CASE REPORT

RJME
Romanian Journal of Morphology & Embryology
http://www.rjme.ro/

Parotid sclerosing mucoepidermoid carcinoma: a case report and immunohistochemical study

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Abstract

Here we report the case of a 63-year-old female with a parotid sclerosing mucoepidermoid carcinoma diagnosed and treated at the Department of Oral and Maxillofacial Surgery, Emergency County Hospital of Craiova, Romania. The clinical and imaging investigation revealed a parotid malignant tumor with central fluid-filled cystic formation. Histopathology found an intermediate grade sclerosing mucoepidermoid carcinoma that invaded the adjacent adipose and striated muscle tissues, but without perineural and lymphovascular invasion. The immunohistochemistry investigated mainly biomarkers involved in the induction of a local aggressive behavior. This case report describes a rare parotid sclerosing mucoepidermoid carcinoma with peculiar clinical and morphological characteristic features. The immunohistochemical study sustained its intermediate grade malignancy highlighting the prognostic value of some of the used biomarkers.

Keywords: sclerosing mucoepidermoid carcinoma, immunohistochemistry, parotid gland, tumor grade malignancy.

Introduction

Mucoepidermoid carcinoma (MEC) is the most common malignant primary salivary gland tumor composed of varying proportions of mucous, intermediate and epidermoid cells, with columnar, clear cell and oncocytoid features [1]. Salivary MEC as a malignant glandular epithelial neoplasia is believed to arise from pluripotent reserve cells of the excretory ducts that are capable of differentiating into such variety of cell types [2]. Exposure to ionizing radiation [3–4] and MECT1-MAML2 translocation and somatic TP53 and POU6F2 mutations [5] seems to be the most important factors involved in the pathogenesis of such salivary gland tumor.

Another important feature of salivary MEC is its variety of biological behaviors that generally is correlated with the histological grade of the tumor. However, there are still many controversies in defining the high-grade MEC variant, none of the proposed MEC grading systems being widely accepted [6–8]. Most studies indicate for patients with salivary MEC a favorable outcome, death being reported in cases with high-grade tumors because of distant metastasis [8, 9].

We report here a case of right parotid MEC developed in a 63-year-old female. A comprehensive immunohistochemical study was performed concerning the local aggressive behavior of this tumor. Written informed consents were obtained from the patient for publication and images usages.

Case presentation

A 63-year-old female presented to our institution with painless swelling in right parotid region for one year, which was slowly grown to the present size. Extraoral examination revealed a mild, firm, oval swelling of about 3 cm in size at the right preauricular area. Patient did not present lymphadenopathy and neither facial nerve involvement. Also, its personal or family records were not significant. Ultrasound examination revealed a solitary mass in the superficial lobe of the right parotid gland with dimensions of 27×20 mm, with heterogeneous echotexture, with peripheral intense vascularization, irregular shape, and with distal acoustic enhancement divided by a vascularized septum (Figure 1, A and B).

A provisional diagnosis of a salivary gland tumor with a potential malignant behavior was considered. Under general anesthesia, following a modified Blair incision a superficial parotidectomy with facial nerve preservation was performed. The tumor was completely excised with negative margins.

On gross examination, a whitish gray, firm mass of 4×2.5×2 cm was identified. On cut section, it appeared as solid mass with poorly defined edges that in the central part had a mucin-filled cyst of 0.7 cm diameter surrounded by other smaller cystic structures.

Histopathological examination

Microscopic examination of the tumor revealed a mixture of malignant epithelial cells with different morphology, respective neoplastic cells with squamoid differentiation, mucous cells (most of goblet-like type), "intermediate" cells, clear cells, columnar apocrine-like cells and sebaceous-like cells (Figure 2, A–F). The neoplastic cells had moderate cellular pleomorphism and rare mitotic figures.

The prevalent neoplastic cell type was the "intermediate" and clear cell types mostly with solid island growth pattern (Figure 3, A and B). However, in the center we noticed a large flattened cavity with eosinophilic, secretory material filling the lumen, lined by varying number of cell layers of variable thickness (Figure 3C). Also, adjacent to it were present few small cystic structures with tick lining noeplastic epithelium. The Periodic Acid–Schiff (PAS) and Alcian blue stainings show the intra- and extracellular mucin secretion (Figure 3, D and E).

One of the histopathological peculiarities of this case was the existence of a large number of neoplastic clear cells admixed with the "intermediate" cells and those with epidermoid feature in the form of variably sized, irregular or rounded cellular aggregates. The clear cells were only focally positive to PAS staining.

Another peculiar histological feature was the presence of an extensive central sclerosis that enclosed the cystic and solid epithelial neoplastic proliferation (Figure 4A). Also, a dense hyalinized fibrous tissue was seen at the tumor periphery along with a rich lymphoid infiltrate with follicles and even formation of germinal centers (Figure 4B).

The tumor edges were infiltrative with invasion of the adjacent adipose and striated muscle tissues, but without perineural and lymphovascular invasion.

Taken together all these histological aspects led us to classify our case as a sclerosing MEC subtype with intermediate grade malignancy.

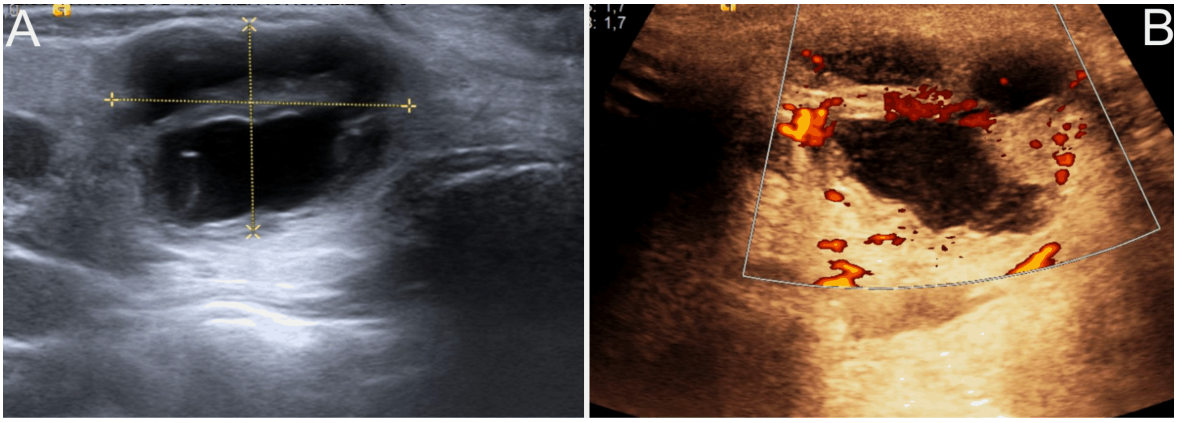

Figure 1 – ***Ultrasonography in B-mode (A) and color Doppler mode (B): a solitary mass in the superficial lobe of the right parotid gland of 27×20 mm dimensions with heterogeneous echotexture, with peripheral intense vascularization, irregular shape, with distal acoustic enhancement divided by a vascularized septum.***

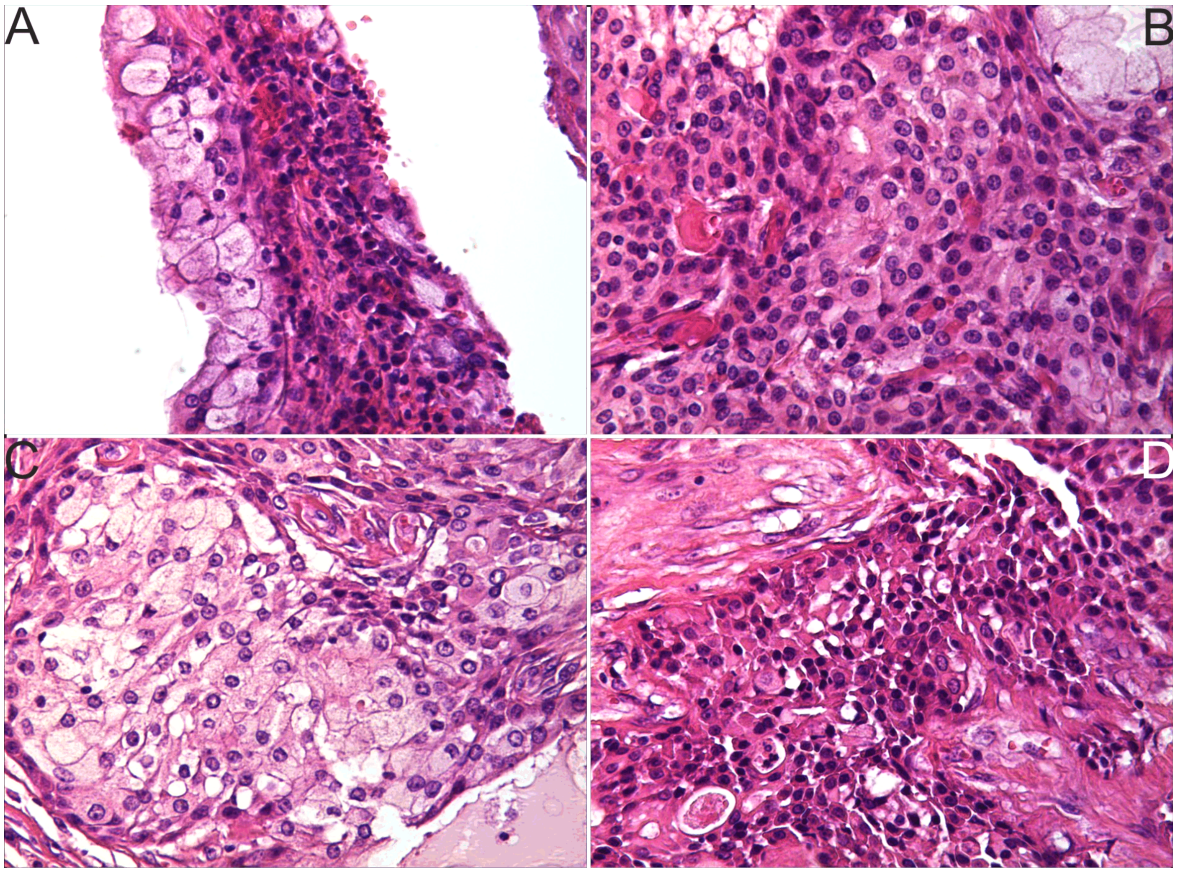

Figure 2 – ***The main histomorphological features of the tumor. The tumor was composed of a mixture of malignant epithelial cells with different morphology: (A) mucous cells (most of goblet-like type), (B) neoplastic cells with squamoid differentiation, (C) clear cells and (D) "intermediate" cells. Hematoxylin–Eosin (HE) staining, ×200.***

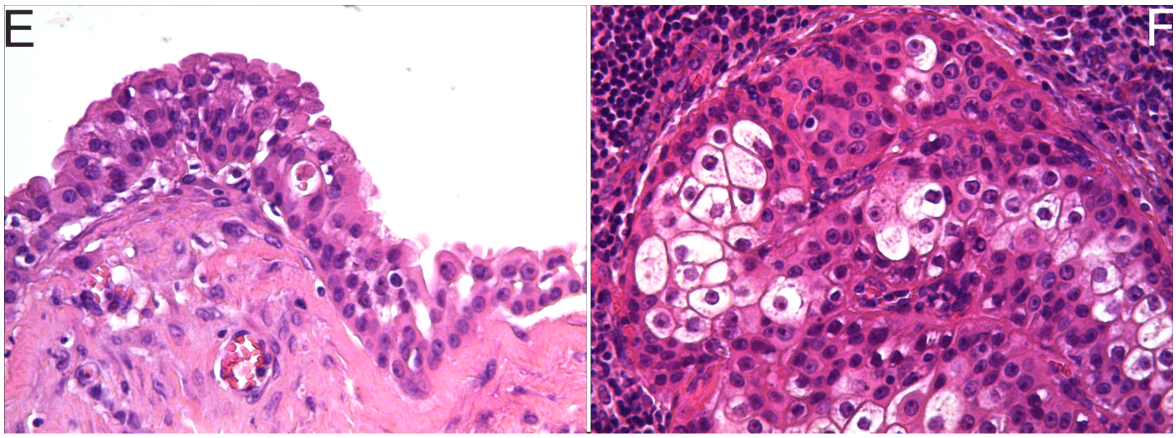

Figure 2 (continued) – ***The main histomorphological features of the tumor. The tumor was composed of a mixture of malignant epithelial cells with different morphology: (E) columnar apocrine-like cells and (F) sebaceous-like cells. HE staining, ×200.***

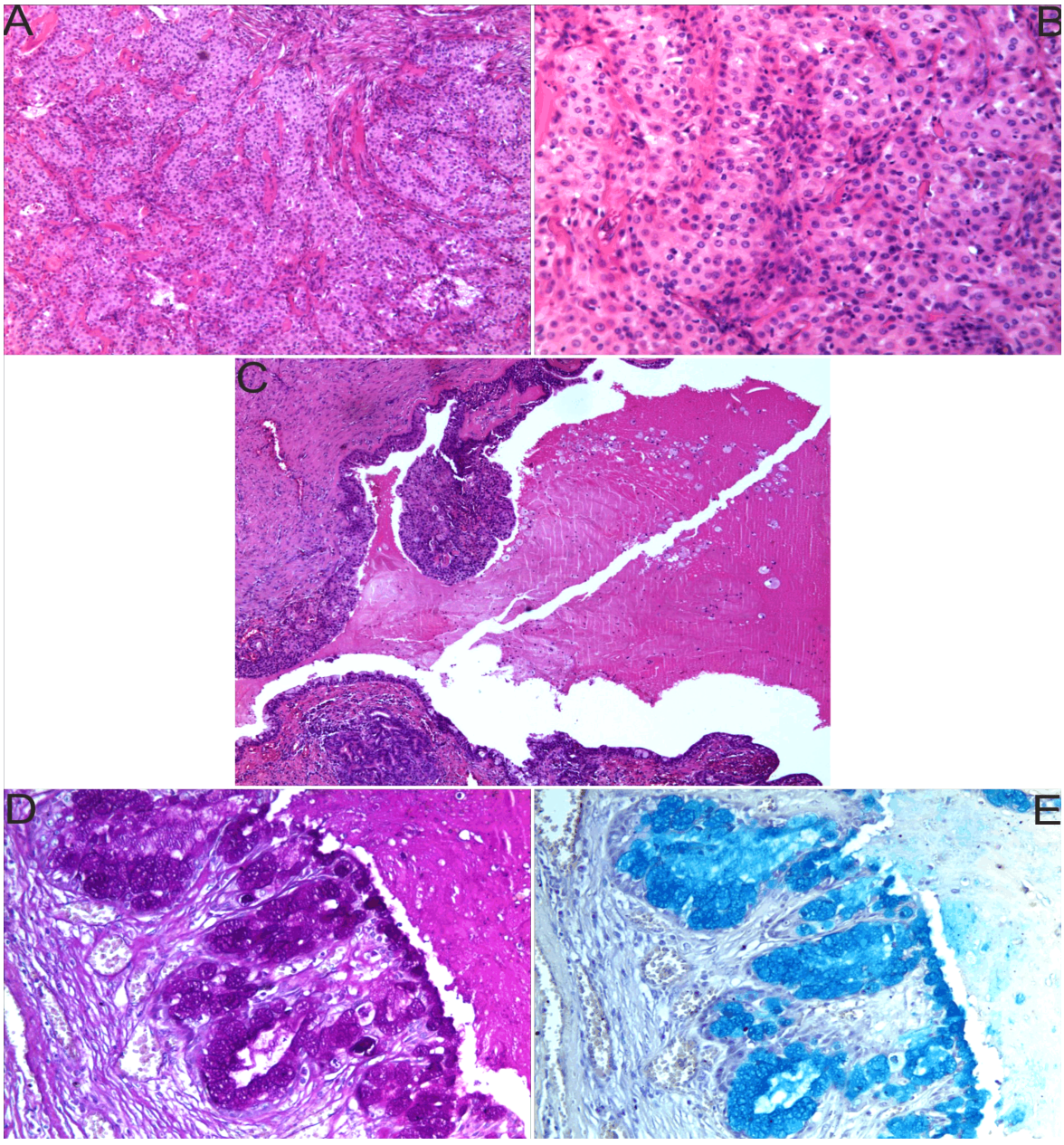

Figure 3 – ***The main histomorphological features of the tumor (continuation): Neoplastic epithelial cells were arranged in solid island (A and B) and a central large cyst (C) (composed of four images of original magnification ×40); (D and E) Some neoplastic cells presented intracellular mucin secretion, mucin that were also present in the cystic lumina. HE staining: (A and C) ×40; (B) ×200. PAS and Alcian blue stains: (D and E) ×100.***

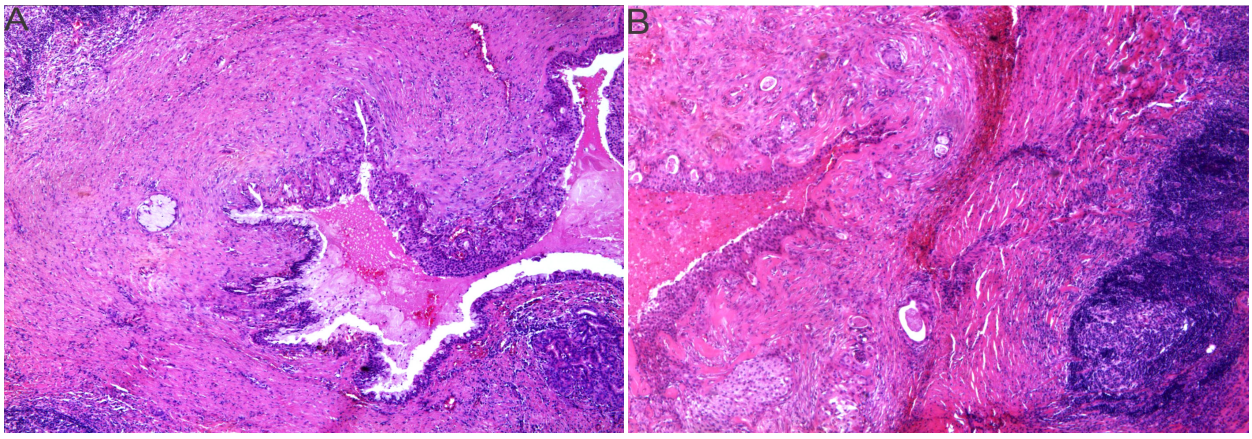

Figure 4 – ***Peculiar histological features of the tumor (continuation) (HE staining): (A) An extensive central sclerosis that enclosed the cystic and solid epithelial neoplastic proliferation (composed of four images of original magnification ×40); (B) A dense hyalinized fibrous tissue was also seen at the tumor periphery along with a rich lymphoid infiltrate with follicles and even germinal centers formation (composed of four images of original magnification ×40).***

Immunohistochemical findings

The mucous and columnar neoplastic cells from our case showed an immunoprofile specific to glandular phenotype by expressing CK7 (Figure 5A), CK8/18, and CEA (Figure 5B). Also, these cells were intensely positive to EMA (Figure 5C) and variable positive for MUC5AC (Figure 5D), CK5 and p63. The "intermediate" and epidermoid neoplastic cells presented variable reactivity for EMA (Figure 5E), CK5 (Figure 5F), p63 and vimentin, and in addition the "intermediate cells" were also positive for CK7 and CK8/18. Also, the neoplastic clear cells had a phenotype close to the epidermoid differentiation with variable positivity for EMA, CK5 and p63. None of the malignant cells showed reactivity for α-SMA and MUC2.

In order to evaluate its malignancy, we investigated tumor cell immunoreactivity for a number of markers used as prognostic markers in other known human malignancies, as listed in Table 1.

Table 1 – ***Characteristics of the antibodies utilized in the study***

Antibody	Clone	Manufacturer	Dilution	Staining pattern
CK5/6	D5/16 B4	Dako	1:50	Cytoplasmic
CK7	OV-TL 12/30	Dako	1:50	Cytoplasmic
CK8/18	EP17/EP30	Dako	1:50	Cytoplasmic/membranous
Vimentin	SP20	Thermo Fisher Scientific, Waltham, USA	1:200	Cytoplasmic
P63	7JUL	Leica Biosystems	1:25	Nuclear
α-SMA	1A4	Dako	1:40	Cytoplasmic
CEA	II-7	Dako	1:50	Cytoplasmic/apical border
EMA	E29	Dako	1:50	Cytoplasmic/apical luminal membrane
MUC2	CCP58	Dako	1:50	Cytoplasmic
MUC5AC	CLH2	Dako	1:50	Cytoplasmic/perinuclear
MMP-9	2C3	Santa Cruz Biotechnology	1:25	Nuclear/cytoplasmic
VEGF	VG1	Dako	1:50	Cytoplasmic/membranous
VEGFR2	A-3	Santa Cruz Biotechnology	1: 50	Cytoplasmic/membranous
EGFR	H11	Dako	1:100	Membranous/cytoplasmic
Her2/neu	Polyclonal	Dako	1:100	Membranous
ER	1D5	Dako	1:60	Nuclear
PgR	PgR 636	Dako	1:50	Nuclear
CD105	OTI3H5	Acris Antibodies GmbH	1:100	Membranous
CD34	QBEnd 10	Dako	1:50	Membranous
CD117	Polyclonal	Dako	1:400	Membranous, cytoplasmic
Ki-67	MIB-1	Dako	1:50	Nuclear
P53	DO-7	Dako	1:50	Nuclear
Bcl-2	124	Dako	1:50	Cytoplasmic
E-cadherin	NCH-38	Dako	1:50	Membranous/cytoplasmic
N-cadherin	6G11	Dako	1:50	Membranous, cytoplasmic, nuclear
CD44	DF1485	Dako	1:50	Membranous, cytoplasmic
CXCR4	Polyclonal	Thermo Scientific	1:500	Membranous, cytoplasmic, nuclear
Galectin-3	9C4	Leica Biosystems	1:30	Nuclear, cytoplasmic

CK: Cytokeratin; α-SMA: α-Smooth muscle actin; CEA: Carcinoembryonic antigen; EMA: Epithelial membrane antigen; MUC: Mucin; MMP: Matrix metalloproteinase; VEGF: Vascular endothelial growth factor; VEGFR: Vascular endothelial growth factor receptor; EGFR: epidermal growth factor receptor; ER: Estrogen receptor; PgR: Progesterone receptor; CXCR: Chemokine receptor.

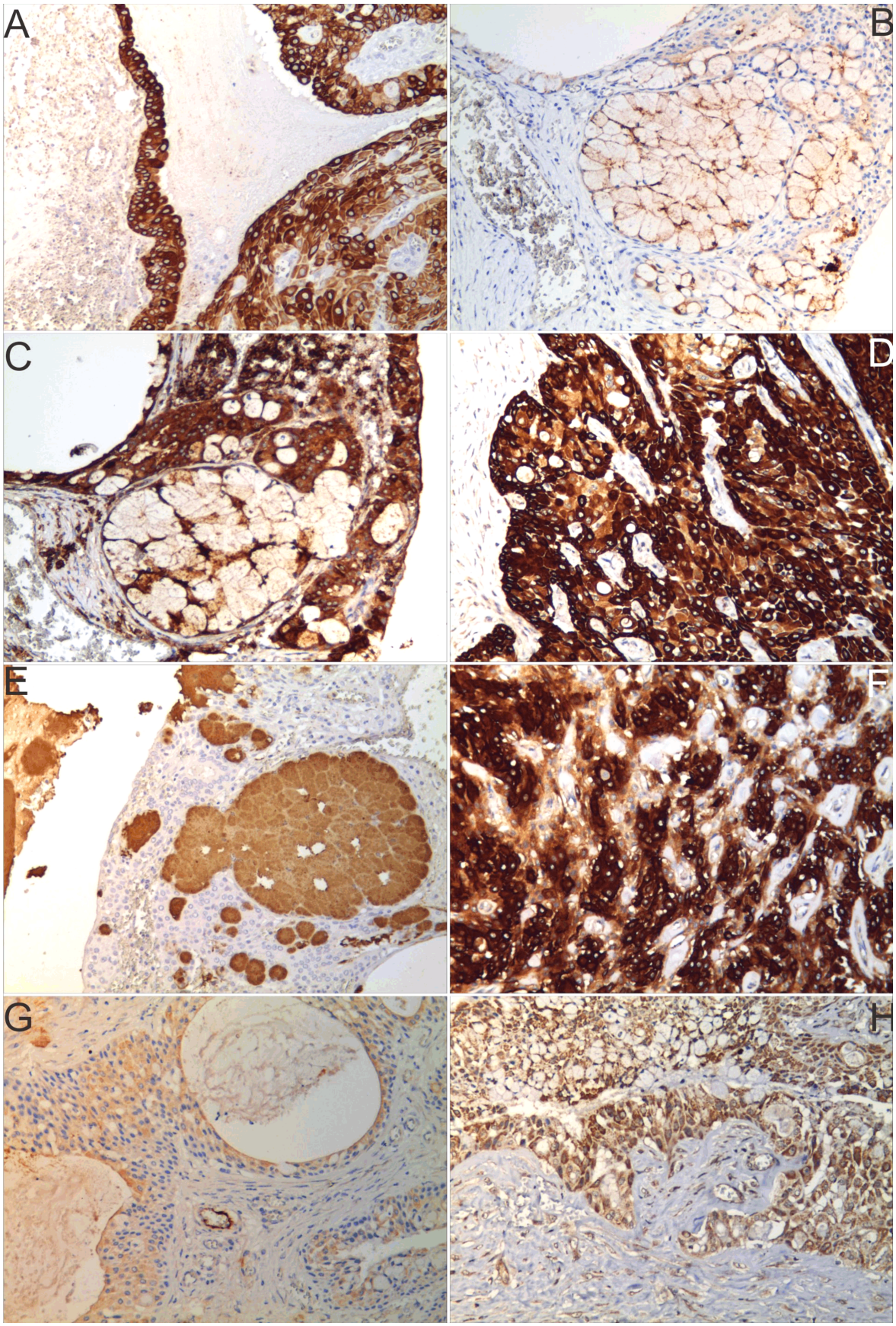

Figure 5 – ***Immunohistochemical patterns of the tumor: (A and B) Neoplastic epithelial cells positive to CK7 and CEA most of them being of mucous or "intermediate" type (immunostaining, ×100); Variable reactivity of tumor cells to EMA (C and D) and respective to MUC5AC (E) mostly being of mucous type (immunostaining, ×100); (F) CK5 was intense positive especially in the intermediate and squamous neoplastic cells type (immunostaining, ×100); (G and H) A weak to moderate positive reaction for VEGF and VEGFR2 was observed in neoplastic cells regardless their morphology (immunostaining, ×100).***

Screening for tumor reactivity to the main growth factor receptors and their ligands we found a weak to moderate positive reaction for VEGF and VEGFR2 (Figure 5, G and H), a similar reactivity for EGFR (Figure 6A) but a negative reaction for Her2/neu, ER and PgR. The VEGFR2 was also positive at the level of endothelial cells and VEGF was also identified in tumor associated stromal cells (Figure 5F).

Regarding the involvement of protein that regulates the cell cycle we found a weak to moderate nuclear reactivity to p53 in more than 50% of tumor cells (Figure 6B) and also a weak cytoplasm reaction to Bcl-2 in the majority of tumor cells compared to more intense reaction of the lymphoid infiltrate from the tumor periphery. The Ki-67 mitotic index was low with less than 5% of tumor cells positive to this marker, without any significant difference regarding the tumor topography.

Investigation of tumor angiogenesis with CD105 and CD34 highlighted the existence of intense active process especially at the tumor periphery (26.43±8.6 *versus* 12.53± 9.3 for CD105 and 41.43±11.6 *versus* 21.35±13.7 for CD34) (Figure 6C). The CD117 staining proved the existence of a great number of mast cells at the tumor periphery, their density being correlated with the microvessels density.

Investigation of markers involved in matrix remodeling and consequently in tumor invasiveness revealed increase reactivity especially for MMP-9, more obviously in cytoplasm of "intermediate" neoplastic cells, especially from the solid proliferation from the tumor periphery (Figure 6D).

Investigation of markers involved in cell adhesion showed a positive reaction with prevailing membranous pattern in the tumor cells for E-cadherin (Figure 6E) and CD44, a more obvious the reactivity being present at the level of neoplastic mucous cells and diminished in the solid proliferations, especially form the tumor edges. In addition, at the tumor advancing edge, the small groups or cords of infiltrating tumor cells were cytoplasm and nuclear positive to N-cadherin.

The chemokine receptor 4 (CXCR4) reactivity was also noticed in tumor cells, with prevailing membrane and cytoplasm pattern (Figure 6F). In addition, we observed intense nuclear and cytoplasmic tumor cells' reactivity for galectin-3, staining that was also noticed in tumor stromal cells (endothelial cells, cancer-associated fibroblasts, myofibroblasts, and mesenchymal stromal cells).

A final diagnosis of sclerosing MEC of intermediate grade malignancy was made. After six months of follow-up, at the time of publication, the patient did not present any clinical or radiographic evidences of recurrence.

Discussion

MECs account for approximately 30% of all malignant tumors of the salivary gland being the most common salivary malignancy in adults and children [1, 10]. The literature data report a 3:2 female predilection with 45 years as the mean patient age for such malignant salivary gland tumor [1, 11]. More than 50% of MECs occur in major glands, the parotid glands being the most affected, while the palate and retromolar area of the mandible are the most commonly involved site for the minor salivary glands [1, 7]. Our case fits in the general epidemiological profile of such salivary tumors, the tumor affecting the right parotid of a 63-year-old female and was the 5th reported case from our Hospital casuistry since 1995.

It seems to have been first described in the literature by Masson & Berger as "epithélioma à double métaplasie" [12]. Subsequently, in 1945, Stewart *et al.* introduced the term "mucoepidermoid tumor" [13]. However, because over time it has been proved that all salivary mucoepidermoid tumors had malignant potential this term was replaced in 1996 by Ellis & Auclair with the term "mucoepidermoid carcinoma" [14].

Our case of parotid MEC presented as a painless, soft mass of 3 cm in diameter that developed during about one year. The literature data shows that the major salivary glands MECs usually had a mean duration of 1.5 years, presenting as solitary painless masses, with variable skin or deeper tissue fixation [10, 15, 16]. Generally, regardless of the MECs location only 13% of patients experience associated pain [10]. Usually, high-grade MEC presents as a rapidly enlarging mass with variable fixation to the surrounding tissues, facial nerve paralysis, and tumor ulceration.

In terms of tumor grading, our case was classified as intermediate grade MEC being composed of few and small neoplastic cysts and solid islands of predominant intermediate neoplastic cells. The neoplastic cells had moderate cellular pleomorphism and rare mitotic figures. Tumor stroma was predominantly of fibrous type and at the periphery presented a chronic inflammatory reaction with lymphoid aggregates formation. Also, we noticed tumor invasion of the adjacent adipose and striated muscle tissues, but without perineural and lymphovascular invasion.

Although, there is no uniformly accepted grading system for MEC, it is generally accepted that a three- level grading approach to classify these tumors it is absolutely necessary to evaluate their prognosis and to establish the most appropriate therapeutic approach. Currently, the salivary MECs are graded as low-, intermediate- and high-grade tumors investigating a series of parameters such as cytomorphologic and architectural aspects, the degree and patterns of invasion, perineural and angiolymphatic invasion, necrosis and mitotic rate [17–19]. However, these grading systems are not infallible considering the fact that the *Armed Forces Institute of Pathology* (AFIP) system [19] tends to downgrade tumors while the Brandwein system [18] seems to upgrade tumors, with an obvious impact on therapeutic attitude to be followed in case of such patients [20].

As histological variant, our case was classified as sclerosing MEC since we noticed extensive central sclerosis that enclosed the cystic and solid epithelial neoplastic proliferation and at the tumor periphery was present a lymphoid infiltrate with follicles and even germinal centers formation. According to the literature data, this MEC variant is very rare with no more than 20 cases that almost exclusively were reported in the major salivary glands [21]. As possible mechanisms of this reactive fibrosis were proposed: (1) tumor infarction and (2) host tissue reaction due to extravasated mucin [22, 23]. Most

of the reported cases where classified as low-grade MEC, but at least in two cases the presence of metastasis were noticed [24]. Other histological variants of MEC described in the literature are: clear cells, oncocytic, sebaceous, spindle cell and with areas mimicking thyroid follicles [1, 18, 19, 25]. Given this variety of histopathological aspects becomes clear that many times the accurate diagnosis of such an entity requires the exclusion of other salivary gland tumors with similar histological aspects such as: squamous cell carcinoma adenosquamous carcinoma, cystadenoma, cystadenocarcinoma, sebaceous carcinoma, and other clear cell tumors such as acinic cell carcinoma, hyalinizing clear cell carcinoma, clear cell oncocytoma, and metastatic renal cell carcinoma [1, 7, 10].

Although it is well known that salivary MEC is an aggressive malignancy, the prognostic factors as well as treatment strategies remain controversial [26–28]. Throughout time there were proposed many prognostic factors for patients with MECs such as: sex, age, tumor location, clinical stage, tumor grade, lymph node status, surgical margins, postoperative radiotherapy and some molecular markers [7, 10, 26, 29].

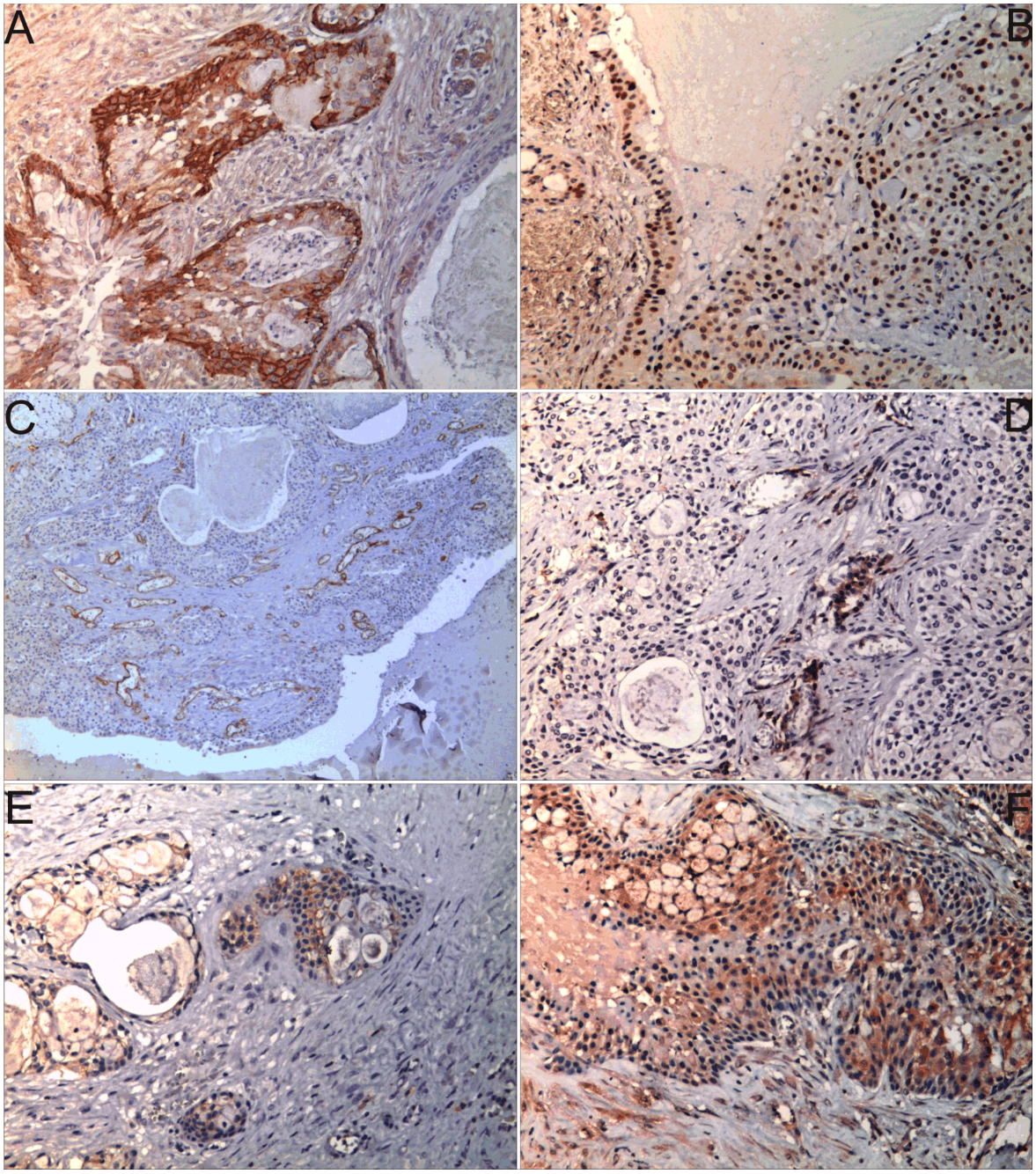

Figure 6 – ***Immunohistochemical patterns of the tumor (continuation): (A) A moderate reactivity mainly in the intermediate and squamous neoplastic cells type was noticed for EGFR (immunostaining, ×100); (B) p53 was positive in more than 50% of tumor cells regardless their morphology (immunostaining, ×100); (C) CD105 positive reaction especially at the tumor periphery as a proof of an intensely active angiogenic process (immunostaining, ×40); (D) A moderate reactivity for MMP-9 noticed mainly at the tumor periphery in the cytoplasm of intermediate and squamous neoplastic cells (immunostaining, ×100); (E) The membranous E-cadherin pattern diminished in the solid proliferations especially form the tumor edges (immunostaining, ×100); (F) A moderate membrane and cytoplasm chemokine receptor 4 (CXCR4) reactivity was also noticed in tumor cells (immunostaining, ×100).***

As we mentioned, our MEC case developed at a 63-year-old female in the superficial lobe of right parotid, presented with a clinical stage II and the pTNM stage III, without perineural and lymphovascular invasion, but with the adjacent adipose and striated muscle tissues invasion, and with tumor free margins. The literature data showed a poor prognosis of MEC among men at older ages (more than 40–50 years old) [19, 30, 32], with submandibular gland involvement [11, 26], with high-grade tumors, in advanced clinical stages (III/IV stage) with lymph node metastasis and positive surgical margins [26, 27, 31–33].

In the last decades, researchers have focused on finding prognostic biomarkers that could guide the therapeutic management in order to increase the survival time of these patients. Thus, investigating the mucin profile of these tumors revealed that high MUC1 expression associated a high histological grade, high rate of recurrence and metastasis and short disease-free interval, while MUC4 expression was related to low grade, low recurrence rate and a long disease-free interval [29, 34]. Another independent prognostic factor for MEC is the Ki-67 index, values higher than 10% suggesting an unfavorable prognosis, associated with high recurrence and metastasizing disease [35]. High Bcl-2 expression was related to low-grade tumors [36], while cyclooxygenase-2 expression was reported to be high in MECs with lymph node metastasis [37]. Also, p27, a universal cyclin-dependent kinase inhibitor, was proved to correlate inversely with histological grade of MEC of the intraoral minor salivary gland and was selected as an independent risk factor for both disease-free and overall survivals [38]. p53 expression is not only considered as an early event in MEC carcinogenesis but also correlates to tumor behavior and local recurrence, high expression being related to high grade tumors [39].

Regarding salivary MECs reactivity to growth factor receptors, their ligands and their prognostic significance the results are inconsistent. Thus, while some studies have shown an association between EGFR and high-grade MECs [40, 41], others reported its overexpression in low-grade tumors [42]. Also, Her2/neu has been shown to be an indicator of poor prognosis in MECs, independent of histological grade, and T or N status [43]. Even if it was shown that this receptor is expressed in less than 20% of MEC cases [44], Her2 and EGFR genomic alterations play a key role in the development of high-grade MEC, favoring also the progression from MAML2 fusion-positive low-/intermediate-grade to high-grade in a subset of MEC [45]. However, it has been proved that activated protein kinase intracellular molecules (phosphorylated ERK-1/ERK-2) expression in MECs did not correlate with Her2/neu or histological grading, but its high reactivity was associated with worse prognosis and high proliferative activity (Ki-67 index) [46]. TGF-β1 was proved to play a key role in the progression of MECs by increased MMP-9 activation in the carcinomatous cells, suggesting that these patients could benefit from targeted therapies blocking effectors of this signaling pathway [47].

On the other hand, although hormonal therapy has been shown to be useful in treatment of breast and prostate cancers, studies regarding ER, PgR and AR (androgen receptor) expression and their prognostic implications in salivary gland malignancies are inconsistent. Thus, concerning ER reactivity in MECs it was showed that three of 10 cases studied by Jeannon *et al.* were positive [48], while Nasser *et al.* reported only one of 10 MEC as being positive for this marker [49] and other authors did not reveal any ER expression in MEC cases [50]. For PgR reactivity, Jeannon *et al.* reported that only one from the 10 studied cases was positive [48], while other authors did not find any reactivity for this hormone receptor in MEC cases [49, 50]. Regarding AR expression, Nasser *et al.* revealed that two of 10 MEC were positive [49], while Ito *et al.* found only two positive cases from 30 MECs [50].

Several studies reported a higher rate of angiogenesis and cellular proliferation in malignant tumors compared to benign tumors, with highest vascularization in the MECs [51, 52]. This fact may reflect the invasiveness potential of such tumors and thus the usefulness of monoclonal anti-human CD105 antibodies in MEC anti-angiogenetic therapy [53].

Moreover, Uchida *et al.*, found CXCR4 overexpression in MECs suggesting its contribution to the metastatic potential of such tumors [54]. Also, galectin-3 expression was related to a more aggressive behavior of salivary glands malignancies, including MECs [55].

Summarizing, our immunohistochemical results have showed a tumor immunoprofile that certified its locally aggressive behavior. Thus, we found an intercellular adhesion loss especially at the tumor periphery with an increased MMP-9 reactivity both for tumor cells and tumor stromal cells at this level. Also, we have proved that at the tumor periphery there was an intense angiogenic process sustained by high value of CD105+ microvessel density and high VEGF and VEGFR2 tumor cell reactivity. In addition, we noticed high tumor CXCR4 reactivity especially at the tumor edges, and also galectin-3 reactivity was observed both in tumor cells and stromal tumor cells.

Conclusions

Here we report a rare case of a parotid sclerosing mucoepidermoid carcinoma with peculiar clinical and morphological characteristic features, reasons that may pose serious diagnosis problems for clinicians and even pathologists. The histopatological and immunohistochemical examinations revealed its intermediate grade malignant behavior highlighting the usefulness investigation of some prognostic markers for such salivary gland tumors.

Conflict of interests

The authors declare no conflict of interests.

Author contribution

All authors contributed equally to the study and the publication.

References

[1] Goode RK, El-Naggar AK. Mucoepidermoid carcinoma. In: Barnes L, Eveson JW, Reichart P, Sidransky D (eds). Pathology and genetics of head and neck tumours. World Health Organization (WHO) Classification of Tumours, International Agency for Research on Cancer (IARC) Press, Lyon, 2005, 219–220.

[2] Batsakis JG. Salivary gland neoplasia: an outcome of modified morphogenesis and cytodifferentiation. Oral Surg Oral Med Oral Pathol, 1980, 49(3):229–232.

[3] Saku T, Hayashi Y, Takahara O, Matsuura H, Tokunaga M, Tokunaga M, Tokuoka S, Soda M, Mabuchi K, Land CE. Salivary gland tumors among atomic bomb survivors, 1950–1987. Cancer, 1997, 79(8):1465–1475.
[4] Whatley WS, Thompson JW, Rao B. Salivary gland tumors in survivors of childhood cancer. Otolaryngol Head Neck Surg, 2006, 134(3):385–388.
[5] Kang H, Tan M, Bishop JA, Jones S, Sausen M, Ha PK, Agrawal N. Whole-exome sequencing of salivary gland mucoepidermoid carcinoma. Clin Cancer Res, 2016, Jun 23.
[6] Boahene DK, Olsen KD, Lewis JE, Pinheiro AD, Pankratz VS, Bagniewski SM. Mucoepidermoid carcinoma of the parotid gland: the Mayo clinic experience. Arch Otolaryngol Head Neck Surg, 2004, 130(7):849–856.
[7] Coca-Pelaz A, Rodrigo JP, Triantafyllou A, Hunt JL, Rinaldo A, Strojan P, Haigentz M Jr, Mendenhall WM, Takes RP, Vander Poorten V, Ferlito A. Salivary mucoepidermoid carcinoma revisited. Eur Arch Otorhinolaryngol, 2015, 272(4):799–819.
[8] Katabi N, Ghossein R, Ali S, Dogan S, Klimstra D, Ganly I. Prognostic features in mucoepidermoid carcinoma of major salivary glands with emphasis on tumour histologic grading. Histopathology, 2014, 65(6):793–804.
[9] Ali S, Sarhan M, Palmer FL, Whitcher M, Shah JP, Patel SG, Ganly I. Cause-specific mortality in patients with mucoepidermoid carcinoma of the major salivary glands. Ann Surg Oncol, 2013, 20(7):2396–2404.
[10] Luna MA. Salivary mucoepidermoid carcinoma: revisited. Adv Anat Pathol, 2006, 13(6):293–307.
[11] Spiro RH. Salivary neoplasms: overview of a 35-year experience with 2,807 patients. Head Neck Surg, 1986, 8(3):177–184.
[12] Masson P, Berger L. Epithélioma à double métaplasie de la parotide. Bull Ass Fr Étud Cancer, 1924, 13:366–373.
[13] Stewart FW, Foote FW, Becker WF. Muco-epidermoid tumors of salivary glands. Ann Surg, 1945, 122(5):820–844.
[14] Ellis GL, Auclair PL. Tumor of the salivary glands. In: ***. Atlas of tumor pathology. Armed Forces Institute of Pathology (AFIP), Washington, D.C., 1996, 155–175, 353–355.
[15] Guzzo M, Andreola S, Sirizzotti G, Cantu G. Mucoepidermoid carcinoma of the salivary glands: clinicopathologic review of 108 patients treated at the National Cancer Institute of Milan. Ann Surg Oncol, 2002, 9(7):688–695.
[16] Spiro RH, Huvos AG, Berk R, Strong EW. Mucoepidermoid carcinoma of salivary gland origin. A clinicopathologic study of 367 cases. Am J Surg, 1978, 136(4):461–468.
[17] Batsakis JG, Luna MA. Histopathologic grading of salivary gland neoplasms: I. Mucoepidermoid carcinomas. Ann Otol Rhinol Laryngol, 1990, 99(10 Pt 1):835–838.
[18] Brandwein MS, Ferlito A, Bradley PJ, Hille JJ, Rinaldo A. Diagnosis and classification of salivary neoplasms: pathologic challenges and relevance to clinical outcomes. Acta Otolaryngol, 2002, 122(7):758–764.
[19] Goode RK, Auclair PL, Ellis GL. Mucoepidermoid carcinoma of the major salivary glands: clinical and histopathologic analysis of 234 cases with evaluation of grading criteria. Cancer, 1998, 82(7):1217–1224.
[20] Seethala RR. An update on grading of salivary gland carcinomas. Head Neck Pathol, 2009, 3(1):69–77.
[21] Lohiya PG, Chaudhary MS, Patil S, Agrawal SA. Sclerosing mucoepidermoid carcinoma of minor salivary gland. Contemp Clin Dent, 2014, 5(4):564–568.
[22] Shinhar SY. Sclerosing mucoepidermoid carcinoma of the parotid gland: case report. Ear Nose Throat J, 2009, 88(11): E29–E31.
[23] Urano M, Abe M, Horibe Y, Kuroda M, Mizoguchi Y, Sakurai K, Naito K. Sclerosing mucoepidermoid carcinoma with eosinophilia of the salivary glands. Pathol Res Pract, 2002, 198(4): 305–310.
[24] Fadare O, Hileeto D, Gruddin YL, Mariappan MR. Sclerosing mucoepidermoid carcinoma of the parotid gland. Arch Pathol Lab Med, 2004, 128(9):1046–1049.
[25] Regezi JA, Sciubba JJ, Jordan CKR. Salivary gland diseases. In: Regezi JA, Sciubba JJ, Jordan CKR (eds). Oral pathology, clinical pathologic correlations. 5th edition, Saunders/Elsevier, Indian Reprint, Philadelphia, 2009, 203–204.
[26] Liu S, Ow A, Ruan M, Yang W, Zhang C, Wang L, Zhang C. Prognostic factors in primary salivary gland mucoepidermoid carcinoma: an analysis of 376 cases in an Eastern Chinese population. Int J Oral Maxillofac Surg, 2014, 43(6):667–673.
[27] Loh KS, Barker E, Bruch G, O'Sullivan B, Brown DH, Goldstein DP, Gilbert RW, Gullane PJ, Irish JC. Prognostic factors in malignancy of the minor salivary glands. Head Neck, 2009, 31(1):58–63.
[28] Nance MA, Seethala RR, Wang Y, Chiosea SI, Myers EN, Johnson JT, Lai SY. Treatment and survival outcomes based on histologic grading in patients with head and neck mucoepidermoid carcinoma. Cancer, 2008, 113(8):2082–2089.
[29] Namboodiripad PC. A review: immunological markers for malignant salivary gland tumors. J Oral Biol Craniofac Res, 2014, 4(2):127–134.
[30] Brandwein MS, Ivanov K, Wallace DI, Hille JJ, Wang B, Fahmy A, Bodian C, Urken ML, Gnepp DR, Huvos A, Lumerman H, Mills SE. Mucoepidermoid carcinoma: a clinicopathologic study of 80 patients with special reference to histological grading. Am J Surg Pathol, 2001, 25(7):835–845.
[31] Chen MM, Roman SA, Sosa JA, Judson BL. Histologic grade as prognostic indicator for mucoepidermoid carcinoma: a population-level analysis of 2400 patients. Head Neck, 2014, 36(2):158–163.
[32] McHugh CH, Roberts DB, El-Naggar AK, Hanna EY, Garden AS, Kies MS, Weber RS, Kupferman ME. Prognostic factors in mucoepidermoid carcinoma of the salivary glands. Cancer, 2012, 118(16):3928–3936.
[33] Pires FR, de Almeida OP, de Araújo VC, Kowalski LP. Prognostic factors in head and neck mucoepidermoid carcinoma. Arch Otolaryngol Head Neck Surg, 2004, 130(2):174–180.
[34] Handra-Luca A, Lamas G, Bertrand JC, Fouret P. MUC1, MUC2, MUC4, and MUC5AC expression in salivary gland mucoepidermoid carcinoma: diagnostic and prognostic implications. Am J Surg Pathol, 2005, 29(7):881–889.
[35] Skalova A, Lehtonen H, von Boguslawsky K, Leivo I. Prognostic significance of cell proliferation in mucoepidermoid carcinomas of the salivary gland: clinicopathological study using MIB 1 antibody in paraffin sections. Hum Pathol, 1994, 25(9):929–935.
[36] Yin HF, Okada N, Takagi M. Apoptosis and apoptotic-related factors in mucoepidermoid carcinoma of the oral minor salivary glands. Pathol Int, 2000, 50(8):603–609.
[37] Zyada MM, Grawish ME, Elsabaa HM. Predictive value of cyclooxygenase 2 and Bcl-2 for cervical lymph node metastasis in mucoepidermoid carcinoma. Ann Diagn Pathol, 2009, 13(5):313–321.
[38] Okabe M, Inagaki H, Murase T, Inoue M, Nagai N, Eimoto T. Prognostic significance of p27 and Ki-67 expression in mucoepidermoid carcinoma of the intraoral minor salivary gland. Mod Pathol, 2001, 14(10):1008–1014.
[39] Abd-Elhamid ES, Elmalahy MH. Image cytometric analysis of p53 and Mdm-2 expression in primary and recurrent mucoepidermoid carcinoma of parotid gland: immunohistochemical study. Diagn Pathol, 2010, 5:72.
[40] Khiavi MM, Vosoughhosseini S, Saravani S, Halimi M. Immunohistochemical correlation of epidermal growth factor receptor and c-erbB-2 with histopathologic grading of mucoepidermoid carcinoma. J Cancer Res Ther, 2012, 8(4):586–590.
[41] Lujan B, Hakim S, Moyano S, Nadal A, Caballero M, Diaz A, Valera A, Carrera M, Cardesa A, Alos L. Activation of the EGFR/ERK pathway in high-grade mucoepidermoid carcinomas of the salivary glands. Br J Cancer, 2010, 103(4):510–516.
[42] Suzuki S, Dobashi Y, Minato H, Tajiri R, Yoshizaki T, Ooi A. EGFR and HER2-Akt-mTOR signaling pathways are activated in subgroups of salivary gland carcinomas. Virchows Arch, 2012, 461(3):271–282.
[43] Press MF, Pike MC, Hung G, Zhou JY, Ma Y, George J, Dietz-Band J, James W, Slamon DJ, Batsakis JG, El-Naggar AK. Amplification and overexpression of HER-2/neu in carcinomas of the salivary gland: correlation with poor prognosis. Cancer Res, 1994, 54(21):5675–5682.
[44] Clauditz TS, Reiff M, Gravert L, Gnoss A, Tsourlakis MC, Münscher A, Sauter G, Bokemeyer C, Knecht R, Wilczak W. Human epidermal growth factor receptor 2 (HER2) in salivary gland carcinomas. Pathology, 2011, 43(5):459–464.
[45] Nakano T, Yamamoto H, Hashimoto K, Tamiya S, Shiratsuchi H, Nakashima T, Nishiyama K, Higaki Y, Komune S, Oda Y. HER2 and EGFR gene copy number alterations are predominant in high-grade salivary mucoepidermoid carcinoma irrespective of MAML2 fusion status. Histopathology, 2013, 63(3):378–392.

[46] Handra-Luca A, Bilal H, Bertrand JC, Fouret P. Extra-cellular signal-regulated ERK-1/ERK-2 pathway activation in human salivary gland mucoepidermoid carcinoma: association to aggressive tumor behavior and tumor cell proliferation. Am J Pathol, 2003, 163(3):957–967.
[47] Wang J, Chen J, Zhang K, Zhao Y, Nör JE, Wu J. TGF-*β*1 regulates the invasive and metastatic potential of mucoepidermoid carcinoma cells. J Oral Pathol Med, 2011, 40(10): 762–768.
[48] Jeannon JP, Soames JV, Bell H, Wilson JA. Immunohistochemical detection of oestrogen and progesterone receptors in salivary tumours. Clin Otolaryngol Allied Sci, 1999, 24(1): 52–54.
[49] Nasser SM, Faquin WC, Dayal Y. Expression of androgen, estrogen, and progesterone receptors in salivary gland tumors. Frequent expression of androgen receptor in a subset of malignant salivary gland tumors. Am J Clin Pathol, 2003, 119(6):801–806.
[50] Ito FA, Ito K, Coletta RD, Vargas PA, Lopes MA. Immunohistochemical study of androgen, estrogen and progesterone receptors in salivary gland tumors. Braz Oral Res, 2009, 23(4):393–398.
[51] Cardoso SV, Souza KC, Faria PR, Eisenberg AL, Dias FL, Loyola AM. Assessment of angiogenesis by CD105 antigen in epithelial salivary gland neoplasms with diverse metastatic behavior. BMC Cancer, 2009, 9:391.
[52] Tadbir AA, Pardis S, Ashkavandi ZJ, Najvani AD, Ashraf MJ, Taheri A, Zadeh MA, Sardari Y. Expression of Ki67 and CD105 as proliferation and angiogenesis markers in salivary gland tumors. Asian Pac J Cancer Prev, 2012, 13(10):5155–5159.
[53] Fonsatti E, Altomonte M, Nicotra MR, Natali PG, Maio M. Endoglin (CD105): a powerful therapeutic target on tumor-associated angiogenetic blood vessels. Oncogene, 2003, 22(42):6557–6563.
[54] Uchida D, Kuribayashi N, Kinouchi M, Ohe G, Tamatani T, Nagai H, Miyamoto Y. Expression and function of CXCR4 in human salivary gland cancers. Clin Exp Metastasis, 2013, 30(2):133–142.
[55] Ferrazzo KL, Neto MM, dos Santos E, dos Santos Pinto D, de Sousa SO. Differential expression of galectin-3, *β*-catenin, and cyclin D1 in adenoid cystic carcinoma and polymorphous low-grade adenocarcinoma of salivary glands. J Oral Pathol Med, 2009, 38(9):701–707.

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Received: February 1, 2016

Accepted: October 30, 2016