Salivary epithelial-myoepithelial carcinoma: clinical, morphological and molecular features

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Key words
Salivary epithelial-myoepithelial carcinoma • Pathologic and molecular features • Prognosis and treatment

Summary
Epithelial-myoepithelial carcinoma (EMC) is a rare biphasic tumor accounting for less than 2% of all salivary gland malignancies. It presents as a slowly growing, asymptomatic small size mass, with ulceration of overlying mucosa in some cases. Microscopically, it is characterized by glands lined by the simultaneous presence of two different cell components, inner epithelial cells and outer myoepithelial cells. Immunohistochemical staining of myoepithelial cells is variably positive for vimentin, Smooth Muscle Actin (SMA), Muscle Specific Actin (MSA), S100, Smooth Muscle Myosin Heavy Chain I(SM-MHC), calponin and p63. Several molecular alterations, mainly point mutations, have been described. Mutations of HRAS, AKT1, CTNNB1 and PIK3CA were highlighted in variable percentage of EMC samples. EMC is considered a low-grade malignant tumor with a 5-year survival rate of 94% that may commonly recur locally after resection in 30-50% of cases. At the moment, adequate resection with negative margins is the minimum recommended and necessary treatment.

Introduction
Epithelial-myoepithelial carcinoma (EMC) is a tumor with a typical biphasic histology: it is defined as “a tumour composed of variable proportions of two cell types which typically form duct-like structures. There is an inner layer of duct-lining cells and an outer layer of clear cells”1.

The duct-lining cells are intercalated duct-like cells, while the outer cells are myoepithelial cells, hence the definition term epithelial-myoepithelial carcinoma (EMC) is descriptive 2. It is supposed to derive from intercalated duct hyperplasia 3.

This tumor was firstly described in 1945 by Bauer WH as adenomyoepithelioma 4, defined as epithelial-myoepithelial carcinomain 1972 by Donath et al. 5 and recognized as a separate entity in the 2nd World Health Organization (WHO) classification in 1991. Different terminologies have been used over the years, e.g. clear cell adenoma 6, monomorphic clear cell tumor 7, glycogen-rich adenoma 8, glycogen-rich adenocarcinoma 9 and clear cell carcinoma 10.

Recently Roy et al confirmed the EMC terminology, identifying a specific subgroup with aggressive behavior 11.

Clinical features
EMCs is a rare neoplasm accounting for less than 2% of all salivary gland malignancies 2, with only 320 cases reported in the literature since its first description in 1972. The majority of cases described in literature are single case reports; one of the larger series in the past gathered 61 patients 12, but the widest series up to date analyzes 246 cases of the major salivary glands, collected from the SEER data-base of Bethesda 13.

EMC more commonly occurs in the parotid gland (75-80%), but can affect also submandibular gland and minor salivary gland throughout the upper aerodigestive tract including oral cavity and the sinonasal tract 14-17.

Acknowledgments
The Authors thank the study group GIPaTeC (Gruppo Italiano di Patologia Testa e Collo).

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EMC has a slight female predominance and is more frequent in older adults, with a peak incidence in the sixth and seventh decades of life, even if some cases have been described in pediatric age\(^\text{16-18}\). EMC is usually unilateral, but rare bilateral cases have been described\(^\text{19-20}\). Clinically, EMC appears as a slowly growing, asymptomatic mass, usually 2-3 cm in size, but also bulky neoplasms have been reported; sometimes, ulceration of overlying mucosa occurs. Rarely, pain or facial nerve paralysis may be present. Instrumental investigation include computed tomography (CT) and magnetic resonance but they appear non-specific\(^\text{21}\), as well as cytological diagnosis (FNAC) that does not allow identification of the specific architectural pattern\(^\text{22}\). A more accurate pre-surgery diagnostic method is Fine-Needle Aspiration Biopsy (FNAB)\(^\text{2}\).

### Pathologic features

Grossly, EMC is a well circumscribed, partially to completely encapsulated, multinodular, whitish tumour (Figg. 1-2 A), with pushing borders; an infiltrative growth pattern is rarely recognized to the naked eye and occurs in only 12% of cases\(^\text{12}\). The great majority of tumours is solid, but a few cystic cases have been described, with endophytic papillary projections\(^\text{2}\).

Microscopically, a multilobular growth pattern can be appreciated for the presence of hyaline stroma separating the lobules (Fig. 2 B). The lobules show the typical bilayered arrangement of the cells, with inner ductal cells and outer myoepithelial cells (Fig. 2 C), that can vary in proportion: usually ducts are clearly evident, but, sometimes, are rare and difficult to find, as in the morphologic variant “EMC with myoepithelial overgrowth”\(^\text{2}\).

The luminal spaces contain eosinophilic, proteinaceous PAS positive and mucicarmine negative material. The ductal cells are cuboidal or cylindrical and show a faint eosinophilic cytoplasm while the myoepithelial cells are polygonal or spindle and show abundant clear, glycogen-rich cytoplasm (Figg. 2 D, E). Myoepithelial cells can show some peculiar features named “Verocay”-like nuclear palisading or squamous metaplasia.

The stroma in which lobules are embedded is, usually, abundant, as the tumour cells produce hyalinized, pinky or loose, bluish, almost myxoid basal lamina material. Cytological atypia in neoplastic cells is mild, but rare cases show marked nuclear pleomorphism, especially in the myoepithelial component, the so called “Ancient” change (similar to “monster” cells in pleomorphic adenomas)\(^\text{2}\).

Usually, mitoses are scarce, till to 2x10 HPF, but, in some cases, they rise to 7/8x10 HPF. The biphasic pattern of EMC is highlighted by immunohistochemistry. The ductal cells intensely stain for Epithelial Membrane Antigen (EMA) and cytokeratins, either pancytokeratin cocktails or low molecular weight keratins (CK\(^\text{19}\))\(^\text{23}\). The myoepithelial cells are variably positive for myoepithelial markers: vimentin, SMA actin, MSA actin (Figg. 3 A, B), S100 (Figg. 3 C, D), smooth muscle myosin heavy chains, calponin and p63 (Fig. 3 E). Among these, from a practical standpoint, p63 is the most useful\(^\text{12}\)\(^\text{2}\). The outer myoepithelial layer is often positive to high molecular weight keratins (negative in ductal cells) and stains for EMA (Fig. 3 F) and low molecular weight keratins, however less intensely than ductal cells.

Recently, DOG1 has been reported expressed in more than 50% of EMCs examined, with a distinctive combined apical ductal and membranous/cytoplasmic myoepithelial staining profile\(^\text{24}\) and also SOX10 has been observed to be positive in EMC as marker of intercalated-duct differentiation\(^\text{25}\).

Many morphologic variants of EMC have been described. Oncocytic and Apocrine variants are defined by extensive oncocytic and apocrine change, present in
more than 50% of the neoplastic cells; they both have clinical indolent behavior.

Oncocytic EMC was firstly described by Savera and Salama in 2005; it onsets in older patients. The ductal component is larger, reminiscent of striated duct epithelium and is characterized by pink, granular, oncocytoid cytoplasm; the myoepithelial component can be clear or oncocytic: in this case, the biphasic nature of the neoplasia could be misunderstood. A papillary growth pattern and luminal calcifications are common.
Oncocytic EMC can be associated to a sebaceous component. Focal sebaceous differentiation has been described in 13% of cases reported by Seethala, but Sebaceous EMC has been recognized as a new histologic variant by Shinozaki et al.

Histologically, tumour cells in Sebaceous EMC show foci of sebaceous differentiation, consisting in small or larger intracytoplasmic vacuoles, sometimes peripherally displacing nuclei and superficially resembling lipoblasts; moreover, the cells stain for EMA, Adipophilin and Perilipin.

Apocrine EMC was reported as a novel variant by Seethala.
in 2013 30; it is characterized by apocrine change of the ductal component with typical apical snouts, frequent overgrowth of the epithelial component in a cribriform or even solid pattern, nuclear pleomorphism, prominent nucleoli and immunohistochemical expression by the neoplastic cells of breast markers as AR, GCDFP15 and HER2.

In Double Clear Cell variant, both the epithelial and myoepithelial components show abundant clear cytoplasm, so this entity is often under recognized because of the loss of the double layered appearance. Moreover, it can be confused with myoepithelioma because of its low mitotic activity and the frequent presence of a capsule 31.

EMC with high grade transformation includes tumours historically defined as dedifferentiated EMC and EMC with anaplasia 32-35 11 12. EMC with anaplasia is characterized by nuclear pleomorphism in more than 20% of myoepithelial cells, with gradual transition between areas with mild and marked atypia, and by increased mitotic activity.

In dedifferentiated EMC, the two carcinomatous components (high-grade carcinoma and low-grade malignant neoplasm) are sharply separated: they differ for growth pattern (solid, nested, sheet-like), comedo-necrosis, high mitotic index, marked nuclear pleomorphism and loss of bilayered architecture, features present in high-grade areas 35.

It is important to recognize EMC with high grade transformation because this variant is associated to poor prognosis 36 11.

The majority of epithelial-myoeipithelial carcinomas arise denovo, but some cases can represent the malignant component in carcinoma ex pleomorphic adenoma (EMC ex pleomorphic adenoma) 32.

EMC may, also, presents as “hybrid tumor”: it can occur as part of a hybrid salivary gland tumor in association with other salivary gland tumors (e.g., adenoid cystic carcinoma [AdCC], salivary duct carcinoma, others) 14.

**Differential diagnosis**

The differential diagnosis of EMC includes malignancies primitively rising in salivary glands and metastatic salivary gland tumors from other sites, like metastatic renal cell carcinoma 2. Among primary malignancies of salivary glands, two groups are included: tumors with biphasic growth pattern, as pleomorphic adenoma and adenoid-cystic carcinoma and tumors rich in clear cells 2.

Pleomorphic adenomas (PA) are well circumscribed and show the characteristic myxochondroid stroma lacking in EMCs; moreover, myoepithelial cells in PA typically merge with surrounding stroma while they are sharply separated from it in EMC.

The biphasic growth pattern is retained in Adenoid-Cystic Carcinoma (AdCC), but myoepithelial cells are smaller and show little, angulated, hyperchromatic nuclei. Both these entities (AdCCandEMC) share some growth patterns like the tubular and the solid ones, but EMC lacks the cribriform growth pattern typical of AdCC.

Among tumours with abundant clear cells, Clear Cell Myoepithelioma (CCM) and Clear Cell Myoepithelial Carcinoma (CCMC) should be considered firstly.

CCM differs from EMC because is often encapsulated and lacks infiltrative margins; on the other hand, CCMC presents as bulky mass, destructively infiltrating surrounding soft tissues. Obviously, both these tumors are monomorphic, being made of solid sheets of clear cells; however, the diagnosis of EMC can be particularly difficult in cases with myoepithelial overgrowth, because the biphasic pattern becomes less evident and, furthermore, a minimal grade of ductal differentiation is acceptable in tumors almost exclusively composed of myoepithelial cells 38 23.

Hyalinizing Clear Cell Carcinoma (HCCC) is characterized by trabecular/cordonal growth pattern, cytokeratins and EMA positivity and limited or absent expression of myoepithelial markers; stromal hyalinization may or may not be present.

Clear cell type of Mucoepidermoid Carcinoma (cc MEC) shows different cell types. Specifically cc MEC is composed ofmucocytes, epidermoid and intermediate cells in addition to predominant clear cells; immunohistochemistry may be helpful because neoplastic cells don’t react for the majority of myoepithelial markers, except for p63.

Oncocytic EMC should be distinguished from benign conditions like oncocytoma, especially clear cell variant and oncocyticystoadenoma. In these last entities, the cytoplasm clearing can be due to fixation and tissue processing artifacts and, also, to glycogen accumulation, but a transition between clear and oncocytic cells can always be found. Immunohistochemistry could be useful in differential diagnosis, but also challenging, because myoepithelial markers in oncocytoma are negative, except p63, due to displacing of mitochondria at the periphery of the cells from the glycogen storage, simulating the typical biphasic pattern of EMC 23.

Apocrine EMC could be confused with Salivary Duct Carcinoma and Sebaceous EMC with Sebaceous Carcinoma when the sebaceous change is extensively diffuse throughout the neoplasia.

Metastasis from Clear Cell Renal Carcinoma should be considered in presence of highly vascularized stroma, CD10, RCC, CAIX, PAX 2 and PAX 8 positivity; metastasis from Thyroid Carcinoma, follicular variant with predominance of clear cells, can be ruled out by negativity of thyroid markers like thyroglobulin and TTF1.

**Biology and genetics**

The biological peculiarities of this tumor is represented by the simultaneous presence of two different cell components, inner epithelial cells and outer myoepithelial cells.

Up to date, molecular and cytogenetic studies of EMC
have been limited. Only recently, genetic profile and molecular events associated to EMC pathogenesis and progression have been analyzed, especially with the advent of more sophisticated analytical technologies (gene microarray, next-generation parallel sequencing, etc). Kleist et al reported LOH at 13q12 and 18q21 in one case of EMC and LOH of microsatellite loci of 9p22-p21 and 10q23-q24 in solid but not in the tubular part of the tumor.

Martins et al have found double clonality in one/two cases of EMC: one clone had gains in chromosomes 2 and 8 and the other had loss of chromosomes 10, 20 and X. This event was interpreted as an expression of the biphasic nature of this tumour.

The first investigated oncogenes were the RAS proteins, encoded by three proto-oncogenes, HRAS, KRAS and NRAS for which activating mutations were abundantly documented as implicated in many human cancer types.

Chiosea et al. analyzed a small EMC case series highlighting the presence of HRAS exon 3, codon 61 mutations, p. Q61R and p. Q61K in 26% of selected patients. Subsequently, sporadic case reports reported the presence of p. 61R mutation in HRAS gene in both primary EMC and associated lung metastasis.

More recently by next generation sequencing (NGS) a mutation screening of 22 different genes was done on a large series of salivary gland cancer (SGC). Mutations of AKT1 gene in exon 4 (p. E17K), CTNNB1 gene in exon 3 (p. 135T) and PIK3CA gene in exon 21 (pG1049R) and in exon 2 (p. K111E) were detected in 20% of EMC, while HRAS mutations in exon 3 (p. Q61R and p. Q61K) were highlighted in 80% of EMC samples (Fig. 4). However, more thorough molecular study was realized by RNA-Seq analysis on 17 patients with diagnosis of EMC. A Sequenome platform of 190 common oncogenic point mutation in 50 genes was performed. The main results highlighted the presence of KRAS mutations in 18% and NRAS mutation in 6% of patients, while, in contrast to what reported in literature, none HRAS mutation was detected. Moreover, no mutation in MET, PIK3CA and BRAF genes was highlighted.

Gene expression profiling realized comparing EMC tumor samples with 6 normal salivary tissue samples identified 220 significantly expressed transcripts, with 36% of upregulated genes and 64% downregulated. In particular, many altered cancer-related genes were identified: CST2 (cystatin 2), BPIFA4P (BPI fold containing family A, member 4 pseudogene), PDK4 (pyruvate dehydrogenase kinase isozyme 4), (the latter is downregulated in colon adenocarcinoma also) PLCG1 (phospholipase C gamma 1) described in colon cancer cells and in breast cancer also, and primarily the up-regulation of FGFR1 (fibroblast growth factor receptor 1), abundantly described also for other tumor types.

Moreover, several non-coding RNA, in particular miRNA, appeared strongly differentially expressed. MiR-663 and miR-3648 were upregulated in tumor samples, both already described in other tumor types.

In particular miR-663 was considered as oncogene in nasopharyngeal carcinoma and in breast cancer. When EMC appears as a hybrid form in association with adenoid cystic carcinoma (AdCC) a translocation of MYB oncogene also be present. The presence of MYB translocation led to hypothesize a common differentiation pathway and therefore that EMC could represent a variant of AdCC.

When EMC is present as hybrid form with SDC a mutation in codon 282 (CGG (Arg) to GGG (Gly)) of p53 gene can also be present.

**Prognosis and treatment**

EMC is a low grade malignancy with a 5-year survival rate of 91% and 10-year survival rate of 90.2%. Local and multiple recurrences may occur after resection, in 30-50% of cases, though, often, many years after initial presentation. Pathologic features correlated with high incidence of recurrence are: positive surgical margins, necrosis within lobules (18% of cases), absence of encapsulation (infiltrative margins), anaplasia in more than 20% of the cells, angiolymphatic invasion and perineural infiltration. EMC with high-grade transformation, in fact, appears to be more aggressive than classic EMC.

Metastases occur more frequently to loco-regional lymphnodes, in 20% of the casesand rarely at distance, in less than 10% of cases, involving lung, kidney and brain.

The treatment of choice is complete surgical excision, with free resection margins. The role of radiotherapy is not well established: it is advisable when there is doubt about completeness of surgical resection, but it seems to bring no survival benefit.

EMCs with high grade transformation need the same...
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