# Advances in Anatomic Pathology
## Clear Cell Neoplasms of Salivary Glands: A Diagnostic Challenge
### --Manuscript Draft--

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| Abstract: | Abstract.<br>This review focuses on the heterogenous group of clear cell neoplasms of salivary glands and attempts to identify major differential diagnostic features. Within the head and neck region, clear cells are found most commonly in salivary gland tumors, but may also be seen in tumors of squamous or odontogenic epithelial origin, primary or metastatic carcinomas, benign or malignant melanocytic lesions, or benign or malignant mesenchymal tumors. Clear cells occur fairly commonly among a wide variety of salivary gland neoplasms, but mostly they constitute only a minor component of the tumor cell population. Clear cells represent a major diagnostic feature in two salivary gland neoplasms, epithelial-myoepithelial carcinoma and hyalinizing clear cell carcinoma. In addition, salivary gland neoplasms composed predominantly of clear cells could also include clear cell variants of other salivary neoplasms, such as mucoepidermoid carcinoma and myoepithelial carcinoma, but their tumor type-specific histological features may only be available in limited non-clear cell areas of the tumor. Diagnosing predominantly clear cell salivary gland tumors is difficult because the immunoprofiles and morphological features may overlap and the same tumor entity may also have a wide range of other histologic presentations. Many salivary gland tumors are characterized by  tumor type-specific genomic alterations, particularly gene fusions of the  ETV6  gene in secretory carcinoma, the  MYB  and  MYBL1  genes in adenoid cystic carcinoma, the  MAML2  gene in mucoepidermoid carcinoma, the |

EWSR1 gene in hyalinizing clear cell carcinoma, and others. Thus, along with conventional histopathologic examination and immunoprofiling, molecular and genetic tests may be important in the diagnosis of salivary gland clear cell tumors by demonstrating genetic alterations specific to them.

Mahul B. Amin, M.D.
Editor-in-Chief
*Advances in Anatomic Pathology*

RE: AAP-22-002, entitled "Clear Cell Neoplasms of Salivary Glands: A Diagnostic Challenge"

Dear Dr. Mahul B. Amin,

Enclosed please find amended manuscript entitled "Clear Cell Neoplasms of Salivary Glands: A Diagnostic Challenge"

writen *by Drs. Ilmo Leivo, Henrik Hellquist, Roderick HW Simpson, Vincent Vander Poorten, Stefan M. Willems, Elaheh Mosaieby, David Slouka, Alfio Ferlito and myself.*

Thank you for your letter dated 01/25/2022. We are most grateful for all comments and suggestions regarding our paper. In the amended manuscript, all concerns of the reviewer have been addressed. In a separate sheet, we have replied.

This manuscript has not been published previously, and it is not under consideration in any other journal. The authors declare no conflicts of interest. Each author has contributed to the work.

I hope that the manuscript will meet the criteria requested by the Editor-in-Chief, and will be published in *Advances in Anatomic Pathology.*

With best wishes,

Sincerely,

Alena Skálová

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Reviewer Comments:

Thank you for your excellent review. The integration of morphology with clinical, IHC and molecular features is valuable. The paper is provisionally accepted for Advances provided you accommodate these minor changes. We request you to make these changes within a week or less so that the manuscript can be sent to the publishers.

**Minor comments.**

1. HCCC: a) HCCC is explained in long form and abbreviation in several places in the manuscript. b) Perhaps use hyalinizing CCC or No abbreviation at all as HCC and CCC can be mistaken for clear cell RCC and hepatocellular carcinoma which can have clear cell features

- *Thank you for the proposal, correction has been made through the manuscript, abbreviation HCCC was replaced by „hyalinizing CCC"*

2. "Benign" oncocytoma: Do you need the qualifier of benign? Suggest using WHO terminology.

- *We agree, „benign oncocytoma" was changed to „oncocytoma"*

3. Page 11: Are there any specific markers for Odontogenic clear cell carcinoma, presuming none, suggest adding the <u>underlined</u> to the text. Metastatic tumors containing clear cells include most likely renal cell carcinoma, clear cell breast carcinoma or thyroid carcinoma and, therefore, in addition to clinical history, the immunomarkers RCC, CD10, PAX8, CAIX, GATA3, ER/PR, TTF-1 are useful to rule metastatic tumors (61).

- *We agree, there are not any specific markers for odontogenic clear cell carcinoma, the sentence was added in the text and the suggested text was added-page 11*

4. Page 13: Add what is **<u>bold and underlined</u>** In addition, immunohistochemistry can help, as clear cell RCC rarely expresses CK7 or and virtually never is positive for p63, which is almost always expressed in HCCC.

- *The suggested sententence was added-page 13*

5. Is it possible to add a simple table with list of clear cell entities with key morphologic, molecular or IHC ancillary testing? Just a recommendation.

- *Table 1. was inserted in the introduction, page 4*

6. Recommend using mainly standard and approved abbreviations as much as possible.

- *Thank you for this comment, we have improved abbreviations*

**Clear Cell Neoplasms of Salivary Glands: A Diagnostic Challenge**

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*There are two senior authors of this manuscript (Ilmo Leivo and Alfio Ferlito), equal contribution

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**Running title:** Clear cell neoplasms in salivary glands

**Abstract.**

This review focuses on the heterogenous group of clear cell neoplasms of salivary glands and attempts to identify major differential diagnostic features. Within the head and neck region, clear cells are found most commonly in salivary gland tumors, but may also be seen in tumors of squamous or odontogenic epithelial origin, primary or metastatic carcinomas, benign or malignant melanocytic lesions, or benign or malignant mesenchymal tumors. Clear cells occur

fairly commonly among a wide variety of salivary gland neoplasms, but mostly they constitute only a minor component of the tumor cell population. Clear cells represent a major diagnostic feature in two salivary gland neoplasms, epithelial-myoepithelial carcinoma and hyalinizing clear cell carcinoma. In addition, salivary gland neoplasms composed predominantly of clear cells could also include clear cell variants of other salivary neoplasms, such as mucoepidermoid carcinoma and myoepithelial carcinoma, but their tumor type-specific histological features may only be available in limited non-clear cell areas of the tumor.

Diagnosing predominantly clear cell salivary gland tumors is difficult because the immunoprofiles and morphological features may overlap and the same tumor entity may also have a wide range of other histologic presentations. Many salivary gland tumors are characterized by tumor type-specific genomic alterations, particularly gene fusions of the *ETV6* gene in secretory carcinoma, the *MYB* and *MYBL1* genes in adenoid cystic carcinoma, the *MAML2* gene in mucoepidermoid carcinoma, the *EWSR1* gene in hyalinizing clear cell carcinoma, and others. Thus, along with conventional histopathologic examination and immunoprofiling, molecular and genetic tests may be important in the diagnosis of salivary gland clear cell tumors by demonstrating genetic alterations specific to them.

## 1. Introduction.

This review focuses on the heterogenous group of clear cell neoplasms of salivary glands and attempts to clarify some of the features that help to differentiate one neoplasm from another. Optically clear cells may be found as incidental histologic findings in any of a multitude of benign or malignant tumors from different cells of origin including epithelial, melanocytic, mesenchymal, or hematopoietic. They may be a result of many different processes, such as fixation artifact, degeneration of cellular organelles, or accumulation of substances within the cells - most commonly glycogen, but sometimes mucopolysaccharides, mucin, or lipids **(1).** Within the head and neck region, clear cells are found most commonly in salivary gland tumors, but may also be seen in tumors of squamous or odontogenic epithelial origin, primary or metastatic carcinomas, benign or malignant melanocytic lesions, or benign or malignant

mesenchymal tumors (**2**). Even metastatic clear cell tumors, such as renal cell carcinoma, can be difficult to differentiate from primary clear cell neoplasms of the salivary glands (**3, 4**).

Clear cells occur fairly commonly among a wide variety of primary salivary gland neoplasms, including pleomorphic adenoma (PA), myoepithelioma/myoepithelial carcinoma, oncocytoma, mucoepidermoid carcinoma (MEC), acinic cell carcinoma, epithelial-myoepithelial carcinoma (EMC), and adenoid cystic carcinoma (AdCC). In most cases, clear cells constitute only a minor component of the cell population of these neoplasms, and the appropriate classification of the tumors is easily established on the basis of typical features that are apparent.

In some tumors, however, clear cells constitute the major cellular component, and it is in this situation that the diagnostic challenge is greatest. Clear cells are usually the principal diagnostic feature in two salivary gland neoplasms, EMC and hyalinizing clear cell carcinoma (CCC). In addition, salivary gland neoplasms composed predominantly of clear cells could also include clear cell variants of well defined salivary neoplasms such as MEC, myoepithelial carcinoma (MC), myoepithelioma and oncocytoma, but their specific histological features can be hidden and only apparent in a limited non-clear cell component of the tumor.

Diagnosing predominantly clear cell salivary gland tumors is difficult because many different tumor types may share similar morphologic features, and the same tumor entity may have a wide range of other histologic presentations (**Table 1**). Moreover, the immunoprofiles of predominantly clear cell salivary tumors of different entities may overlap, such as p63/p40/HMW cytokeratin positive and S100/SOX10 negative immunostaining in hyalinizing CCC, MEC, and squamous cell carcinoma (SCC) (**5**).

Over the past decade, a significant development in molecular techniques and understanding of the genomic landscape of salivary gland neoplasms has taken place (**6-8**). Several salivary gland tumors were characterized by recurrent genomic alterations, including gene fusions involving the *ETV6* gene in secretory carcinoma (**9-11**), the *MYB* and *MYBL1* genes in adenoid cystic carcinoma (**12**), the *MAML2* gene in MEC (**13-14**), and the fusion *EWSR1::ATF1* in hyalinizing CCC (**15**). In addition, *HRAS* exon 3 mutations were seen in most cases of EMC (**16**), and rearrangement of the gene *EWSR1* was described in a significant proportion of clear cell myoepithelial carcinomas (**17-18**), respectively. Thus, along with conventional histologic examination and immunoprofiling, molecular and genetic tests can facilitate the diagnosis of salivary gland clear cell tumors by demonstrating genetic alterations specific to them.

## 2. Major primary clear cell neoplasms of salivary glands

### 2.1. Hyalinizing clear cell carcinoma

Hyalinizing CCC is a low-grade salivary gland malignancy that was originally described by Batsakis in 1980 **(19),** and the concept was later refined by Simpson et al **(20)** and Milchgrub et al **(21).** While it is possible that hyalinizing CCC rarely arises also in major salivary glands, it occurs most commonly in minor salivary glands, usually in the palate or the base of the tongue **(21)** **(Fig. 1).** Until recently, hyalinizing CCC was regarded not as a distinct entity but rather as a diagnosis of exclusion. Uncertainty about the true nature and identity of hyalinizing CCC was reflected in the frequently changing designations of this tumor type. Originally described as "clear cell carcinoma" **(19, 20)**, it was later designated as "hyalinizing clear cell carcinoma" **(21).** Then, in the 2005 World Health Organization Classification (WHO) of Head and Neck Tumors it was renamed as "clear cell carcinoma, not otherwise specified" **(22),** and in the 2008 Armed Forces Institute of Pathology Fascicle of Salivary Gland Tumors as "clear cell adenocarcinoma" **(23).** The term "clear cell carcinoma" then re-appeared in the 2017 WHO Classification of Head and Neck Tumors **(24)**, and finally the neoplasm is called "hyalinizing clear cell carcinoma" again in the upcoming 5th edition of WHO classification in 2022 **(25)**.

The discovery that most hyalinizing CCCs harbor *EWSR1::ATF1* fusion that is not found in any other type of salivary gland tumor, strongly supports the present view that hyalinizing CCC is a distinct tumor entity **(15).** Microscopically, hyalinizing CCC is typically composed of clear cells, arranged in anastomosing trabeculae, cords, nests, or solid sheets surrounded by a stroma of spindle-shaped fibroblasts and dense hypocellular hyalinized tissue sometimes with myxoid foci **(Fig. 2)**. Hyalinizing CCCs have invasive borders, even occasionally exhibiting perineural infiltration, and the tumor cells display minimal nuclear pleomorphism with a very low mitotic and proliferative index. Histologically, there is a relatively wide range of appearances of hyalinizing CCC tumor cells: whilst a predominance of clear cells is seen in most examples, sometimes a variable proportion of the tumor cells may have pale eosinophilic rather than clear cytoplasm. Generally however, many hyalinizing CCCs display a mixture of both cell types. Tumors with virtually no clear cells can occasionally be seen, but they usually retain the same overall growth pattern as typical clear cell examples of hyalinizing CCC **(5).** A very characteristic feature of hyalinizing CCC is the appearance of the stroma comprising abundant hyalinized basement membrane-like material. It is often sharply

demarcated from the desmoplastic or fibrocellular stroma that sometimes appears myxoid and may mimic a pleomorphic adenoma (**5**). The presence of these two stroma types is essentially pathognomonic for the diagnosis of hyalinizing CCC and is seen in most, but not all cases. The finding of hyalinized stroma in a tumor with a cribriform architecture may mimic other more common salivary gland tumors, such as AdCC and PA, particularly if the hyalinizing CCC has only a minor clear cell component.

The presence of a diagnostic molecular marker, the *EWSR1::ATF1* fusion, in hyalinizing CCC has allowed for a more complete appreciation of its histologic spectrum. This, in turn, has disclosed pitfalls in diagnosing carcinomas with clear cell morphology on the basis of histology alone (**Fig. 2**). For example, hyalinizing CCC sometimes exhibits overt squamous differentiation (**26**), and they may not be hyalinized, and they may not even be dominated by clear cells. Thus, without molecular confirmation, the diagnosis of hyalinizing CCC would hardly be possible (**5**). Immunophenotypically hyalinizing CCC has similarities to SCC and MEC with positive immunostaining for high–molecular-weight keratins and p63 and negative immunostaining for markers of myoepithelial differentiation. Focal mucinous differentiation is known to occur in up to 50% of cases of hyalinizing CCC (**27-28**). The distinction of hyalinizing CCC from clear cell mucoepidermoid carcinoma (MEC) can be truly challenging, and hyalinizing CCC may be misclassified as the more common MEC (**27-28**). In one recent study, the presence of an alternative *EWSR1::CREM* fusion in a salivary clear cell carcinoma with a very prominent mucinous component and an original diagnosis of MEC, finally convinced pathologists of a revised diagnosis of hyalinizing CCC (**29**). This is a critical distinction as a solid nested MEC would be classified as high-grade malignancy with a worse prognosis and more aggressive treatment, while hyalinizing CCC usually behaves as a low-grade tumor.

## 2.2. Epithelial-myoepithelial carcinoma

Epithelial-myoepithelial carcinoma (EMC) is a rare salivary gland malignancy that comprises about 1–2 % of all salivary gland tumors and 2–5 % of malignant salivary gland tumors (**30**). Initially described by Donath et al. in 1972 (**31**), EMC was likely recognized as early as 1956 and reported under a variety of names such as adenomyoepithelioma, clear cell adenoma, tubular solid adenoma, monomorphic clear cell tumor, glycogen-rich adenoma, glycogen-rich adenocarcinoma, and clear cell carcinoma (**32-34**).

EMC represents a typical example of a biphasic salivary gland neoplasm. Histologically, it is composed of a tubular to nested bi-layered arrangement of inner (luminal) ductal cells, and

outer (abluminal) myoepithelial cells. The ductal component is typically composed of small lightly eosinophilic cuboidal cells forming tubules. The abluminal myoepithelial component consists of larger polygonal cells, usually with clear cytoplasm. This outer layer in turn is surrounded by a basement membrane of varying thickness; this can be so marked that the predominant histological appearance of the tumor becomes that of a paucicellular hyaline mass with only scanty bi-layered neoplastic ducts. (**35**) The vast majority of EMCs have low grade cytomorphologic features, but up to one third may show perineural invasion **(36).** Angiolymphatic invasion and necrosis, which appear to correlate with local recurrence, are less frequent **(36).**

Most EMCs have a predominant clear cell abluminal myoepithelial layer with a well delineated luminal ductal non-clear cell component. A double-clear variant of EMC is rare (3.3%) **(36)**; in this subtype, the cytoplasm of both the epithelial and myoepithelial cells is clear, (hence "double-clear"), thus obviously making morphological distinction from hyalinizing CCC difficult. However, while the clear cells of hyalinizing CCC may look similar to the clear cells in EMC, the latter will have, at least focally, also a population of small dark ductal epithelial cells and a clear cell abluminal component with myoepithelial phenotype. The latter population may be identified with actin, smooth muscle myosin heavy chain, calponin, p63, or (less specifically) S100 antibodies **(36)**. EMC can on occasions lack clear cells altogether; then immunohistochemistry for CK7 and p63 will reveal an unexpected biphasic pattern in a challenging undiagnosed carcinoma, which is then rightfully recognized as EMC. One other pointer in differentiating EMC from hyalinizing CCC is that the former with its biphasic cell population occurs most frequently in the parotid gland, whereas hyalinizing CCC composed of one neoplastic cell type only usually arises in minor salivary glands, particularly the palate.

In distinguishing EMC from other salivary gland tumors with biphasic differentiation immunohistochemistry is of limited value. However, a molecular test that can lead to an accurate diagnosis of EMC is now available **(16, 37)**. Mutations in codon 61 of *HRAS* gene have been detected in a vast majority of EMC, independent of histologic variants, the anatomic location and clinicopathologic parameters **(16, 37)**. This genetic alteration was consistently lacking from the histologic mimics of EMC. Thus, the assessment of *HRAS* mutations can contribute to correct diagnosis of EMC in challenging histopathological settings.

### 3. Clear cell variants of well defined salivary neoplasms

### 3.1. Clear cell mucoepidermoid carcinoma

Mucoepidermoid carcinoma (MEC) is the most common type of salivary gland carcinoma and it is found in both the major and minor salivary glands. It is typically composed of varying numbers of epidermoid, intermediate, and mucin-producing cells. Rarely, clear cells predominate over the other cell types, and these most often represent intermediate cells; tumors where this occurs are termed the clear cell variant of MEC **(38-40)**. This variant is almost exclusively composed of cells with abundant optically clear cytoplasm, only mild to moderate nuclear enlargement, and a nested growth pattern (**Fig. 3**). In the clear cell variant of MEC tumor cells containing cytoplasmic mucin are rare and difficult to discern. The clear cytoplasm of the tumor cells is stained by periodic acid–Schiff (PAS) with some granularity indicating the presence of glycogen, while the mucinous cells are stained by PAS even after diastase digestion indicating mucin **(40).** Intracellular mucins are also stained blue by Alcian blue. Immunohistochemical positivity for p63 and p40 and negativity for S100 and SOX10 may be helpful in differential diagnosis between MEC and the clear-cell variant of myoepithelial carcinoma and myoepithelioma. However, the immunoprofile of hyalinizing CCC is identical to that of MEC. Approximately 60-80% of MECs harbor a tumor type-specific translocation t(11;19)(q21;p13) and its *CRTC1::MAML2* fusion gene or a rare variant translocation t(11;15)(q21;q26) with *CRTC3::MAML2* fusion **(13, 41-42).** Among the various salivary gland tumors, *CRTC1/3::MAML2* fusion is specific for MEC and it can be used in diagnostic workup (**Fig. 3**). In selected problematic cases, demonstration of *MAML2* rearrangement is recommended to confirm the diagnosis of MEC, particularly in examples of the oncocytic or clear cell variants of MEC, or in differential diagnosis from pleomorphic adenomas with extensive oncocytic or mucinous metaplasia **(40, 43-44).** In such cases, demonstration of *MAML2* rearrangement may be critical for the correct diagnosis. Obviously, only positive results are informative.

### 3.2. Clear cell myoepithelial carcinoma

Myoepithelial carcinoma (MC) is a malignant salivary neoplasm that is almost exclusively composed of myoepithelial cells and has an invasive growth pattern **(45).** Most MCs occur in the parotid gland followed by the palate, and the submandibular gland **(45, 18)**. In earlier studies, MC was reported to account for < 2% of all salivary gland malignancies **(46-**

47), but currently the incidence of MC is suspected to be higher. This is mainly because MC is easily underrecognized given its broad histologic spectrum and overlapping morphologic features with other salivary gland tumors, benign or malignant **(48).** MC may arise in the context of a preexisting pleomorphic adenoma (MC ex-PA) or de novo, and it may affect major or minor salivary glands.

Microscopically, morphologic heterogeneity is a typical histologic feature of MC, with tumors mostly displaying a mixture of different cell types and growth patterns, and this spectrum includes a clear cell variant **(17-18, 49)**. The neoplastic myoepithelial cells in MC exhibit considerable variation, and although most tumors show a mixture of different cells, one type often predominates. Most commonly, this is the epithelioid cell type (79%), followed by spindle cells (19%), basaloid-type cells with high nuclear to cytoplasmic ratio (15%), and plasmacytoid cells (15%). Clear cells as the predominant cell type are relatively rare (8%) **(45).**

Myoepithelial cells in various salivary gland neoplasms commonly undergo clear cell transformation, for example in EMC, but the clear cell variant of myoepithelial carcinoma (CCMC) composed exclusively of neoplastic cells with water clear cytoplasm is rare **(17-18, 49)**. CCMCs are composed of compact nests of large polyhedral cells with abundant clear cytoplasm divided by fibrous septa (**Fig. 4**). Histologically, the most characteristic feature of CCMC is its multinodular architecture and its zonal cellular arrangement. The latter consists of a hypercellular peripheral rim of tumor cells surrounding a hypocellular sometimes necrotic center of the tumor islands. These two features help differentiate MC from benign tumors like pleomorphic adenoma and myoepithelioma, and malignancies such as hyalinizing CCC. CCMC characteristically is stained variably positive with p40, p63, SOX10, S-100 protein, high molecular weight cytokeratin, muscle specific actin, and alpha smooth muscle actin antibodies. Calponin, which is considered the most sensitive and specific marker of myoepithelial cells, is however rarely expressed in CCMC **(17).**

In the differential diagnosis of CCMC, hyalinizing CCC must be considered. This distinction is important since CCMC tends to behave more aggressively with a 50% recurrence rate and 40% metastatic rate **(50).** In one recent study, patients with CCMC developed distant and lymph node metastases in 33% and 24% of cases, respectively **(18).** Positive immunostaining for cytokeratin, p40, and p63 is shared by CCMC and hyalinizing CCC. Although the immunoprofile may vary considerably between cases, the minimal requirement for diagnosis of myoepithelial carcinoma of salivary gland is co-expression of cytokeratin/EMA, SOX10, S100 protein, and/or at least one other myoepithelial marker.

*EWSR1* gene rearrangement has been identified in approximately one third of MCs, which have predominantly clear cell morphology and aggressive clinical behavior (**17, 51).** However, it was reported recently that none of the MCs with *EWSR1* rearrangement identified by FISH, showed an *EWSR1* fusion transcript in sequencing, and consequently this type of *EWSR1* abnormality in MCs may actually represent a passenger mutation with minor effect (**18).** The most common fusion transcripts in all subtypes of MC included *FGFR1::PLAG1* and *TGFBR3::PLAG1* (**52**), and *LIFR::PLAG1, CTNNB1::PLAG1, FGFR1::PLAG1*, and *CHCHD7::PLAG1* (**18),** respectively.

### 3.3.    Clear cell variant of oncocytoma

Oncocytomas are benign encapsulated neoplasms composed of large epithelial cells with abundant eosinophilic granular cytoplasm due to the accumulation of mitochondria (oncocytes). p63 positive basal-like cells are an obligate component in oncocytoma. Oncocytomas may be multifocal and bilateral and constitute approximately 1% of all salivary gland tumors with marked tendency for parotid gland involvement and a much lower occurrence in the minor glands (**53**). Clear cells may occasionally predominate in some lesions and may be attributed to intracytoplasmic glycogen deposition or to fixation artifact (**54**). Clear cell oncocytoma is composed almost entirely of oncocytes with clear cytoplasm (**55**). It is important to mention that clear cell oncocytoma of the salivary gland is a benign tumor with excellent prognosis, when compared to other salivary tumors with exclusively or predominantly clear cell features, which are mostly malignant (**55).**

## 4. Non-salivary clear cell neoplasms in differential diagnosis

### 4.1. Odontogenic clear cell carcinoma

Antonescu et al (**15**) reported a recurrent *EWSR1::ATF1* fusion in hyalinizing CCC of minor salivary glands. Identical *EWSR1* and *ATF1* gene rearrangements have also been identified in clear cell odontogenic carcinoma (CCOC), providing molecular evidence for a link between hyalinizing CCC and CCOC (**56).** CCOC is an odontogenic carcinoma characterized by nests, sheets, and cords of clear cells in a fibrocellular or hyalinized stroma. These tumors develop in the jaw bones with three quarters occurring in the mandible, most frequently in the posterior body and lower ramus (**57**). *EWSR1* rearrangements have been reported in over 80%

of cases of CCOC (54). *ATF1* is the most common fusion partner of *EWSR1*, while *CREB1* and *CREM* have been found less frequently (**56, 58-59**). CCOCs are histomorphologically and immunohistochemically similar to salivary gland hyalinizing CCC, and the two tumor types share *EWSR1* rearrangement suggesting that they share a related form of pathogenesis (**15**). There are not any specific markers for odontogenic clear cell carcinoma. The differential diagnosis of CCOC includes other clear cell-rich gnathic neoplasms such as clear cell calcifying epithelial odontogenic tumor, amyloid-rich central odontogenic fibroma, and both primary and metastatic intraosseous salivary tumors such as intraosseous clear cell mucoepidermoid carcinoma (**60**). Metastatic tumors containing clear cells include most likely renal cell carcinoma, clear cell breast carcinoma or thyroid carcinoma and, therefore, in addition to clinical history, the immunomarkers RCC, CD10, PAX8, CAIX, GATA3, ER/PR, TTF-1 are useful to rule out metastatic tumors (**61**).

### 4.2. Primary cutaneous tumors with clear cell morphology

Primary salivary squamous cell carcinoma (SCC) is very rare, and the diagnosis can only be established by excluding metastatic SCC. A clear cell variant of cutaneous squamous cell carcinoma (SCC), also referred to as hydropic SCC, is a very rare variant of SCC with extensive hydropic changes of keratinocytes (**62**). The hydropic degeneration of neoplastic cells and the accumulation of intracellular fluid and not the accumulation of glycogen, lipid, or mucin, results in its clear cell appearance. All cases reported so far have been in the head and neck region with the mandible being the most common site (**63**). Other differential diagnoses of cutaneous origin may include clear cell acanthoma, clear cell hidradenoma, clear cell hidradenocarcinoma, tricholemmoma, and pilar tumor. In the latter tumor the clear cells have a high content of cytoplasmic glycogen. These tumors are, however, found in to the skin and do not represent a major differential diagnostic problem with salivary clear cell tumors.

### 4.3. Clear cell malignant melanoma and other soft tissue clear cell neoplasms

Clear cell sarcoma is an rare aggressive malignant neoplasm with morphologic and immunohistochemical similarities to malignant melanoma. Although both malignant melanoma and clear cell sarcoma display melanin pigment and melanocytic markers, the two disorders are genetically distinct (**64**). Cases of malignant melanoma may contain BRAF mutations (**65**), whereas clear cell sarcoma lacks this mutation (**66**) and characteristically exhibits the reciprocal translocation t(12;22)(q13;q12) resulting in a rearrangement of the EWS RNA binding protein 1 (*EWSR1*) gene. Clear cell sarcomas of the head and neck are uncommon (**67-68**). Tumors

arising from the salivary glands are extremely rare but have been described in the parotid and submandibular glands, respectively **(69, 64).**

## 5. Metastatic neoplasms with clear cell morphology

Between 15–35% of all parotid gland tumors are malignant and 21– 42% of these represent metastatic disease. The majority of metastatic parotid tumors are derived from skin malignancies of the head and neck, usually squamous cell carcinomas in 45% and melanomas in 37%, respectively. A carcinoma metastatic to salivary glands and originating from a primary tumor located below the clavicle is uncommon, but the kidney is one of the more common infraclavicular primary sites of such tumors. The most common clear cell malignancy metastatic to the oral mucosa and the jaws is renal cell carcinoma. However, metastases of melanoma and malignant clear cell tumors of the prostate, bowel, thyroid, and liver must also be considered, and in the absence of clinical information, excluded with appropriate immunohistochemical markers. Distinguishing primary salivary tumors from metastatic tumors with clear cell features has important diagnostic, therapeutic, and decision-making considerations.

## 5.1. Metastatic renal cell carcinoma

Several metastatic renal cell carcinomas (RCC) have been reported in salivary glands, and the discovery of a metastasis may be the first indication of a primary RCC **(3-4).** Although the similarity with primary hyalinizing CCC is in most cases minimal, occasionally distinguishing it or even clear cell oncocytoma from metastatic RCC in the salivary gland can be challenging. RCC is considerably more vascular and displays generally either solid growth or has a crowded nested pattern. Small capillaries may be evident in hyalinizing CCC, but in RCC, the vascular channels are often conspicuous, dilated, and even sinusoidal. Hemorrhage and hemosiderin are generally more prominent in metastatic RCC. The nuclei are larger and more atypical than in hyalinizing CCC, and there is never squamous differentiation, or the dual type of stroma of hyalinizing CCC. Nevertheless, both primary hyalinizing CCC and metastatic RCC may be glycogen positive, and they may have a solid, organoid growth pattern, exhibit infiltrative growth, have little cytological atypia and few mitotic figures, and may be composed

almost entirely of clear cells. Mucicarmine positivity would favor a salivary gland primary tumor, but primary hyalinizing CCC of salivary gland is usually negative for intracytoplasmic mucin as well. In addition, immunohistochemistry can help, as clear cell RCC rarely expresses CK7 and virtually never is positive for p63, which is almost always expressed in hyalinizing CCC (70). Overall, RCC usually presents a more heterogeneous architecture and is more vascular than primary salivary clear cell carcinomas. The more pleomorphism and cytological atypia, the less likely the tumor is a primary hyalinizing CCC. Despite this, in some cases, it may not be possible confidently to differentiate between primary and metastatic carcinoma, and a clinical and imaging evaluation for a renal primary tumor should be performed (3, 4). Finally, it must never be forgotten that a metastatic RCC is always included in a list of clear cell salivary differential diagnosis (5, 21).

## 6. Conclusion

Clear cell neoplasms of salivary glands are diagnostic challenges. They comprise a diverse group of benign and malignant tumors with variable clinicopathological characteristics. The distinction between different tumors of this group and this differential diagnosis from metastatic disease is essential and can be facilitated by a combination of thorough clinical evaluation, histological, immunohistochemical stainings as well as molecular genetic characteristics in selected cases.

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**Figure Legeds.**

**Fig. 1. Hyalinizing clear cell carcinoma (HCC) of left base of the tongue.**

A: Gadolinium-enhanced MRI of clinical stage cT3N1 HCCC. The left panel shows the primary tumor and a level II lymphadenopathy on axial images. The right panel shows a sagittal T1-weighted image of the base of tongue tumor filling the vallecula and pushing the epiglottis down.

B: Transoral robotic resection and ipsilateral comprehensive neck dissection resulted in a pathological stage pT3N2b HCCC.

**Fig. 2. Hyalinizing clear cell carcinoma with *EWSR1::ATF or EWSR1::CREM fusion***

A: Hyalinizing clear cell carcinoma with *EWSR1::ATF1* gene fusion shows solid-alveolar nests of uniform clear cells with round to oval nuclei and decent chromatin, separated by gently cellular and hyalinized septa.

B: Tumor cells are positive for p63.

C: Tumor cells are negative for S100 protein, peripheral nerves serve as positive control

D: The fusion joining of *EWSR1* gene exon 8 with *ATF1* gene exon 4 is illustrated. Protein domains are depicted.

E: The fusion joining of *EWSR1* gene exon 14 with *CREM* gene exon 6 is illustrated. Protein domains are depicted.

**Fig. 3. Clear Cell Mucoepidermoid Carcinoma**

A: The tumor was composed predominantly of cells with large and watery clear cytoplasm although more classic areas of mucoepidermoid carcinoma in the form of cystic structures with mucous cells are present as well.

B: The presence of scattered mucous cells is highlighted Periodic-acid-Shiff positivity, while clear cells showed loss of PAS staining.

C: p63 was positive predominantly positive in clear cells.

D: The fusion joining of *CRTC1* gene exon 1 with *MAML2* gene exon 2 is illustrated. Protein domains are depicted.

E: The fusion joining of *CRTC3* gene exon 1 with *MAML2* gene exon 2 is illustrated. Protein domains are depicted.

**Fig. 4. Clear Cell Myoepithelial Carcinoma (CCMC)**

A: CCMC with *CTNNB*::*PLAG1* fusion is composed of compact nests of large polyhedral cells with abundant clear cytoplasm divided by fibrous septa

B: A case of CCMC with *LIFR*::*PLAG1* fusion shows multifocal squamous metaplasia.

C: The fusions joining of *CTNNB* gene, exon 1, *CHCHD7* gene, exon 1, and *LIFR* gene, exon 1 with *PLAG1* gene, exon 2 are illustrated. Protein domains are depicted.

**Clear Cell Neoplasms of Salivary Glands: A Diagnostic Challenge**

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**Running title:** Clear cell neoplasms in salivary glands

**Abstract.**

This review focuses on the heterogenous group of clear cell neoplasms of salivary glands and attempts to identify major differential diagnostic features. Within the head and neck region, clear cells are found most commonly in salivary gland tumors, but may also be seen in tumors of squamous or odontogenic epithelial origin, primary or metastatic carcinomas, benign or malignant melanocytic lesions, or benign or malignant mesenchymal tumors. Clear cells occur

fairly commonly among a wide variety of salivary gland neoplasms, but mostly they constitute only a minor component of the tumor cell population. Clear cells represent a major diagnostic feature in two salivary gland neoplasms, epithelial-myoepithelial carcinoma and hyalinizing clear cell carcinoma. In addition, salivary gland neoplasms composed predominantly of clear cells could also include clear cell variants of other salivary neoplasms, such as mucoepidermoid carcinoma and myoepithelial carcinoma, but their tumor type-specific histological features may only be available in limited non-clear cell areas of the tumor.

Diagnosing predominantly clear cell salivary gland tumors is difficult because the immunoprofiles and morphological features may overlap and the same tumor entity may also have a wide range of other histologic presentations. Many salivary gland tumors are characterized by tumor type-specific genomic alterations, particularly gene fusions of the *ETV6* gene in secretory carcinoma, the *MYB* and *MYBL1* genes in adenoid cystic carcinoma, the *MAML2* gene in mucoepidermoid carcinoma, the *EWSR1* gene in hyalinizing clear cell carcinoma, and others. Thus, along with conventional histopathologic examination and immunoprofiling, molecular and genetic tests may be important in the diagnosis of salivary gland clear cell tumors by demonstrating genetic alterations specific to them.

## 1. Introduction.

This review focuses on the heterogenous group of clear cell neoplasms of salivary glands and attempts to clarify some of the features that help to differentiate one neoplasm from another. Optically clear cells may be found as incidental histologic findings in any of a multitude of benign or malignant tumors from different cells of origin including epithelial, melanocytic, mesenchymal, or hematopoietic. They may be a result of many different processes, such as fixation artifact, degeneration of cellular organelles, or accumulation of substances within the cells - most commonly glycogen, but sometimes mucopolysaccharides, mucin, or lipids **(1).** Within the head and neck region, clear cells are found most commonly in salivary gland tumors, but may also be seen in tumors of squamous or odontogenic epithelial origin, primary or metastatic carcinomas, benign or malignant melanocytic lesions, or benign or malignant

mesenchymal tumors (**2**). Even metastatic clear cell tumors, such as renal cell carcinoma, can be difficult to differentiate from primary clear cell neoplasms of the salivary glands (**3, 4**).

Clear cells occur fairly commonly among a wide variety of primary salivary gland neoplasms, including pleomorphic adenoma (PA), myoepithelioma/myoepithelial carcinoma, oncocytoma, mucoepidermoid carcinoma (MEC), acinic cell carcinoma, epithelial-myoepithelial carcinoma (EMC), and adenoid cystic carcinoma (AdCC). In most cases, clear cells constitute only a minor component of the cell population of these neoplasms, and the appropriate classification of the tumors is easily established on the basis of typical features that are apparent.

In some tumors, however, clear cells constitute the major cellular component, and it is in this situation that the diagnostic challenge is greatest. Clear cells are usually the principal diagnostic feature in two salivary gland neoplasms, EMC and hyalinizing clear cell carcinoma (CCC). In addition, salivary gland neoplasms composed predominantly of clear cells could also include clear cell variants of well defined salivary neoplasms such as MEC, myoepithelial carcinoma (MC), myoepithelioma and oncocytoma, but their specific histological features can be hidden and only apparent in a limited non-clear cell component of the tumor.

Diagnosing predominantly clear cell salivary gland tumors is difficult because many different tumor types may share similar morphologic features, and the same tumor entity may have a wide range of other histologic presentations (**Table 1**). Moreover, the immunoprofiles of predominantly clear cell salivary tumors of different entities may overlap, such as p63/p40/HMW cytokeratin positive and S100/SOX10 negative immunostaining in hyalinizing CCC, MEC, and squamous cell carcinoma (SCC) (**5**).

Over the past decade, a significant development in molecular techniques and understanding of the genomic landscape of salivary gland neoplasms has taken place (**6-8**). Several salivary gland tumors were characterized by recurrent genomic alterations, including gene fusions involving the *ETV6* gene in secretory carcinoma (**9-11**), the *MYB* and *MYBL1* genes in adenoid cystic carcinoma (**12**), the *MAML2* gene in MEC (**13-14**), and the fusion *EWSR1::ATF1* in hyalinizing CCC (**15**). In addition, *HRAS* exon 3 mutations were seen in most cases of EMC (**16**), and rearrangement of the gene *EWSR1* was described in a significant proportion of clear cell myoepithelial carcinomas (**17-18**), respectively. Thus, along with conventional histologic examination and immunoprofiling, molecular and genetic tests can facilitate the diagnosis of salivary gland clear cell tumors by demonstrating genetic alterations specific to them.

## 2. Major primary clear cell neoplasms of salivary glands

### 2.1. Hyalinizing clear cell carcinoma

Hyalinizing CCC is a low-grade salivary gland malignancy that was originally described by Batsakis in 1980 (**19**)**,** and the concept was later refined by Simpson et al (**20**) and Milchgrub et al (**21**)**.** While it is possible that hyalinizing CCC rarely arises also in major salivary glands, it occurs most commonly in minor salivary glands, usually in the palate or the base of the tongue (**21**) (**Fig. 1**)**.** Until recently, hyalinizing CCC was regarded not as a distinct entity but rather as a diagnosis of exclusion. Uncertainty about the true nature and identity of hyalinizing CCC was reflected in the frequently changing designations of this tumor type. Originally described as "clear cell carcinoma" (**19, 20**), it was later designated as "hyalinizing clear cell carcinoma" (**21**)**.** Then, in the 2005 World Health Organization Classification (WHO) of Head and Neck Tumors it was renamed as "clear cell carcinoma, not otherwise specified" (**22**)**,** and in the 2008 Armed Forces Institute of Pathology Fascicle of Salivary Gland Tumors as "clear cell adenocarcinoma" (**23**)**.** The term "clear cell carcinoma" then re-appeared in the 2017 WHO Classification of Head and Neck Tumors (**24**), and finally the neoplasm is called "hyalinizing clear cell carcinoma" again in the upcoming 5th edition of WHO classification in 2022 (**25**).

The discovery that most hyalinizing CCCs harbor *EWSR1::ATF1* fusion that is not found in any other type of salivary gland tumor, strongly supports the present view that hyalinizing CCC is a distinct tumor entity (**15**)**.** Microscopically, hyalinizing CCC is typically composed of clear cells, arranged in anastomosing trabeculae, cords, nests, or solid sheets surrounded by a stroma of spindle-shaped fibroblasts and dense hypocellular hyalinized tissue sometimes with myxoid foci (**Fig. 2**). Hyalinizing CCCs have invasive borders, even occasionally exhibiting perineural infiltration, and the tumor cells display minimal nuclear pleomorphism with a very low mitotic and proliferative index. Histologically, there is a relatively wide range of appearances of hyalinizing CCC tumor cells: whilst a predominance of clear cells is seen in most examples, sometimes a variable proportion of the tumor cells may have pale eosinophilic rather than clear cytoplasm. Generally however, many hyalinizing CCCs display a mixture of both cell types. Tumors with virtually no clear cells can occasionally be seen, but they usually retain the same overall growth pattern as typical clear cell examples of hyalinizing CCC (**5**)**.** A very characteristic feature of hyalinizing CCC is the appearance of the stroma comprising abundant hyalinized basement membrane-like material. It is often sharply

demarcated from the desmoplastic or fibrocellular stroma that sometimes appears myxoid and may mimic a pleomorphic adenoma (**5**). The presence of these two stroma types is essentially pathognomonic for the diagnosis of hyalinizing CCC and is seen in most, but not all cases. The finding of hyalinized stroma in a tumor with a cribriform architecture may mimic other more common salivary gland tumors, such as AdCC and PA, particularly if the hyalinizing CCC has only a minor clear cell component.

The presence of a diagnostic molecular marker, the *EWSR1::ATF1* fusion, in hyalinizing CCC has allowed for a more complete appreciation of its histologic spectrum. This, in turn, has disclosed pitfalls in diagnosing carcinomas with clear cell morphology on the basis of histology alone (**Fig. 2**). For example, hyalinizing CCC sometimes exhibits overt squamous differentiation (**26**), and they may not be hyalinized, and they may not even be dominated by clear cells. Thus, without molecular confirmation, the diagnosis of hyalinizing CCC would hardly be possible (**5**). Immunophenotypically hyalinizing CCC has similarities to SCC and MEC with positive immunostaining for high–molecular-weight keratins and p63 and negative immunostaining for markers of myoepithelial differentiation. Focal mucinous differentiation is known to occur in up to 50% of cases of hyalinizing CCC (**27-28**). The distinction of hyalinizing CCC from clear cell mucoepidermoid carcinoma (MEC) can be truly challenging, and hyalinizing CCC may be misclassified as the more common MEC (**27-28**). In one recent study, the presence of an alternative *EWSR1::CREM* fusion in a salivary clear cell carcinoma with a very prominent mucinous component and an original diagnosis of MEC, finally convinced pathologists of a revised diagnosis of hyalinizing CCC (**29**). This is a critical distinction as a solid nested MEC would be classified as high-grade malignancy with a worse prognosis and more aggressive treatment, while hyalinizing CCC usually behaves as a low-grade tumor.

## 2.2. Epithelial-myoepithelial carcinoma

Epithelial-myoepithelial carcinoma (EMC) is a rare salivary gland malignancy that comprises about 1–2 % of all salivary gland tumors and 2–5 % of malignant salivary gland tumors (**30**). Initially described by Donath et al. in 1972 (**31**), EMC was likely recognized as early as 1956 and reported under a variety of names such as adenomyoepithelioma, clear cell adenoma, tubular solid adenoma, monomorphic clear cell tumor, glycogen-rich adenoma, glycogen-rich adenocarcinoma, and clear cell carcinoma (**32-34**).

EMC represents a typical example of a biphasic salivary gland neoplasm. Histologically, it is composed of a tubular to nested bi-layered arrangement of inner (luminal) ductal cells, and

outer (abluminal) myoepithelial cells. The ductal component is typically composed of small lightly eosinophilic cuboidal cells forming tubules. The abluminal myoepithelial component consists of larger polygonal cells, usually with clear cytoplasm. This outer layer in turn is surrounded by a basement membrane of varying thickness; this can be so marked that the predominant histological appearance of the tumor becomes that of a paucicellular hyaline mass with only scanty bi-layered neoplastic ducts. (**35**) The vast majority of EMCs have low grade cytomorphologic features, but up to one third may show perineural invasion (**36**). Angiolymphatic invasion and necrosis, which appear to correlate with local recurrence, are less frequent (**36**).

Most EMCs have a predominant clear cell abluminal myoepithelial layer with a well delineated luminal ductal non-clear cell component. A double-clear variant of EMC is rare (3.3%) (**36**); in this subtype, the cytoplasm of both the epithelial and myoepithelial cells is clear, (hence "double-clear"), thus obviously making morphological distinction from hyalinizing CCC difficult. However, while the clear cells of hyalinizing CCC may look similar to the clear cells in EMC, the latter will have, at least focally, also a population of small dark ductal epithelial cells and a clear cell abluminal component with myoepithelial phenotype. The latter population may be identified with actin, smooth muscle myosin heavy chain, calponin, p63, or (less specifically) S100 antibodies (**36**). EMC can on occasions lack clear cells altogether; then immunohistochemistry for CK7 and p63 will reveal an unexpected biphasic pattern in a challenging undiagnosed carcinoma, which is then rightfully recognized as EMC. One other pointer in differentiating EMC from hyalinizing CCC is that the former with its biphasic cell population occurs most frequently in the parotid gland, whereas hyalinizing CCC composed of one neoplastic cell type only usually arises in minor salivary glands, particularly the palate.

In distinguishing EMC from other salivary gland tumors with biphasic differentiation immunohistochemistry is of limited value. However, a molecular test that can lead to an accurate diagnosis of EMC is now available (**16, 37**). Mutations in codon 61 of *HRAS* gene have been detected in a vast majority of EMC, independent of histologic variants, the anatomic location and clinicopathologic parameters (**16, 37**). This genetic alteration was consistently lacking from the histologic mimics of EMC. Thus, the assessment of *HRAS* mutations can contribute to correct diagnosis of EMC in challenging histopathological settings.

### 3. Clear cell variants of well defined salivary neoplasms

## 3.1. Clear cell mucoepidermoid carcinoma

Mucoepidermoid carcinoma (MEC) is the most common type of salivary gland carcinoma and it is found in both the major and minor salivary glands. It is typically composed of varying numbers of epidermoid, intermediate, and mucin-producing cells. Rarely, clear cells predominate over the other cell types, and these most often represent intermediate cells; tumors where this occurs are termed the clear cell variant of MEC **(38-40)**. This variant is almost exclusively composed of cells with abundant optically clear cytoplasm, only mild to moderate nuclear enlargement, and a nested growth pattern (**Fig. 3**). In the clear cell variant of MEC tumor cells containing cytoplasmic mucin are rare and difficult to discern. The clear cytoplasm of the tumor cells is stained by periodic acid–Schiff (PAS) with some granularity indicating the presence of glycogen, while the mucinous cells are stained by PAS even after diastase digestion indicating mucin **(40).** Intracellular mucins are also stained blue by Alcian blue. Immunohistochemical positivity for p63 and p40 and negativity for S100 and SOX10 may be helpful in differential diagnosis between MEC and the clear-cell variant of myoepithelial carcinoma and myoepithelioma. However, the immunoprofile of hyalinizing CCC is identical to that of MEC. Approximately 60-80% of MECs harbor a tumor type-specific translocation t(11;19)(q21;p13) and its *CRTC1::MAML2* fusion gene or a rare variant translocation t(11;15)(q21;q26) with *CRTC3::MAML2* fusion **(13, 41-42).** Among the various salivary gland tumors, *CRTC1/3::MAML2* fusion is specific for MEC and it can be used in diagnostic workup (**Fig. 3**). In selected problematic cases, demonstration of *MAML2* rearrangement is recommended to confirm the diagnosis of MEC, particularly in examples of the oncocytic or clear cell variants of MEC, or in differential diagnosis from pleomorphic adenomas with extensive oncocytic or mucinous metaplasia **(40, 43-44).** In such cases, demonstration of *MAML2* rearrangement may be critical for the correct diagnosis. Obviously, only positive results are informative.

## 3.2. Clear cell myoepithelial carcinoma

Myoepithelial carcinoma (MC) is a malignant salivary neoplasm that is almost exclusively composed of myoepithelial cells and has an invasive growth pattern **(45).** Most MCs occur in the parotid gland followed by the palate, and the submandibular gland **(45, 18)**. In earlier studies, MC was reported to account for < 2% of all salivary gland malignancies **(46-**

**47**), but currently the incidence of MC is suspected to be higher. This is mainly because MC is easily underrecognized given its broad histologic spectrum and overlapping morphologic features with other salivary gland tumors, benign or malignant **(48).** MC may arise in the context of a preexisting pleomorphic adenoma (MC ex-PA) or de novo, and it may affect major or minor salivary glands.

Microscopically, morphologic heterogeneity is a typical histologic feature of MC, with tumors mostly displaying a mixture of different cell types and growth patterns, and this spectrum includes a clear cell variant **(17-18, 49)**. The neoplastic myoepithelial cells in MC exhibit considerable variation, and although most tumors show a mixture of different cells, one type often predominates. Most commonly, this is the epithelioid cell type (79%), followed by spindle cells (19%), basaloid-type cells with high nuclear to cytoplasmic ratio (15%), and plasmacytoid cells (15%). Clear cells as the predominant cell type are relatively rare (8%) **(45).**

Myoepithelial cells in various salivary gland neoplasms commonly undergo clear cell transformation, for example in EMC, but the clear cell variant of myoepithelial carcinoma (CCMC) composed exclusively of neoplastic cells with water clear cytoplasm is rare (**17-18, 49**). CCMCs are composed of compact nests of large polyhedral cells with abundant clear cytoplasm divided by fibrous septa (**Fig. 4**). Histologically, the most characteristic feature of CCMC is its multinodular architecture and its zonal cellular arrangement. The latter consists of a hypercellular peripheral rim of tumor cells surrounding a hypocellular sometimes necrotic center of the tumor islands. These two features help differentiate MC from benign tumors like pleomorphic adenoma and myoepithelioma, and malignancies such as hyalinizing CCC. CCMC characteristically is stained variably positive with p40, p63, SOX10, S-100 protein, high molecular weight cytokeratin, muscle specific actin, and alpha smooth muscle actin antibodies. Calponin, which is considered the most sensitive and specific marker of myoepithelial cells, is however rarely expressed in CCMC **(17).**

In the differential diagnosis of CCMC, hyalinizing CCC must be considered. This distinction is important since CCMC tends to behave more aggressively with a 50% recurrence rate and 40% metastatic rate **(50).** In one recent study, patients with CCMC developed distant and lymph node metastases in 33% and 24% of cases, respectively **(18).** Positive immunostaining for cytokeratin, p40, and p63 is shared by CCMC and hyalinizing CCC. Although the immunoprofile may vary considerably between cases, the minimal requirement for diagnosis of myoepithelial carcinoma of salivary gland is co-expression of cytokeratin/EMA, SOX10, S100 protein, and/or at least one other myoepithelial marker.

*EWSR1* gene rearrangement has been identified in approximately one third of MCs, which have predominantly clear cell morphology and aggressive clinical behavior **(17, 51).** However, it was reported recently that none of the MCs with *EWSR1* rearrangement identified by FISH, showed an *EWSR1* fusion transcript in sequencing, and consequently this type of *EWSR1* abnormality in MCs may actually represent a passenger mutation with minor effect **(18).** The most common fusion transcripts in all subtypes of MC included *FGFR1::PLAG1* and *TGFBR3::PLAG1* **(52)**, and *LIFR::PLAG1, CTNNB1::PLAG1, FGFR1::PLAG1*, and *CHCHD7::PLAG1* **(18),** respectively.

### 3.3.    Clear cell variant of oncocytoma

Oncocytomas are benign encapsulated neoplasms composed of large epithelial cells with abundant eosinophilic granular cytoplasm due to the accumulation of mitochondria (oncocytes). p63 positive basal-like cells are an obligate component in oncocytoma. Oncocytomas may be multifocal and bilateral and constitute approximately 1% of all salivary gland tumors with marked tendency for parotid gland involvement and a much lower occurrence in the minor glands **(53)**. Clear cells may occasionally predominate in some lesions and may be attributed to intracytoplasmic glycogen deposition or to fixation artifact **(54)**. Clear cell oncocytoma is composed almost entirely of oncocytes with clear cytoplasm **(55)**. It is important to mention that clear cell oncocytoma of the salivary gland is a benign tumor with excellent prognosis, when compared to other salivary tumors with exclusively or predominantly clear cell features, which are mostly malignant **(55).**

## 4. Non-salivary clear cell neoplasms in differential diagnosis

## 4.1. Odontogenic clear cell carcinoma

Antonescu et al **(15)** reported a recurrent *EWSR1::ATF1* fusion in hyalinizing CCC of minor salivary glands. Identical *EWSR1* and *ATF1* gene rearrangements have also been identified in clear cell odontogenic carcinoma (CCOC), providing molecular evidence for a link between hyalinizing CCC and CCOC **(56).** CCOC is an odontogenic carcinoma characterized by nests, sheets, and cords of clear cells in a fibrocellular or hyalinized stroma. These tumors develop in the jaw bones with three quarters occurring in the mandible, most frequently in the posterior body and lower ramus **(57)**. *EWSR1* rearrangements have been reported in over 80%

of cases of CCOC (54). *ATF1* is the most common fusion partner of *EWSR1*, while *CREB1* and *CREM* have been found less frequently (**56, 58-59**). CCOCs are histomorphologically and immunohistochemically similar to salivary gland hyalinizing CCC, and the two tumor types share *EWSR1* rearrangement suggesting that they share a related form of pathogenesis **(15).** There are not any specific markers for odontogenic clear cell carcinoma. The differential diagnosis of CCOC includes other clear cell-rich gnathic neoplasms such as clear cell calcifying epithelial odontogenic tumor, amyloid-rich central odontogenic fibroma, and both primary and metastatic intraosseous salivary tumors such as intraosseous clear cell mucoepidermoid carcinoma **(60).** Metastatic tumors containing clear cells include most likely renal cell carcinoma, clear cell breast carcinoma or thyroid carcinoma and, therefore, in addition to clinical history, the immunomarkers RCC, CD10, PAX8, CAIX, GATA3, ER/PR, TTF-1 are useful to rule out metastatic tumors **(61).**

## 4.2. Primary cutaneous tumors with clear cell morphology

Primary salivary squamous cell carcinoma (SCC) is very rare, and the diagnosis can only be established by excluding metastatic SCC. A clear cell variant of cutaneous squamous cell carcinoma (SCC), also referred to as hydropic SCC, is a very rare variant of SCC with extensive hydropic changes of keratinocytes **(62).** The hydropic degeneration of neoplastic cells and the accumulation of intracellular fluid and not the accumulation of glycogen, lipid, or mucin, results in its clear cell appearance**.** All cases reported so far have been in the head and neck region with the mandible being the most common site **(63).** Other differential diagnoses of cutaneous origin may include clear cell acanthoma, clear cell hidradenoma, clear cell hidradenocarcinoma, tricholemmoma, and pilar tumor. In the latter tumor the clear cells have a high content of cytoplasmic glycogen. These tumors are, however, found in to the skin and do not represent a major differential diagnostic problem with salivary clear cell tumors.

## 4.3. Clear cell malignant melanoma and other soft tissue clear cell neoplasms

Clear cell sarcoma is an rare aggressive malignant neoplasm with morphologic and immunohistochemical similarities to malignant melanoma. Although both malignant melanoma and clear cell sarcoma display melanin pigment and melanocytic markers,  the two disorders are genetically distinct **(64)**. Cases of malignant melanoma may contain BRAF mutations **(65)**, whereas clear cell sarcoma lacks this mutation **(66)** and characteristically exhibits the reciprocal translocation t(12;22)(q13;q12) resulting in a rearrangement of the EWS RNA binding protein 1 (*EWSR1)* gene. Clear cell sarcomas of the head and neck are uncommon **(67-68).** Tumors

arising from the salivary glands are extremely rare but have been described in the parotid and submandibular glands, respectively **(69, 64).**

## 5. Metastatic neoplasms with clear cell morphology

Between 15–35% of all parotid gland tumors are malignant and 21– 42% of these represent metastatic disease. The majority of metastatic parotid tumors are derived from skin malignancies of the head and neck, usually squamous cell carcinomas in 45% and melanomas in 37%, respectively. A carcinoma metastatic to salivary glands and originating from a primary tumor located below the clavicle is uncommon, but the kidney is one of the more common infraclavicular primary sites of such tumors. The most common clear cell malignancy metastatic to the oral mucosa and the jaws is renal cell carcinoma. However, metastases of melanoma and malignant clear cell tumors of the prostate, bowel, thyroid, and liver must also be considered, and in the absence of clinical information, excluded with appropriate immunohistochemical markers. Distinguishing primary salivary tumors from metastatic tumors with clear cell features has important diagnostic, therapeutic, and decision-making considerations.

## 5.1. Metastatic renal cell carcinoma

Several metastatic renal cell carcinomas (RCC) have been reported in salivary glands, and the discovery of a metastasis may be the first indication of a primary RCC **(3-4).** Although the similarity with primary hyalinizing CCC is in most cases minimal, occasionally distinguishing it or even clear cell oncocytoma from metastatic RCC in the salivary gland can be challenging. RCC is considerably more vascular and displays generally either solid growth or has a crowded nested pattern. Small capillaries may be evident in hyalinizing CCC, but in RCC, the vascular channels are often conspicuous, dilated, and even sinusoidal. Hemorrhage and hemosiderin are generally more prominent in metastatic RCC. The nuclei are larger and more atypical than in hyalinizing CCC, and there is never squamous differentiation, or the dual type of stroma of hyalinizing CCC. Nevertheless, both primary hyalinizing CCC and metastatic RCC may be glycogen positive, and they may have a solid, organoid growth pattern, exhibit infiltrative growth, have little cytological atypia and few mitotic figures, and may be composed

almost entirely of clear cells. Mucicarmine positivity would favor a salivary gland primary tumor, but primary hyalinizing CCC of salivary gland is usually negative for intracytoplasmic mucin as well. In addition, immunohistochemistry can help, as clear cell RCC rarely expresses CK7 and virtually never is positive for p63, which is almost always expressed in hyalinizing CCC (**70**). Overall, RCC usually presents a more heterogeneous architecture and is more vascular than primary salivary clear cell carcinomas. The more pleomorphism and cytological atypia, the less likely the tumor is a primary hyalinizing CCC. Despite this, in some cases, it may not be possible confidently to differentiate between primary and metastatic carcinoma, and a clinical and imaging evaluation for a renal primary tumor should be performed (**3, 4**). Finally, it must never be forgotten that a metastatic RCC is always included in a list of clear cell salivary differential diagnosis (**5, 21**).

## 6. Conclusion

Clear cell neoplasms of salivary glands are diagnostic challenges. They comprise a diverse group of benign and malignant tumors with variable clinicopathological characteristics. The distinction between different tumors of this group and this differential diagnosis from metastatic disease is essential and can be facilitated by a combination of thorough clinical evaluation, histological, immunohistochemical stainings as well as molecular genetic characteristics in selected cases.

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**Figure Legeds.**

**Fig. 1. Hyalinizing clear cell carcinoma (HCC) of left base of the tongue.**

A: Gadolinium-enhanced MRI of clinical stage cT3N1 HCCC. The left panel shows the primary tumor and a level II lymphadenopathy on axial images. The right panel shows a sagittal T1-weighted image of the base of tongue tumor filling the vallecula and pushing the epiglottis down.

B: Transoral robotic resection and ipsilateral comprehensive neck dissection resulted in a pathological stage pT3N2b HCCC.

**Fig. 2. Hyalinizing clear cell carcinoma with *EWSR1::ATF or EWSR1::CREM fusion***

A: Hyalinizing clear cell carcinoma with *EWSR1::ATF1* gene fusion shows solid-alveolar nests of uniform clear cells with round to oval nuclei and decent chromatin, separated by gently cellular and hyalinized septa.

B: Tumor cells are positive for p63.

C: Tumor cells are negative for S100 protein, peripheral nerves serve as positive control

D: The fusion joining of *EWSR1* gene exon 8 with *ATF1* gene exon 4 is illustrated. Protein domains are depicted.

E: The fusion joining of *EWSR1* gene exon 14 with *CREM* gene exon 6 is illustrated. Protein domains are depicted.

**Fig. 3. Clear Cell Mucoepidermoid Carcinoma**

A: The tumor was composed predominantly of cells with large and watery clear cytoplasm although more classic areas of mucoepidermoid carcinoma in the form of cystic structures with mucous cells are present as well.

B: The presence of scattered mucous cells is highlighted Periodic-acid-Shiff positivity, while clear cells showed loss of PAS staining.

C: p63 was positive predominantly positive in clear cells.

D: The fusion joining of *CRTC1* gene exon 1 with *MAML2* gene exon 2 is illustrated. Protein domains are depicted.

E: The fusion joining of *CRTC3* gene exon 1 with *MAML2* gene exon 2 is illustrated. Protein domains are depicted.

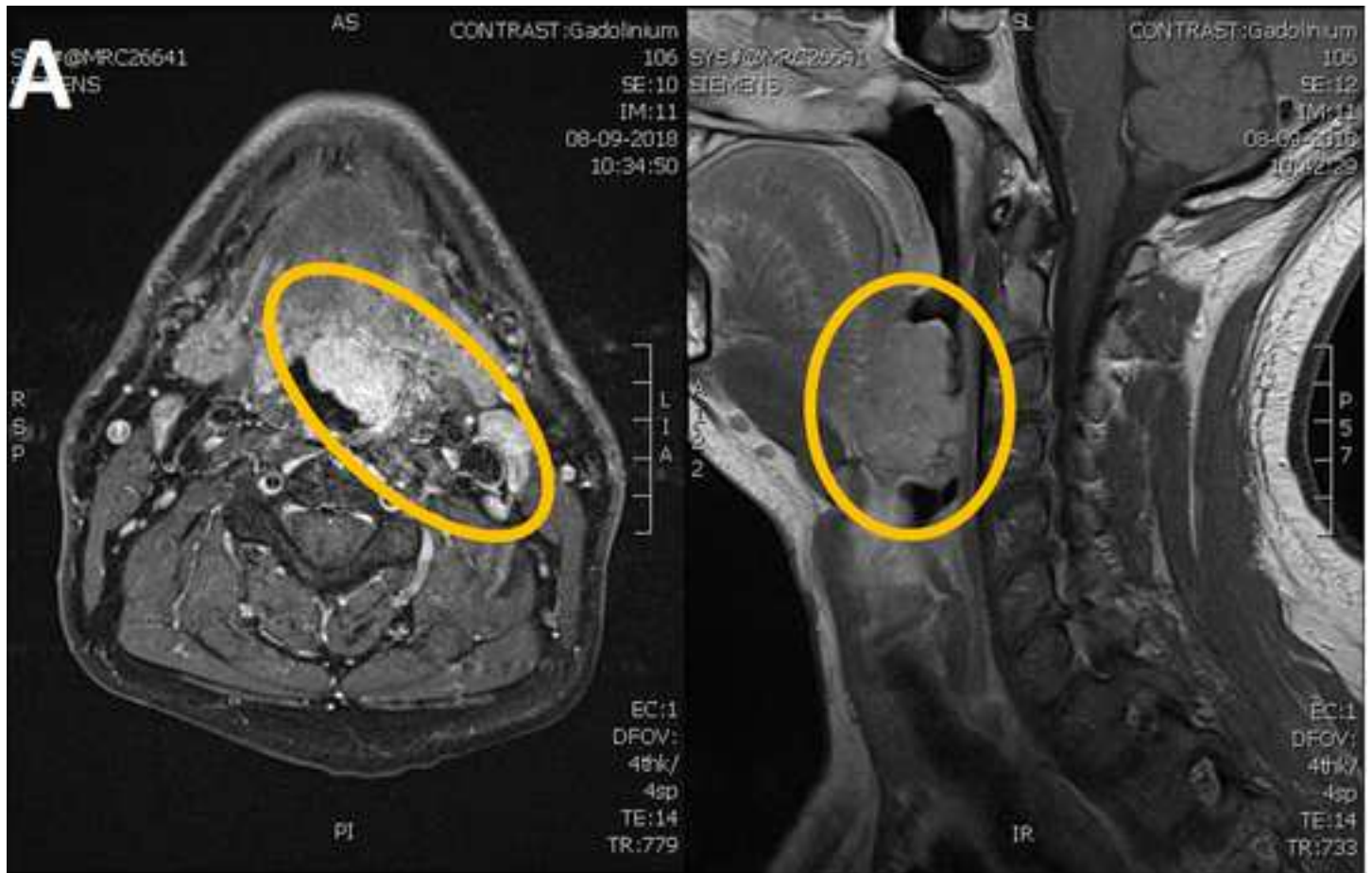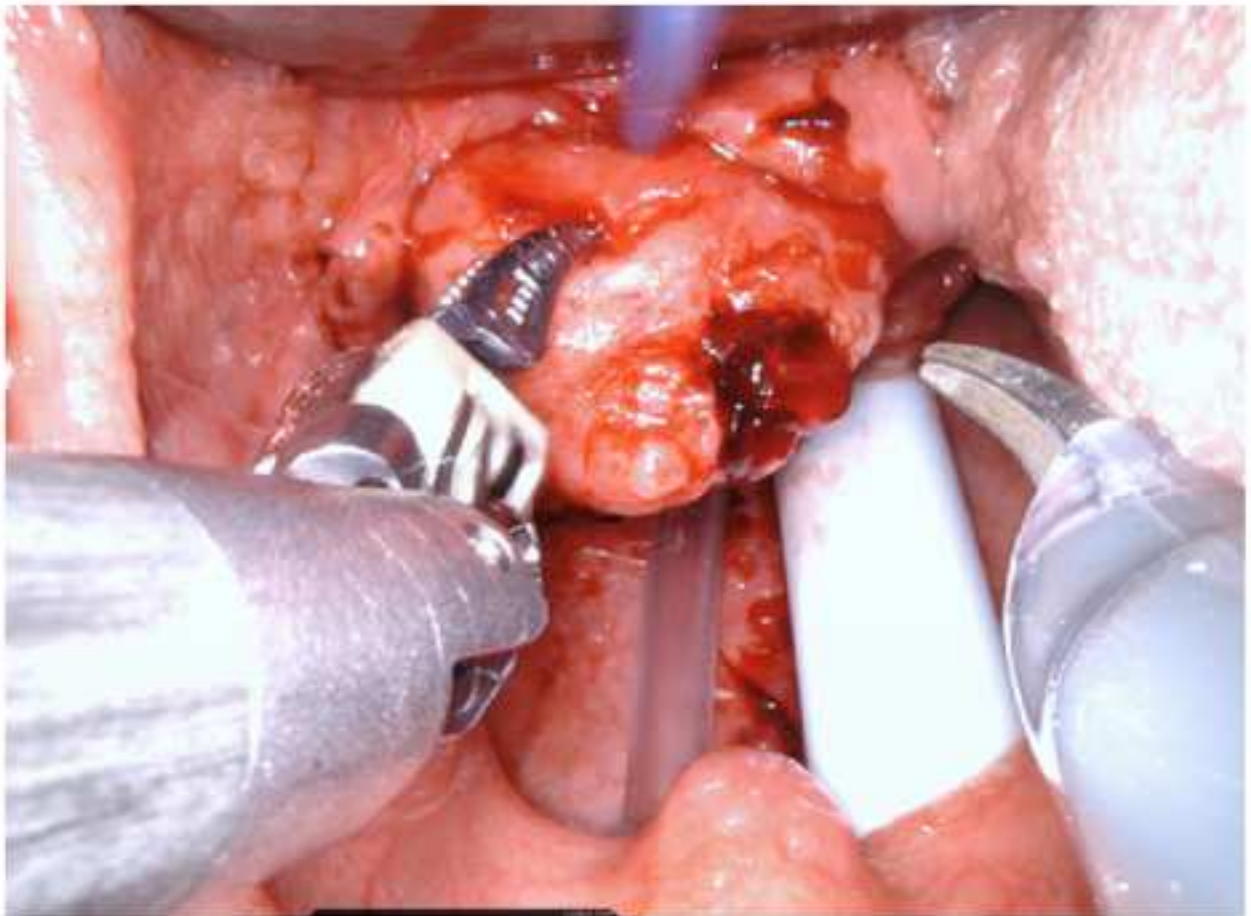**Fig. 4. Clear Cell Myoepithelial Carcinoma (CCMC)**

A: CCMC with *CTNNB::PLAG1* fusion is composed of compact nests of large polyhedral cells with abundant clear cytoplasm divided by fibrous septa

B: A case of CCMC with *LIFR::PLAG1* fusion shows multifocal squamous metaplasia.
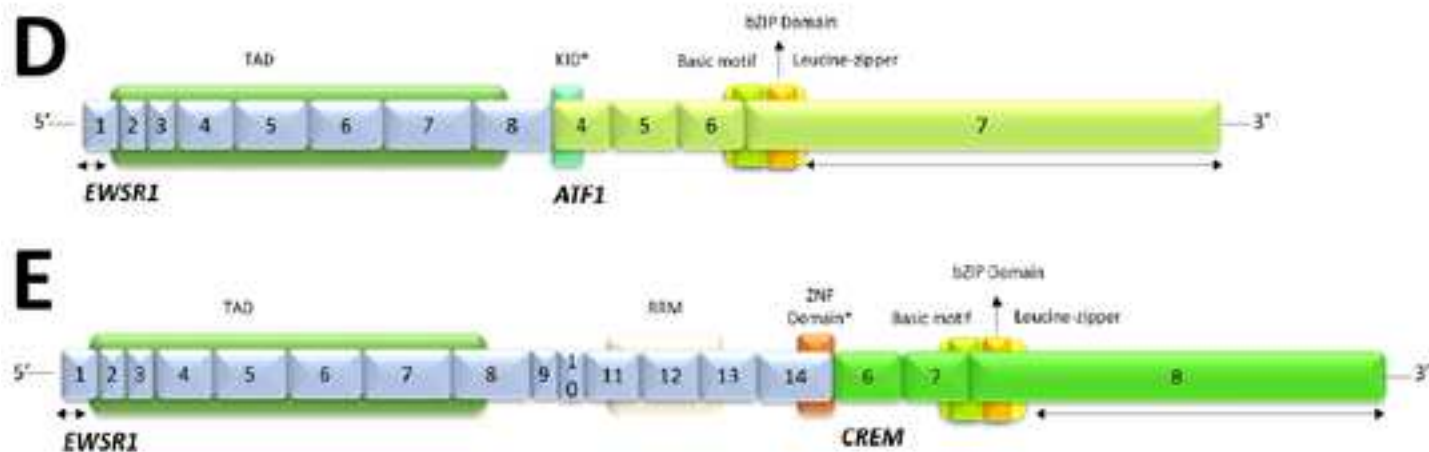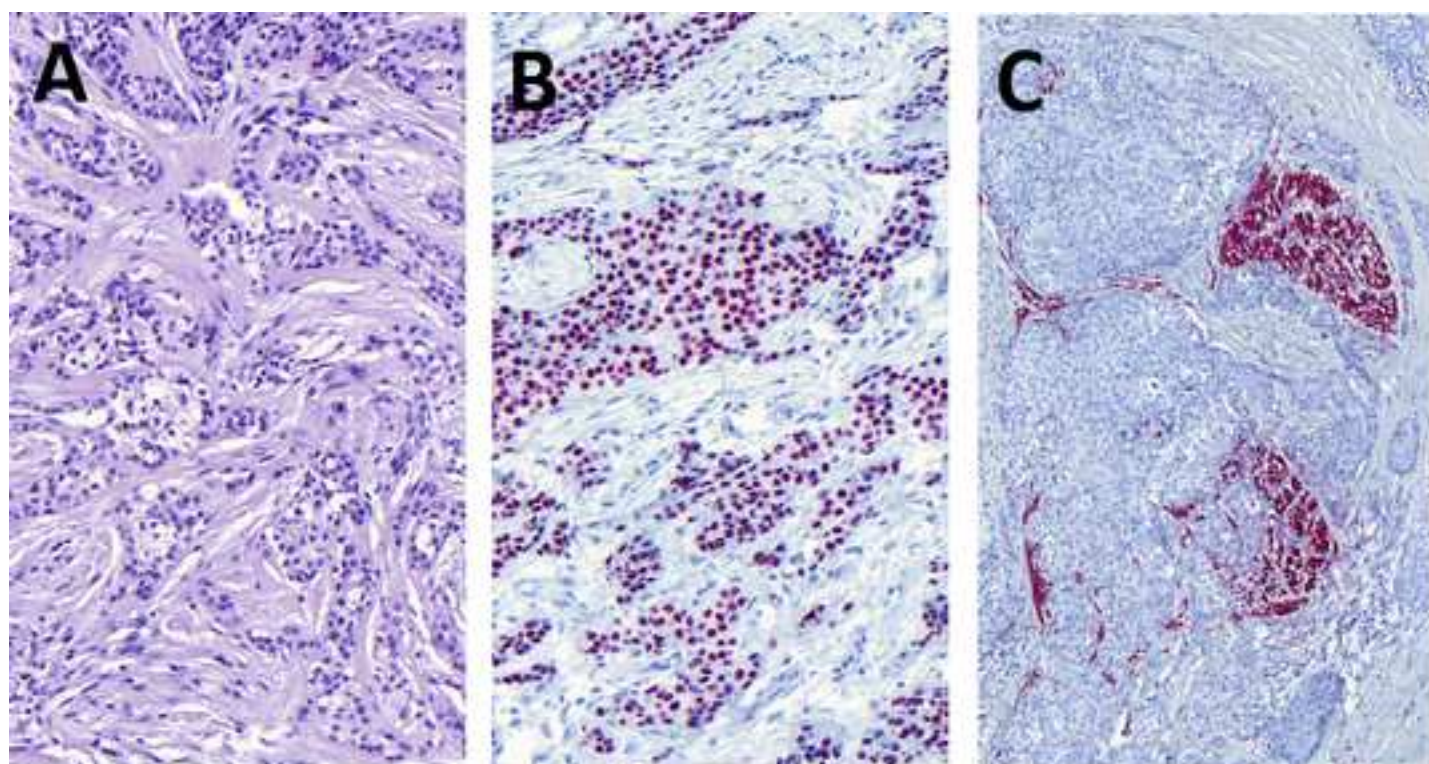
C: The fusions joining of *CTNNB* gene, exon 1, *CHCHD7* gene, exon 1, and *LIFR* gene, exon 1 with *PLAG1* gene, exon 2 are illustrated. Protein domains are depicted.

Table 1

**Table 1. Key morphologic, immunohistochemical and molecular findings in differential diagnosis of clear cell neoplasms of salivary glands.**

|  | Morphology | Immunoprofile | Molecular profiling |
|---|---|---|---|
| Hyalinizing CCC | Trabecular, diffuse or glandular pattern of clear/eosinophilic epithelial cells with hyalinizing collagenous and/or fibrocellular stroma | Cytokeratin+, p63+, SMA-, calponin- S-100- SOX10- | *EWSR1::ATF1 EWSR1::CREM* |
| Epithelial-myoepithelial carcinoma | Biphasic pattern of luminal epithelial and abluminal myoepithelial cells with invasive growth | CK7+ in epithelium; p63+, SMA+, calponin+, SMMHC+, S-100+ in myoepithelium | *HRAS* codon 61 mutations |
| Myoepithelial carcinoma | Multinodular pattern of myoepithelial cells with invasive growth | Variably p40+, p63+, SOX10+, S-100+, MSA+, SMA+, cytokeratin+ | *EWSR1* rearrangements and mutations *PLAG1* fusions with variable partners |
| Mucoepidermoid carcinoma | Mucous cells, intermediate cells, squamoid cells | p63+, p40+, S-100-, SOX10- | *CRTC1::MAML2 CRTC3::MAML2* |
| Oncocytoma | Solid pattern of oncocytic cells | p63+ in basal-like cells |  |
| Clear cell odontogenic carcinoma (CCOC) | Nests, sheets or cords of clear cells in hyaline stroma | Cytokeratin+, p63+, SMA-, calponin- S-100- | *EWSR1::ATF1 EWSR1::CREB EWSR1::CREM* |
| Squamous cell carcinoma | Squamous, often keratinizing cells with invasive growth | p40+, p63+ | Multiple somatic mutations, esp. *TP53* mutations frequent |
| Malignant melanoma | Pleomorphic clear cells with invasive pattern | Melan A+, S-100+, cytokeratin- | *BRAF* mutations |
| Metastatic renal cell carcinoma | Solid or nested pattern of clear cells with conspicuous blood vessels | RCC+, CD10+, vimentin+, CK7-, p63-, | Multiple somatic mutations |

Fig. 1

Click here to access/download;Figure;Fig. 1.tif

NLS: Nuclear localization signal motif
C2H2-type ZNF 1-7: C2H2-type (classical) zinc fingers
MDT: Massively decrease transcription activity
MAT: Massively activates transcription
◄──► : Untranslated region / 5' and 3' UTR