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High-grade epithelial carcinoma arising in a low-grade epithelial myoepithelial carcinoma of the parotid gland: a rare case report with immunohistochemical and molecular analysis

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Summary. A case of high-grade epithelial carcinoma arising in a low-grade epithelial-myoepithelial carcinoma of the parotid gland is described. The patient was a 52 year-old male who presented a parotideal lump of approximately 20 mm in diameter and underwent excisional surgery. Histologically a typical low-grade epithelial myoepithelial carcinoma was evidenced and, in its context, an area of 7 x 4 mm showing focal aspects of a high-grade adenocarcinoma. The immunohistochemical pattern of the ductal epithelial cells of the low-grade component was similar to that of the epithelial cells of the high-grade carcinoma. The Ki-67 labelling index of the epithelial-myoepithelial carcinoma was 5%, whereas that of the high-grade lesion was 30%. EGFR, p53 and HER-2 genes seem to play no role in the biological behaviour of the tumour, as well as p16CDKN2A, BRAF, NRAS and C-KIT genes studied with biomolecular methods in both the high-grade and low-grade components. No local recurrence occurred after surgery, but multiple bone, cutaneous and lung metastases were detected 10 months later. The patient died 19 months after diagnosis.

Key words: parotid, myoepithelial, high-grade carcinoma

«Carcinoma epiteliale ad alto grado insorto nel contesto di carcinoma epiteliale-mioepiteliale a basso grado della ghiandola parotide. Raro case report con analisi immunoistochimica e biomolecolare»

Riassunto. Descriviamo in questo articolo un raro caso di carcinoma epiteliale ad alto grado insorto nel contesto di un carcinoma epiteliale - mioepiteliale a basso grado della parotide. Il paziente era un maschio di 52 anni con una lesione parotidea di circa 20 mm, la quale è stata asportata chirurgicamente. L'esame istologico ha evidenziato un carcinoma epiteliale-mioepiteliale a basso grado con un area di 7 x 4 mm con aspetti focali di adenocarcinoma ad alto grado. L'analisi immunoistochimica ha messo in evidenza una certa similitudine tra le cellule epiteliali duttali della componente a basso grado e le cellule epiteliali della componente ad alto grado. L'indice Ki-67 era 5% nella prima e 30% nella seconda. I geni EGFR, p53 e HER-2 non sembrano rivestire alcun ruolo nel determinare il comportamento biologico della neoplasia, come del resto i geni p16CDKN2A, BRAF, NRAS e C-KIT, esaminati con tecniche di biologia molecolare in entrambe le componenti. Non c'è stata recidiva locale in seguito all'intervento chirurgico, ma circa 10 mesi dopo sono state evidenziate multiple metastasi a livello osseo, cutaneo e polmonare. Il paziente è deceduto 19 mesi dopo la diagnosi.

Parole chiave: parotide, mioepiteliale, carcinoma ad alto grado

Introduction

Epithelial-myoepithelial carcinomas (EMC) are rare malignancies of the salivary glands, first described by Donath et al. in 1972 (1). EMC account for 1% of all salivary gland tumours that primarily involve the parotid, but also involve the minor salivary glands (2-4). Histologically ECM are composed of two cellular components: luminal ductal cells and surrounding peripheral myoepithelial differentiated cells. EMC is a low-grade malignant neoplasia with, generally, a good prognosis and a 10-year disease-free survival of 81.8% (4). Factors that increase the aggressiveness of EMC include infiltration of resection margins, vascular invasion, necrosis, and myoepithelial anaplasia. Cases with severe myoepithelial anaplasia have also been referred to as myoepithelial carcinoma arising in EMC (5).

The epithelial ductal component has recently been described as evolving into a high-grade carcinoma, showing greater aggressiveness and a worse prognosis (3, 4). This transition has been called "dedifferentiated EMC" (DEMC) and can involve only a small focal area. To our knowledge, only a few cases of DEMC of the salivary glands have been reported in the literature to date (4, 6-13). The small number of cases described makes comprehension of the biological features of the neoplasia difficult and, consequently, the appropriate clinical management is problematic. Recently, it has been suggested that the term "dedifferentiation" has to be abandoned, a term that has also been applied in other salivary gland malignancies, in favour of "high-grade transformation" (HGT) (14, 15).

In this report, we describe a case of typical low-grade EMC with areas of abrupt HGT and a rapid, fatal outcome.

Case Report

A 52-year-old Caucasian male was admitted to the Otolaryngology Unit of the University of Sassari (Sassari, Italy) complaining of a painless mass in the left parotid area. He was a chemist, non-smoker and with no family history of salivary gland pathologies. He has regularly used mobile phones for 16 years and cordless phone devices for approximately 20 years, performing or receiving 5 to 10 calls a day, with a cumulative daily usage time of 10-20 minutes. He predominantly was using the right side of the head as he was right-handed. The patient did not report other signs or symptoms. Physical examination and computed tomography (CT) scan of the head and neck area revealed a lump measuring 25 x 20 x 14.5 mm in the left parotid gland, which was firm and partially fixed on adjacent tissues. Routine hematologic and biochemical tests, as well as a chest radiogram, were normal. Fine needle aspiration cytology (FNAC) was performed and revealed a neoplasia of the salivary gland, with aspects suggestive of a pleomorphic adenoma.

The patient underwent surgery, and an intraoperative frozen section examination was performed; a malignant tumour to be further characterised was diagnosed. Subsequently, the whole parotid gland measuring 40 x 25 x 20 mm was removed, along with perilesional lymphatic and adipose tissue. The postoperative course was uneventful.

Based on the microscopic morphology and immunohistochemical profile, a final diagnosis of EMC with a small focus of HGT was made. A micrometastasis was found in one of five lymph nodes removed.

In light of these findings, oncological staging was completed with a total body CT scan, without evidence of distant metastases. The patient was referred for oncological counselling and underwent adjuvant radiotherapy and multi-chemotherapy. Ten months after surgery, positron emission tomography (PET) and CT scans were performed for follow-up purposes and revealed multiple pulmonary and bone metastases. In spite of adjuvant therapies, the clinical condition of the patient showed a progressive deterioration; multiple subcutaneous nodules of about 5 mm in diameter in the scalp and cheek region appeared. Two of these nodules were surgically excised, and pathological examination showed metastatic carcinoma with the same morphologic features of the high-grade component without any evidence of EMC. The evolution of the disease continued with additional multiple metastases in the liver, brain, adrenal glands, kidney, and bone, and the patient died 19 months after diagnosis.

Histology, immunohistochemistry, and molecular biology

Grossly, the tumour measured 25 x 20 x 14 mm and had a firm consistency and a white-greyish colour. It was fixed in 10% neutral buffered formalin, routinely processed, and paraffin embedded. Three micron-thick sections were prepared for hematoxylin and eosin staining and histoimmunological analysis. The lesion was entirely processed, and multiple sections were examined.

Histological examination showed most of the tumour areas as typical EMC. These areas were numerous, circumscribed, partially encapsulated, and composed of tubules. These tubules were composed of a double layer of epithelial-myoepithelial cells, consisting internally of intercalated eosinophilic duct-like luminal cells and peripherally surrounded by clear myoepithelial cells.

The nuclei were regular and round or ovoid. The tubules were surrounded by diffuse and abundant hyalinised basement membrane-like material. Some of the tubules showed cystic structures. No significant mitotic activity or necrosis was seen in the typical EMC areas. No areas showing clear cellular or oncocytic changes, sebaceous differentiation, or pleomorphic adenoma components were present. Furthermore, areas of myoepithelial overgrowth consisting of sheets of clear cells arranged in solid nests with no ducts were evident. Perineural infiltration was seen. In the context of this morphological picture, a small 7 x 4 mm area composed of sheets of atypical epithelial cells with marked nuclear pleomorphism, large nucleoli, brisk mitotic activity (11/10 high-powered fields), and necrosis was detected. No evident transition area was observed; however, an abrupt transformation into a high-grade carcinoma with features morphologically suggesting a poorly differentiated ductal carcinoma of the salivary gland was detected (Figure 1a, Ins). The cells were of luminal type and organised in highly atypical glands and tubules. Wide perineural and vascular invasion, as well as extraglandular extensions, were detected.

Five small lymph nodes were found close to the tumour; a micrometastasis of 1 mm or greater in diameter was observed in the marginal sinus in one of them (Figure 1b). The metastatic subcutaneous tumour showed a poorly differentiated carcinoma, without any myoepithelial component.

Immunohistochemical stains were performed by the avidin-biotin-peroxidase complex (ABC) technique. The antibodies, sources, and dilutions used, as well as the results obtained, are listed in Table 1. Immunohistochemistry was semi-quantitatively scored, on the basis of the proportion of immunereactive cells and the intensity of reactivity.

The typical EMC showed the inner cells of the ducts to be positive for cytokeratin, CAM5.2 (Figure 1c), HMWK (34BE12), EMA (Figure 1d), CK7, CK14, and very weak for CK5/6. These cells were negative for MSA, SMA, CD10, p63, Calponin, HER2, p53, BCL1, GFAP, Vimentin, AR, and ER. The outer cells were positive for S100 protein, SMA, CD10, p63, Calponin (Figure 1e), CK5/6, p53, BCL1, Vimentin, and HMWK (34BE12), negative for CAM5.2, EMA, MSA, CK7, and CK14, and very weak for CK5/6.

The high-grade component presented immunohistochemical findings similar to the luminal ductal cells of the EMC and showed diffuse and strong staining with CAM5.2 (Figure 1c), HMWK (34BE12), EMA, CK7, and CK14 and very weak staining with CK5/6. These cells were negative for MSA, SMA, CD10, p63, Calponin, HER2, p53, BCL1, GFAP, Vimentin, AR, and ER staining.

Molecular analysis of p16CDKN2A, BRAF, NRAS, and cKIT mutations was performed, and the results were negative in both the low- and high-grade components.

Discussion

EMCs are rare tumours mainly occurring in the parotid gland (3,7). Cases showing high-grade transformation of the neoplastic cells are exceedingly rare (6-12). This transformation may affect myoepithelial cells or, rarely, the epithelial ductal component, and may be gradual and progressive or extremely rapid and invasive.

This case report is a typical EMC of the parotid containing a small area of high-grade undifferentiated

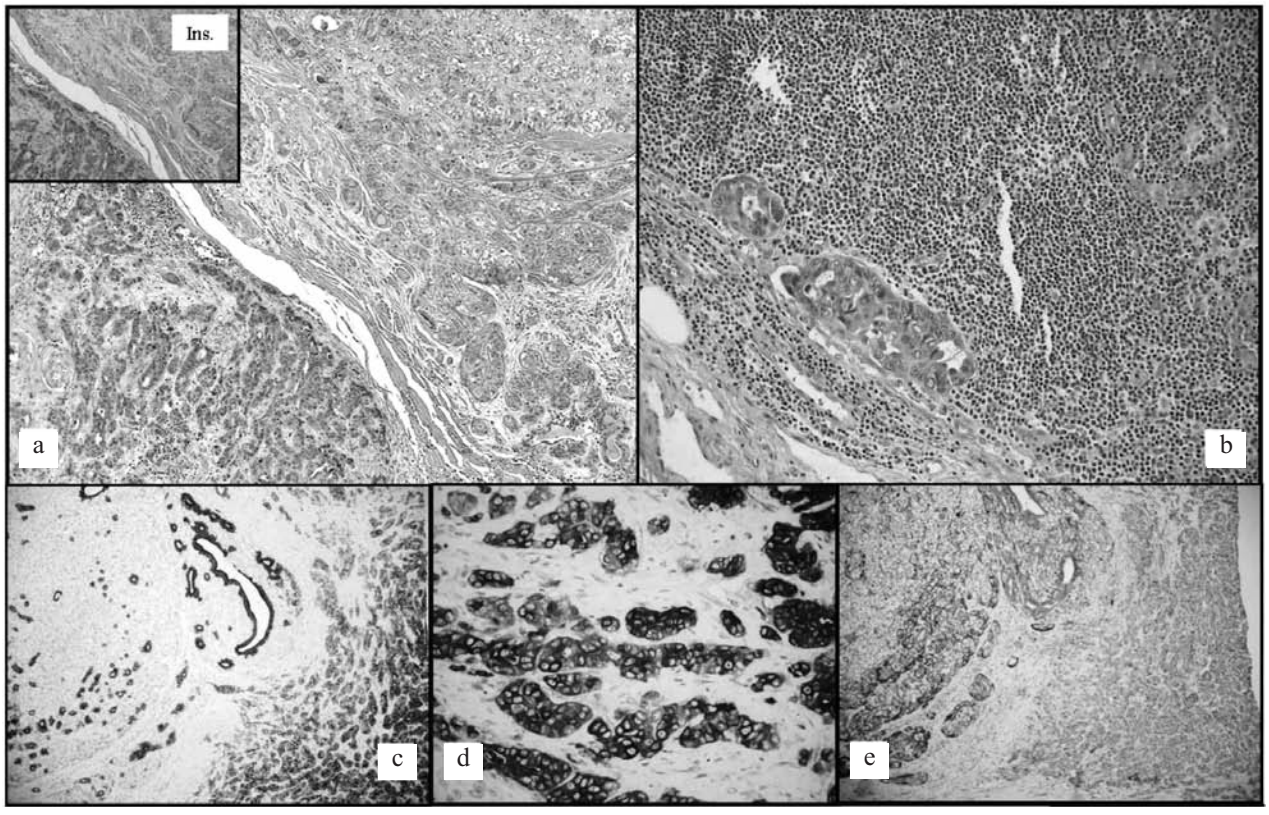

Figure 1. a) of the tumour depicting both the low and high-grade component, Ins) major magnification of the same image, b) hematoxylin and eosin-stained section of a lymph node showing neoplastic invasion of the marginal zone, c) immunoreactivity for CAM 5.2 in the ductal cells of the low-grade component (on the left) and in the high-grade component (on the right), e) immunoreactivity for Calponin in the myoepithelial cells of the low-grade component, d) intense immunoreactivity for EMA in the ductal cells of the low-grade component.

carcinoma, without a gradual transition or morphologically intermediate area. These characteristics could suggest the presence of a collision tumour; however, the immunohistochemical characteristics of the epithelial ductal cells of the EMC and those of the HGT component were similar. This condition supported the hypothesis that the high-grade component developed through dedifferentiation of the ductal epithelial cells of the low-grade EMC.

The cellular features of the high-grade area were consistent with a poorly differentiated ductal carcinoma. Nevertheless, intraductal necrosis suggesting breast comedocarcinoma was absent, and BRST2, AR, ER, and HER2 immunohistochemical staining, often described in ductal carcinoma, was lacking.

Transformation of a low-grade malignant lesion, or even a benign tumour, in a high-grade carcinoma is a well-known event in the context of salivary gland tumours. Dedifferentiation of low-grade carcinomas has been described in adenoid cystic carcinoma (14, 16), acinic cell carcinoma (15, 17), polymorphous low-grade adenocarcinoma (18, 19), low-grade mucoepidermoid carcinoma (20), malignant myoepithelioma (21), and intraductal carcinoma (low-grade cribriform cystadenocarcinoma) (22). In addition, it is well known that a long standing pleomorphic adenoma might evolve into a so-called carcinoma ex pleomorphic adenoma (23).

In our case, a very small area of dedifferentiated carcinoma arose in the context of a small parotid tumour and was associated with a very aggressive clinical course. This is of great importance in the clinical practice because failure to recognize such areas may result in oncological and clinical mismanagement of

Table 1. Immunohistochemical results in both EMC and HGT areas of the lesion.

Antibody	Source	Diluition	Clone	Results		
				EMC		HGC
				Inner	Outer	
CAM 5.2	Novocastra	1:100	5D3	+	-	+
CK14	Biogenex	1:50	LL002	+	-	+
CK7	Novocastra	1:100	RN7	+	-	+
CK5/6	Biocare Medical	1:100	CK5/6.007	+/-	+	+/-
HMWK	Novocastra	1:100	34βE12	+	-	-/+
EMA	Dako	1:100	E29	+	-	+
CD10	Novocastra	1:100	56C6	-	+	-
AR	Novocastra	1:100	AR27	-	-	-
ER	Novocastra	1:80	6F11	-	-	-
BRST2	Novocastra	1:50	23A3	-	-	-
S100	Novocastra	1:3500	Polyclonal	-	+	-
MSA	Biogenex	1:50	1A4	-	-	-
SMA	Biogenex	1:50	HHF35	-	+	-
BCL1	Histo-line	1:150	SP4	-	+	-
Vimentin	Novocastra	1:100	V9	-	+	-
Ki-67	Novocastra	1:100	MM1	+ 5%	+ 5%	+ 30%
GFAP	Biogenex	1:100	Polyclonal	-	-	-
HER2	Novocastra	1:100	10A7	-	-	-
p53	Novocastra	1.50	DO-1	-	+	-
EGFR	Novocastra	KIT	EGFR.25	+	-	-
Calponin	Novocastra	1:50	CALP	-	+	-
P 63	Novocastra	1:50	7JUL	-	-	-

CAM: anti-cytokeratin; CK: cytokeratin, HMWK: high molecular weight keratin, EMA: epithelial membrane antigen, CD: cluster of designation, S100: protein S100, AR: androgen receptor, ER: estrogen receptor, BRST: prolactin-inducible protein, MSA: muscle-specific actin, SMA: smooth muscle actin, BCL: B-cell lymphoma, GFAP: glial fibrillary acidic protein, HER: human epidermal growth factor receptor, EGFR: epidermal growth factor receptor, +: positive for few cells; -: no significant reactivity.

patients. This suggests that EMC parotid tumours must be routinely subject to extensive and complete sampling for careful microscopic evaluation in order to avoid missing small dedifferentiated foci. Even if the number of cases reported is small, the prognosis is significantly worse than that of typical EMC. This suggests that treatment must also be more radical, including wider excision, lymph node dissection of the neck, and possibly adjuvant radiation therapy. However, our patient showed no local recurrence and died from distant widespread neoplasm; this suggests a tendency for very early systemic dissemination via the bloodstream.

From a biological point of view, it is well known that cancer derives from genetic somatic alterations, which generally accumulate progressively during life with reference to intrinsic biological factors and exposure to mutagens. These events determine loss of proliferation control by cells. Oncogenic mutations may occur in normal tissue, but they more frequently occur within a pre-existing neoplasm. This means that any type of tumour may undergo dedifferentiation with an accumulation of genetic changes. These changes might lead to highly aggressive neoplastic features, improving the capacity of vascular invasion and the metastatic potential of the tumour, as occurred in the present case.

The identification of molecular alterations in HGT lesions is of great interest in understanding the underlying mechanisms and in establishing, in some cases, the best therapeutic approach. Among several mutations known to induce aggressive neoplastic behaviour, overexpression of HER-2/neu has been described in high-grade areas of carcinoma ex pleomorphic adenoma (24) as well as in some cases of ductal carcinoma (25). However, HER-2/neu overex-

pression was not present in our case or in other reported cases of dedifferentiated EMC. Strong immunoreactivity for p53 protein has been sporadically reported in cases of dedifferentiated EMC (7). In our case, p53 was immunohistologically negative in both the EMC and HGT areas. In addition, EGFR in our case was negative, at both the immunohistochemical and molecular levels. An increased Ki-67 labelling index in the HGT component was not surprising, given the higher level of cellular anaplasia. Furthermore, molecular analysis of p16CDKN2A, BRAF, NRAS, and cKIT mutations show negative results in both neoplastic areas.

Conclusions

EMC is a rare malignancy accounting for 1% of all salivary gland tumours, with generally a good prognosis. Nevertheless, it may be the substratum of high-grade dedifferentiation, which considerably worsens the prognosis. This must always be kept in mind by healthcare specialists, especially pathologists who have to perform complete and meticulous sampling of the tumour in order to avoid missing small dedifferentiated foci. Knowledge of the morphological and immunohistochemical features of the different grade areas, extensively described in this report as well as by others in the literature, is of great importance for diagnosis. Further contributions are necessary to better comprehend the molecular mechanisms of dedifferentiation.

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