A case of hyalinizing clear cell carcinoma (HCCC) of the minor salivary glands of the oral cavity is reported. A 52-year-old woman presented with a growing mass at the base of the tongue. The patient underwent complete resection of the tumour. The histological picture was characterized by trabeculae or solid nests of proliferating cells with a clear cytoplasm, surrounded by a hyalinizing stroma. Tumour cells were immunoreactive for Cytokeratins 5, 6, 7, 8, 14, 17 and 18. No reactivity was observed for cytokeratin 20, vimentin, S-100 protein, smooth-muscle actin, muscle-specific actin, and calponin. These findings confirmed the diagnosis of HCCC of minor salivary glands of the oral cavity. The clinical presentation, the immunohistochemical pattern and the role of cytokeratins in the differential diagnosis of HCCC are discussed with a review of the literature.

Key words: clear cell carcinoma, salivary glands, cytokeratins, immunocytochemistry.

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and carcinoembryonic antigen (CEA). Vimentin, S-100 protein, Smooth-muscle actin, Muscle-specific actin, and calponin are not expressed (Milchgrub et al., 1994; Wang et al., 2002; Sicurella et al., 2004; Urban et al., 1996).

Several entities should be considered in the differential diagnosis of HCCC, including other primary clear cell carcinomas of the salivary glands, like epithelial-myoepithelial carcinoma (EMCC) (Milchgrub et al., 1994; Wang et al., 2002; Batsakis 1980; Batsakis et al., 1983), low-grade mucoepidermoid carcinoma (Milchgrub et al., 1994; Wang et al., 2002), oncocytoma (Milchgrub et al., 1994), and metastases from renal cell carcinoma and melanoma (Milchgrub et al., 1994).

In this study we report a case of hyalinizing clear cell carcinoma of the minor salivary glands arising at the base of the tongue. The clinical, pathological and immunohistochemical features are analyzed, with particular attention to the role of cytokeratins in the differential diagnosis of this tumour entity.

Materials and Methods

A 52 year-old caucasian woman presented to our hospital with a history of an asymptomatic mass which had been localized at the base of the tongue for several months. On oral cavity observation, a well localized mass, reddish in colour, about 2 cm in diameter, was observed. At clinical examination, the tumour showed a parenchimatous consistence. It was covered by a slightly hyperemic mucosa, and was not painful. No cervical lymph nodes were palpable. The patient underwent complete resection of the tumour. Multiple tumour samples were formalin-fixed and routinely processed. 3 micron paraffin sections were stained with Hematoxylin and Eosin and immunostained with commercial antibodies against CK5, CK6, CK7, CK8/18, CK14, CK17, CKAE1/AE3, CK19, CK20, Vimentin, S100 protein, Alfa-SMA, Calponin and Ki67.

Results

Histopathological examination revealed a uniform population of polymorphic, round to polygonal tumour cells with a clear cytoplasm, polymorphic vacuolated nuclei with evident nucleoli, arranged in trabeculae or solid nests, surrounded by a hyalinized stroma (Figure 1). Focally cystic spaces containing eosinophilic, serous material were observed. PAS stain showed the presence of glycogen in the cytoplasm of clear cells. Apoptotic bodies were seen, scattered among tumour cells (Figure 2). No mitotic figures were observed. The tumour was surrounded by a fibrous capsule which resulted focally infiltrated. Multiple foci of vascular invasion were detected outside the tumour capsule (Figure 3). The immunohistochemical pattern was characterized by immunoreactivity for CK5, CK6, CK7, CK8/18, CK14, CK17, CK19, CKAE1/AE3 (Figures 4, 5, 6, 7, 8). Immunoreactivity for CK7 was strong and diffuse while immunostaining for the other markers was focal. No reactivity was detected for CK20, Vimentin, S100, Alfa-SMA, Calponin. Ki67 showed nuclear immunoreactivity in less than 5% of tumour cells. Further clinical examinations were undertaken to check for the presence of other primary tumours: CT-scan of kidneys did not show any renal mass. Based on these findings, the tumour was diagnosed as HCCC.

At 12 months follow-up there is no evidence of recurrent disease or metastases.

Discussion

Primary clear cell tumours of salivary glands comprise a subgroup of salivary neoplasias which are distinct in terms of histogenesis, tumour biology and clinical behaviour (Wang et al., 2002). The differential diagnosis of clear cell salivary neoplasms encompasses a broad range of possibilities (Seifert 1996), including epithelial-myoepithelial carcinoma (EMEC) (Batsakis et al., 1992; Kawahara et al., 2004), clear cell variants of myoepithelial carcinoma (Savera et al., 2000), acinic cell carcinoma (Ellis 1998), oncocytoma (Ellis 1988), and low and intermediate grade mucoepidermoid carcinoma (Brandwein et al., 2000). Metastases of renal cell tumour should also be excluded (Simpson et al., 1990). In clinical practice, the diagnostic term “clear cell carcinoma” is a diagnosis of exclusion, applied only after other specific tumours with clear cell morphology are excluded (Suzuki et al., 2007).

In the differential diagnosis among different clear cell tumours, histology is often of little use mainly when no definitive evidence of myoepithelial differentiation is found, due to the high morphological similarities observed in the different “clear cell” entities.
Figure 1. H-E; original magnification x 100. Tumour cells arranged in solid nest or pod-like structures surrounded by abundant hyalinizing stroma. Figure 2. H-E; original magnification x 250. Diffuse proliferation of clear cells with apoptotic bodies.

Figure 3. H-E; original magnification x 250. Vascular invasion outside the tumour capsule.

Figure 4. CK17; original magnification x 400. Focal cytoplasmic immunostaining of tumour cells.

Figure 5. CK14; original magnification x 400. Granular cytoplasmic immunoreactivity in the majority of tumour cells.

Figure 6. CK5; original magnification x 400. Strong cytoplasmic and membranous immunostaining associated with focal perinuclear Golgian reactivity.

Figure 7. CK7; original magnification x 400. Strong cytoplasmic immunohistochemical staining.

Figure 8 CK18; original magnification x 250. Diffuse membranous immunoreactivity.

(Kawahara et al., 2004). On the other hand, immunocitochemistry may be helpful in revealing the cell of origin of the tumour. Myoepithelial carcinoma shows immunoreactivity of tumour cells for S-100 protein and for vimentin in 100% of cases, variably associated with immunolabelling for CK7, CK14, SMA and calponin (Savera et al., 2000). Epithelial-myoeoepithelial carcinoma shows a positive reaction to S-100 protein, muscle specific actin, associated with immunoreactivity for EMA and cytokeratins AE1/AE3 (Wang et al., 2002; Kawahara et al., 2004). Acinic cell carcinoma cells strongly react with CK7, while CK14 is typically not expressed (Chu and Weiss, 2002). Mucoepidermoid carcinoma shows immunoreactivity for CK7, CK8/18, CK14, CK17 and CK19 (Chu and Weiss, 2002).

The immunohistochemical pattern of the present case was characterized by a diffuse reactivity for
CK5, CK7, CK8/18 and by focal reactivity for CK6, CK17 and CK14; only scattered cells showed immunoreactivity for CK19, in the absence of immunolabelling for CK20, S-100 protein, actin and vimentin. This pattern appears specific for HCCC due to the unique association of basal cell markers (CK5) with myoepithelial markers (CK6, CK14), cytokeratins typical of duct epithelia (CK19), markers of glandular origin (CK7), and with markers of secretory and parenchymatous cells (CK8/18). The diagnosis of myoepithelial carcinoma was ruled out by the lack of expression of actin (Nagao et al., 1998, Savera et al., 2000). The diagnosis of acinic cell carcinoma was unlikely because of immunoreactivity in the present case, for CK14, never expressed in acinic cell carcinoma. The possibility of a solitary metastasis from a tumour arising from kidney was excluded by the expression, in our case, of CK7, constantly not expressed in this tumour (Chu and Weiss, 2002; Nikitakis et al., 2004). The possibility of a squamous-cellular carcinoma of the oral cavity spreading to the salivary glands was ruled out by diffuse immunoreactivity in the present case for CK7, normally absent or focally and weakly expressed in squamous-cellular carcinoma (Nikitakis et al., 2004; Regauer et al., 2000). Mucoepidermoid carcinoma remains the only tumour of the salivary glands to lack of expression of actin (Nagao et al., 2000). The diagnosis of acinic cell carcinoma was unlikely because of immunoreactivity in the present case, for CK14, never expressed in acinic cell carcinoma. The possibility of a solitary metastasis from a tumour arising from kidney was excluded by the expression, in our case, of CK7, constantly not expressed in this tumour (Chu and Weiss, 2002; Nikitakis et al., 2004). The diagnosis of an acinic cell carcinoma was unlikely because of immunoreactivity in the present case, for CK14, never expressed in acinic cell carcinoma. The possibility of a solitary metastasis from a tumour arising from kidney was excluded by the expression, in our case, of CK7, constantly not expressed in this tumour (Chu and Weiss, 2002; Nikitakis et al., 2004). The possibility of a squamous-cellular carcinoma of the oral cavity spreading to the salivary glands was ruled out by diffuse immunoreactivity in the present case for CK7, normally absent or focally and weakly expressed in squamous-cellular carcinoma (Nikitakis et al., 2004; Regauer et al., 2000). Mucoepidermoid carcinoma remains the only tumour of the salivary glands to lack of expression of actin (Nagao et al., 1998, Savera et al., 2000). The absence of globet cells, negativity of tumour cells for mucin staining and the absence of areas of squamous differentiation in our case allowed us to exclude this diagnosis (Milchgrub et al., 1994; Suzuki et al., 2007).

In conclusion, for diagnostic purposes, the immunohistochemical pattern of the present case could be suggested, when confirmed in a large series of HCCC, as a useful tool in the diagnosis of HCCC of minor salivary glands.

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