under a stereomicroscope, and mounted onto blank resin stubs. Ultrathin sections were cut with an ultratome (Nova, LKB, Bromma, Sweden) and mounted on mesh grids (6–8 sections/grid). They were then counterstained with