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ORIGINAL ARTICLE

Pedunculated oncocytic carcinoma in buccal mucosa: immunohistochemical and ultrastructural studies

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PURPOSE: In this study we evaluated pedunculated oncocytic carcinoma (OC) in the buccal mucosa via immunohistochemical and ultrastructural studies.
PATIENT AND METHODS: An 84-year-old man was referred to our clinic with a pedunculated mass about 4 cm in diameter in the right buccal mucosa. An incision biopsy revealed the diagnosis of oncocytic tumor, and enucleation was performed. The tumor was stained for immunohistochemical analysis using the ABC method and antibodies against cytokeratin (CK), epithelial membrane antigen (EMA), desmin, S-100 protein and muscle-specific actin, respectively. The tumor was stained with uranyl acetate and lead citrate for visualization by electron microscopy.
RESULTS: Histopathology results revealed that the tumor consisted of oncocytic cells, characterized by eosinophilic and granular cytoplasm, and atypical nuclei. These cells had infiltrated local blood vessels, salivary glands and muscles. Immunohistochemical staining indicated that these cells were positive for CK and EMA, and negative for desmin, muscle-specific actin and S-100 protein. Electron microscopy revealed numerous dilated cytoplasmic mitochondria, and the cell contours and nucleic shapes of tumor cells were often irregular.
CONCLUSIONS: Because the histopathologic features of OC are similar to those of benign oncocytoma, the diagnosis of OC must be confirmed by a combination of clinical and ultrastructural characteristics.
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Keywords: immunohistochemical study; oncocytic carcinoma; oncocytoma; ultrastructural study

Introduction

Oncocytes have a round nucleus with a prominent nucleolus, granular, eosinophilic cytoplasm, and abundant mitochondria with oxidative enzymes. Generally detected in older adults, oncocytes occur in many organs including the salivary glands, larynx, trachea, esophagus, thyroid and parathyroid, and have historically been called 'oxyphilic cells' in the parathyroid and 'Askanazy cells' in the thyroid; the tumor comprised of these cells was known as 'Hürthle-cell tumor' (Ashley, 1978). Hamperl (1950) maintained that the characteristic eosinophilic tumor cells resembled oncocytes of the salivary glands, pancreas, adrenal, parathyroid and liver, and therefore recommended replacing the term 'Hürthle-cell tumor' with adenoma or carcinoma with oncocytic transformation (Hamperl, 1950). Benign tumors consisting of oncocytic cells have been called 'oncocytoma' by Ackerman (1943) or 'oxyphilic granular cell adenoma' by Meza-Chávez (1949). In 1972 WHO grouped 'oxyphilic adenoma' as one of the monomorphic adenomas (Thackray and Sobin, 1972) but later changed it to 'oncocytoma or oncocytic adenoma' (Seifert and Sobin, 1991). 'Malignant oncocytoma' in the salivary gland was first reported by Bauer and Bauer (1953), which was later termed 'oncocytic carcinoma' by WHO (1991) (Seifert and Sobin, 1991).

Oncocytic carcinoma (OC) is an uncommon malignant tumor diagnosed with two sets of criteria (Seifert and Sobin, 1991). An OC consists of large, brightly stained oncocytic cells with an eosinophilic and granular cytoplasm and an atypical nucleus. Ultrastructurally and histochemically, the tumor cells are like non-neoplastic oncocytes with abundant mitochondria and oxidative enzyme activity. The majority of clinical OCs have been reported in the parotid gland, while they are uncommon in the minor salivary glands (Gray *et al*, 1976). Most cases have been treated with surgery, and a few cases have also been treated with postoperative radiotherapy, which improved local control (Scher *et al*, 1991). The recurrence rate varies between 10% and 52% (Brandwein and Huvos, 1991; Scher *et al*, 1991; Sugimoto *et al*, 1993). Prognosis is correlated with distant metastases (Nakada *et al*, 1998) and tumor size (Goode and Corio, 1988).

Only four cases of malignant oncocytoma have been reported in the minor salivary glands (Briggs and Evans,

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1967; Goode and Corio, 1988; Ruby and Kish, 1996; Gavilanes *et al*, 2000), while 51 cases of OC have been reported in the major gland (Coli *et al*, 1998). Here we report for the first time a pedunculated OC in the buccal mucosa, along with an immunohistochemical and ultrastructural description of the tumor.

Patient and methods

An 84-year-old man was referred to our department in April 2000 with a pedunculated mass in the right cheek. He was receiving therapy for cerebral thrombosis, prostatomegaly and chronic renal failure. The patient reported that the lesion had gradually enlarged over the last 7 months with no untoward symptoms. Intraoral examination revealed a pedunculated mass measuring about 4 cm in the right buccal mucosa (Figure 1A). The tumor was soft and elastic and the surface was smooth with slight ulceration. The border between the base of the pedicle and surrounding tissue was obscure. Computed tomography and magnetic resonance imaging showed a mass lesion in the right cheek area, not associated with the parotid (Figure 1B). None of the regional lymph nodes showed any swelling. Both ^{99m}Tc and ^{67}Ga scintiphotography revealed no distant metastases. Incision biopsy results revealed the diagnosis of oncocytic tumor (suspicious low grade malignancy), and enucleation was performed under local anesthesia. An incision with a 1 cm marginal distance from the base of the tumor pedicle was made in the buccal mucosa. Because of diffuse infiltration of the tumor to the submucosal layer, it was resected *en block* with surrounding tissue up to the fascia of the masseter muscle. There was no communication between the tumor and the parotid duct. The cut surface of the tumor was solid and whitish-yellow in color. Four years after surgery, there has been no evidence of recurrence of the primary tumor or metastases.

For light microscopic examination, specimens were fixed in 10% neutral buffered formalin and embedded in paraffin, and 5 μm-thick sections were then stained with hematoxylin and eosin. For immunohistochemistry, sections were stained using the ABC method and antibodies against cytokeratin (CK), epithelial membrane antigen (EMA), desmin, S-100 protein and muscle-specific actin (HHF35), respectively (Table 1). Each of the sections was incubated for 32 min. As negative controls, normal IgG was used instead of the primary antibody. For electron microscopy, the specimens were cut into 1 mm cubes and fixed in 2.5% glutaraldehyde. After postfixing in 1% OsO_4, the specimens were dehydrated and embedded in Epon 812. Ultra-thin sections were stained with uranyl acetate and lead citrate and then examined with a Hitachi H-800 transmission electron microscope.

Results

The tumor was composed of many oncocytic cells. The tumor cells had infiltrated normal minor salivary glands and the surrounding muscles (Figure 2a). In the pedicular region, the tumor was surrounded by a thin fibrous capsule (Figure 2b) and the tumor had infiltrated local blood vessels (Figure 2c). The tumor cells had a small nucleus with condensed chromatin and a prominent nucleolus. In addition, the cytoplasm was eosinophilic and granular. There was a high degree of nuclear pleomorphism and cellular atypia (Figure 2d). Immunohistochemically, the tumor cells reacted strongly to antibodies against CK and EMA, which are epithelial phenotypic markers (Figure 2e,f). The tumor cells did not stain with antibodies to desmin, muscle-specific actin (HHF35) or S-100 protein.

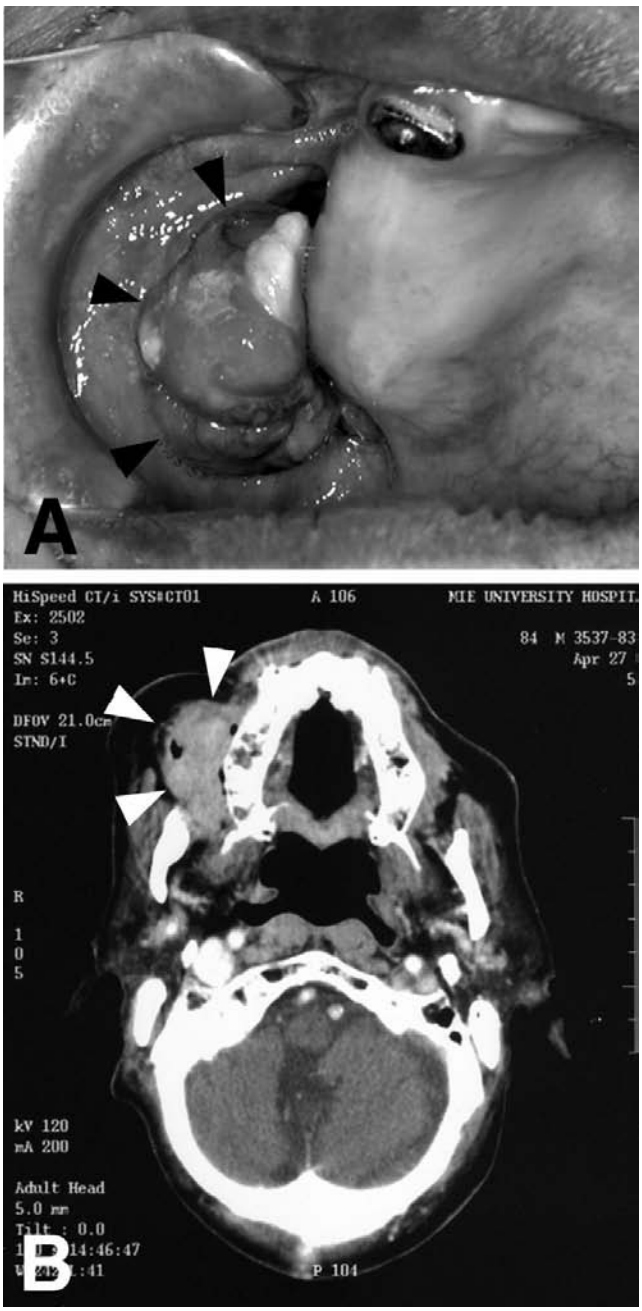

Figure 1 (**A**) Photograph of a 4 cm diameter pediculate mass (arrowheads) in the right buccal mucosa. (**B**) Computed tomography showing the mass lesion (arrowheads) adjacent to the lateral pterygoid muscle.

Table 1 The immunoreagents used in the study

Antibody	*Clone*	*Dilution*	*Source*
CK (No. 5,6,8,18)	5D3 + LP34	1:100	Novocastra
EMA	E29	1:50	Dako
Desmin	D33	1:50	Dako
S-100	poly	1:100	Dako
MSA	HHF35	1:50	Dako

CK, cytokeratin; EMA, epithelial membrane antigen; MSA, muscle-specific actin

The cytoplasm of most of the tumor cells contained numerous dilated mitochondria, often with many broken cristae. Some cells contained a few lipid droplets and lysosomes in the cytoplasm. There was no evidence of intercellular adherent junctions (e.g. desmosomes) or luminal structures, even in areas of high cell crowding (Figure 3a). In the pedicular region of the tumor, fibroblasts of the residual surrounding capsule were suppressed by tumor cells and often broken (Figure 3b). Tumor cells had diffusely infiltrated into submucosal connective tissues at the base of the tumor pedicle. These cells were round in shape and had a dilated cytoplasm, a prominent nucleolus, microvilli, and characteristics indicative of oncocytes. These cells often had condensed chromatin, irregularly shaped nuclei, and spindle-shaped or irregular cell contours, suggesting malignant transformation (Figure 3c,d).

Discussion

Oncocytic carcinoma is an uncommon malignant neoplasm that constitutes only 5% of all oncocytomas (Hartwick and Batsakis, 1990). Diagnostic criteria have been outlined by the WHO (Seifert and Sobin, 1991). However, the diagnosis of malignancy should be based on a combination of clinical and histopathological features because benign oncocytomas occasionally share the diagnostic features of OC. Specifically, such common characteristics include: (1) scattered mitosis and focal cellular pleomorphism, (2) local extension outside

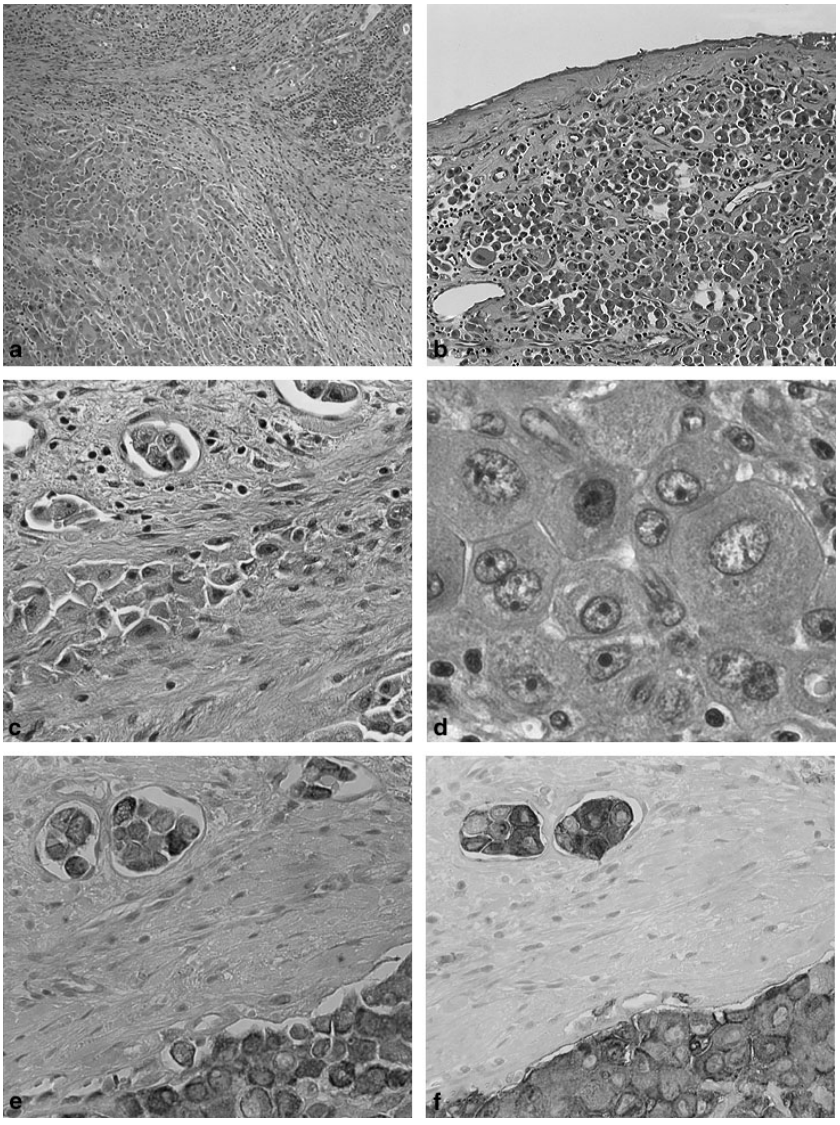

Figure 2 Light micrographs of the oncocytic carcinoma of the minor salivary gland. (**a**–**d**) hematoxylin and eosin staining; (**e**, f) immunostaining for cytokeratin (**e**) and epithelial membrane antigen (**f**). (**a**) A histological section composed of sheets of oncocytic cells. These cells have infiltrated surrounding muscles and the minor salivary glands (original magnification ×40). (**b**) In the outgrowing region, oncocytic cells have pushing margins and are surrounded by a thick, fibrous capsule that appeared to be focally invaded by nests of neoplastic cells (original magnification ×40). (**c**) High-power photomicrograph showing oncocytic cells within vascular spaces (original magnification ×100). (**d**) High-power photomicrograph showing degree of oncocytic histomorphology with dysplastic change (original magnification ×300). (**e**, **f**) A strong positive reaction can be found in oncocytic cells (original magnification ×100).

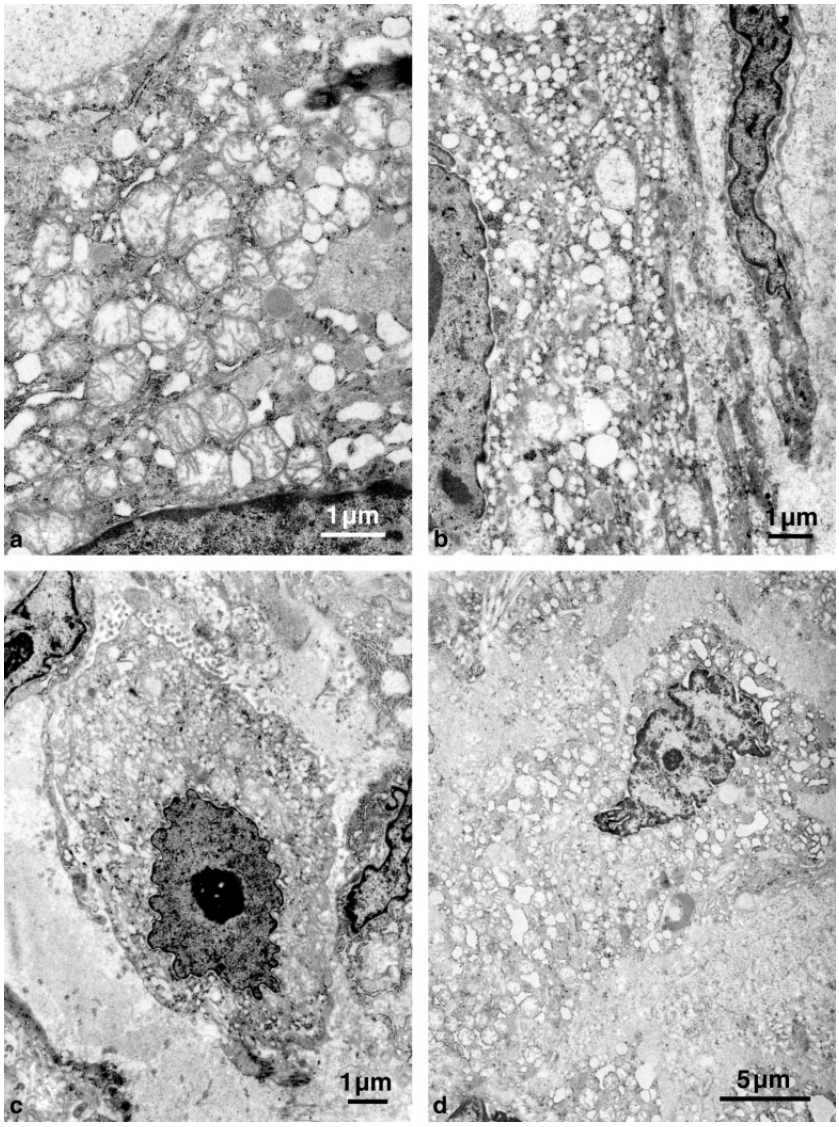

Figure 3 Electron micrographs of the oncocytic carcinoma of the minor salivary gland. (**a**) The region of the tumor with high cell crowding. The cytoplasm of the oncocytic cells contained numerous dilated mitochondria often with broken cristae (original magnification ×10 000). (**b**) The outgrowth region of the tumor. Fibroblasts in the capsule often appear to be broken (original magnification ×6000). (**c**, **d**) Oncocytic cells showing chromatin condensed, irregularly shaped nuclei, and spindle or irregular cell contour (original magnification **c** ×5000, **d** ×3000).

the capsule into adjacent soft tissue and bone, and (3) local recurrence (Gray *et al*, 1976). The cellular phenotype of oncocytomas does not vary in benign or malignant tumors. According to Batsakis (1974), solid oncocytomas warrant classification as low-grade malignancies, despite their benign histopathologic appearance, because of their behavior. The tumor in this case was considered malignant based on the findings of infiltration of tumor cells to blood vessels, minor salivary glands and surrounding muscles, and based on cellular atypia. OCs may arise from oncocytes *de novo* or from benign oncocytomas after a long interval (Nakada *et al*, 1998). Six cases of OC have reportedly originated from benign oncocytoma, three of which involved relapse while the others occurred after a long clinical course. In the present case, the small pediculate lesion became enlarged over the last 7 months; however, the origin of the malignant oncocytic cells still remains obscure. Three case reports of pediculate benign oncocytomas in the salivary gland have been reported (Capo, 1965; Mori *et al*, 1986).

The most prominent characteristic of OC is the abundance of mitochondria in tumor cells. This cellular change may result from compensation for defects in cellular respiration and other metabolic pathways (Hartwick and Batsakis, 1990) or from mitochondrial division (Tremblay and Pearse, 1959). Mitochondrial oxidative enzymes, such as succinate dehydrogenase (SDH) (Damiani *et al*, 1998), are highly active in oncocytic cells and stain positive immunohistochemically for CK (Tatemoto *et al*, 1987; Ruby and Kish, 1996; Gavilanes *et al*, 2000) and EMA (Tatemoto *et al*, 1987; Ruby and Kish, 1996), and negatively for S-100 protein (Sugimoto *et al*, 1993; Ruby and Kish, 1996; Coli *et al*, 1998; Gavilanes *et al*, 2000) and HHF35 (Sugimoto *et al*, 1993). Ductal cells in normal salivary glands have SDH activity and stain positively for CKs (Tremblay and Pearse, 1959). EMA is useful for determining the epithelial nature of neoplastic cells, as it concentrates at the luminal side of ductal cells in normal salivary glands (Ninomiya *et al*, 1989). S-100 protein reacted to modified or neoplastic myoepithelial

cells of pleomorphic adenomas (Weiss *et al*, 1991). Consequently, these findings suggest that oncocytic cells may originate histogenically from ductal epithelial cells.

An increased number of mitochondria have also been reported in melanoma, adenocarcinoma (Carlsöö *et al*, 1979) and in apocrine cells (Tatemoto *et al*, 1987). Damiani *et al* (1998) described the accumulation of normal mitochondria at the base or at the luminal pole of tumor cells and around the nuclei of apocrine cells. In contrast, oncocytes have swollen mitochondria scattered irregularly throughout the cytoplasm. Carlsöö *et al* (1979) proposed as a diagnostic standard for oncocytoma that mitochondria occupy 60% of the total area of the cytoplasm. Other ultrastructural features of malignant oncocytic cells have been reported including microvilli (Tatemoto *et al*, 1987), desmosomes (Jalisi, 1968; Gavilanes *et al*, 2000), lipid droplets, lysosomes (Sugimoto *et al*, 1993) and chromatin accumulation in the nuclei (Gavilanes *et al*, 2000). In the present case, the tumor cells had spherical swollen mitochondria with frequently broken cristae that could be found evenly dispersed over more than 60% of the total area of the cytoplasm. Microvilli on the cell membrane and chromatin accumulation in nuclei were observed in some tumor cells. In general, these cells contained few cytoplasmic organelles, except for numerous mitochondria. Malignant transformation of oncocytes is often suspected upon notable changes in cell nuclei and cell contour and destruction of fibroblasts in the surrounding capsule.

In conclusion, immunohistochemical findings suggest that oncocytic cells may originate from ductal epithelial cells. The biological behavior of oncocytomas cannot be accurately predicted based on histopathological features alone; the diagnosis of benign or malignant tumors should be based on a combination of clinical, histopathological and ultrastructural findings.

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