Pathology - Research and Practice xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Pathology - Research and Practice

journal homepage: www.elsevier.com/locate/prp

Hyalinizing clear cell carcinoma of salivary glands: A retrospective study focused on uncommon morphology, immunohistochemistry, and detection of gene fusion using fluorescence in situ hybridization

Yi-nuo Zhao[a], Xiao Wang[a], Fen-hua Liang[a], Wen-jie Zhang[a], Xiang-tao Song[b,*]

[a] People's Hospital of Rizhao, Shandong, China
[b] Yantai Hospital of Traditional Chinese Medicine, Shandong, China

ARTICLE INFO

Keywords:
Hyalinizing clear cell carcinoma
Salivary glands
Morphology
Immunohistochemistry
EWSR1
FISH

ABSTRACT

Aims: To investigate histological, immunohistochemical, and molecular features, especially uncommon morphology of hyalinizing clear cell carcinoma (HCCC) to expand the morphological spectrum of HCCC.
Methods and results: We examined 5 cases of HCCC by histological, immunohistochemical, and molecular analysis. Generally, 5 HCCC cases shared similar characteristics, exhibiting clear to slightly eosinophilic cells arranged in cords, nests, islands, or trabeculae with a hyalinized stroma, while myxoid stroma, perineural invasion, and polygonal cells with high-grade nuclei were observed in 3 cases. Immunohistochemically, 5 cases were entirely immunoreactive for CKpan, whereas 80% HCCC cases were positive for P63, and CK14. None expressed immunoreactivity for S-100, Calponin, or GFAP. The positive rate of Ki-67 staining was about 5% in the classic area of case 3, but 40% in the high-grade area. As for the result of FISH findings, EWSR1 gene break was detected in all 5 HCCC cases.
Conclusions: Our study has expanded the morphological spectrum of HCCC, and proposed the diagnosis of HCCC should be confirmed by fully analyzing histological, immunohistochemical, and molecular features practically.

1. Introduction

Hyalinizing clear cell carcinoma (HCCC) was first described by Milchgrub et al. [1]. It is an extremely rare tumor entity of salivary gland origin, which is recognized as a low-grade carcinoma mainly arising from minor salivary glands with a female predominance [2–5]. Generally, HCCC has an indolent clinical course with a good overall outcome, and rarely appears lymph node or distant metastasis [3,6–8]. Morphologically, this tumor characteristically has cords, trabeculae, or nests of clear to pale eosinophilic cells within a hyalinized stroma. HCCC is occasionally confused in morphology with clear cell variant of mucoepidermoid carcinoma, monomorphic variant of epithelial-myoepithelial carcinoma, as well as clear cell variant of myoepithelial carcinoma, etc. Given the overlap with these tumors of salivary glands, the diagnosis of HCCC may be challenging [9–12]. Fortunately, in 2011, Antonescu et al. found the presence of *EWSR1* rearrangement by fluorescence in situ hybridization (FISH), and the presence of *EWSR1-ATF1* fusion transcript by reverse transcriptase-polymerase chain reaction (RT-PCR) in HCCC [13]. Due to the extremely low incidence of *EWSR1-ATF1* fusion transcript in other clear cell tumors, the finding of this specific and recurrent translocation leads to additional help in the diagnosis of HCCC, which has also been proven by following small case series and case reports [14–18].

Inspired by original studies on HCCC, we carry out a clinicopathological study on 5 additional genetically confirmed cases of this rare tumor entity with emphasis on uncommon morphology, immunophenotypic status, and detection of *EWSR1* rearrangement by FISH analysis.

2. Materials and methods

2.1. Case selection

After reviewing all primary tumors overlapping with HCCC in salivary glands and other related sites, 5 cases of HCCC were screened out from 2000 to 2016 at the Department of Pathology in People's Hospital of Rizhao, in accordance with the characteristic features described by *WHO classification of Head and Neck Tumors* and the recent literature on HCCC. The haematoxylin and eosin (H&E) slides were reviewed by two experienced doctors, and clinical histories of each patient were obtained whenever available. This retrospective study was approved by the Institute Research Ethics Committee of People's Hospital of Rizhao.

* Corresponding author at: Yantai Hospital of Traditional Chinese Medicine, Shandong, 264000, China.
E-mail address: xiangtaosong@163.com (X.-t. Song).

https://doi.org/10.1016/j.prp.2017.12.021
Received 13 November 2017; Received in revised form 11 December 2017; Accepted 31 December 2017

Table 1
Clinical findings of 5 HCCC cases.

Case	Age/Sex	Size(cm)	Site	Original stage	Margin status	Perineural invasion	Vascular invasion	Follow-up(month)
1	56/F	1.5	base of tongue	$pT_1N_0M_0$, I	Neg	No	No	78; NED
2	49/F	2.3	hard palate	$pT_2N_0M_0$, II	Neg	No	No	106; NED
3	78/M	4.1	parotid gland	$pT_3N_0M_0$, III	Neg	No	No	11; DOD
4	38/F	0.8	base of tongue	$pT_1N_0M_0$, I	Neg	No	No	46; NED
5	62/M	1.8	nasopharynx	$pT_{4a}N_0M_0$, IV_A	Neg	Yes	No	8; NED

F indicates female; M, male; Neg, negative; NED, no evidence of disease; DOD, dead of disease.

2.2. Immunohistochemical staining

The excised specimens were fixed in 10% formaldehyde, and embedded in paraffin. Sections of 3 μm thickness from each block were subjected to immunohistochemical staining, using the antibodies as follows: CKpan (1:100 dilution; Dako, Glostrup, Denmark), P63 (1:200 dilution; Abcam, Cambridge, UK), CK14 (1:100 dilution; Abcam, Cambridge, UK), S-100 (1:200 dilution; Dako, Glostrup, Denmark), Calponin (1:50 dilution; Dako, Glostrup, Denmark), GFAP (1:1000 dilution; Dako, Glostrup, Denmark), Ki-67 (1:300 dilution; Abcam, Cambridge, UK). The immunoreaction was performed by the labelled streptavidin-biotin method and overnight incubation, when 3,3′-Diaminobenzidine was used as the chromogen.

The immunoreactivity was interpreted using a semiquantitative method. The resulting score was calculated by multiplying the staining intensity (0 = no staining; 1 = mild staining; 2 = moderate staining; 3 = strong staining) by the percentage of positive tumor cells (0–100). Immunostaining was considered 0 or negative when the score was < 25; 1+ or weak when 26–100; 2+ or moderate when 101–200; 3+ or strong when 201–300. For all antibodies above, a minimum of 100 tumor cells were meticulously examined.

2.3. FISH analysis

Before hybridization, each H&E slide was reviewed to confirm areas containing cell clusters for counting cells. Sections of 3 μm thickness were proposal from buffered formalin-fixed, paraffin-embedded tissue blocks. The slides were then deparaffinized by three 15-min xylene washes, flushed in absolute ethanol for 10 min, and air-dried. Subsequently, slides deparaffinized were subjected to heat preprocessing in distilled water, and digested using 0.25% pepsin and 0.01 M HCl for 15 min at 37 °C. After washing twice in 2 × sodium saline citrates (SSC), the slides were dehydrated by gradient immersion in 70%, 85% and 100% ethanol for 1 min each at room temperature, and air-dried afterwards. The FISH analysis was performed using *EWSR1* gene break-apart probe (Abbott Molecular, IL, USA), which was appropriately applied on each slide, covered with a glass coverslip, and sealed with a rubber cement. The slides were codenatured in an in situ thermocycler (System 1000, Perkin Elmer, Baesweiler, Germany) at 88 °C for 9 min, annealed at 37 °C, and hybridized in a humid chamber at 37 °C overnight. After posthybridization wash in 0.4 × SCC at 70 °C for 2 min and 2 × SCC at room temperature for 2 min, the slides were air-dried in the dark, counterstained with 4,6-diamino-2-phenylindole, coverslipped, and immediately evaluated.

For the FISH evaluation, signals were considered to be split, when the green and red signals were separated by a distance ≥2 signal diameters. A positive result was reported, when ≥ 10% of tumor nuclei demonstrated the split-signal pattern on the basis of *EWSR1* break-apart FISH assays [3,4,13,16,19–21].

3. Results

3.1. Patients

Prior to the description of HCCC in *WHO Classification of Head and Neck Tumors* as a novel entity, 5 HCCC cases were presumably diagnosed as myoepithelial carcinoma (case 1), mucoepidermoid carcinoma (case 2, 4), and clear cell carcinoma, not otherwise specified (case 3, 5). The patients were 3 female and 2 male, when the ages at diagnosis ranged from 38 to 78 years (median, 56y; mean, 56.6y). The symptoms at presentation varied, including asymptomatic, dysphagia, and ulceration, and the size of the tumors ranged from 0.8 to 4.1 cm (median, 1.8 cm; mean, 2.1 cm). Also, the locations of the tumors were diverse, including base of tongue, hard palate, parotid gland, and nasopharynx. The patients totally underwent tumor resection or enlargement resection, while 2 of 5 patients received radiotherapy after the surgery. During the follow-up period, 4 patients were alive with no evidence of disease, whereas 1 of 5 died of disease. Approximately 6 months after the initial resection, this patient underwent a biopsy confirming recurrence at the primary site, and the CT imaging then confirmed the development of lung metastases as well. Unfortunately, after 5 months of symptomatic treatment, this patient died. In addition, more details of 5 HCCC cases were summarized in Table 1.

3.2. Morphology

Histologically, 3 tumors (case 1, 2, 4) were well-circumscribed masses composed of cells arranged in cords, trabeculae, and nests structures. The tumor cells displayed abundant clear to slightly eosinophilic cytoplasm with centrally placed nuclei, and inconspicuous nucleoli. 2 of 3 cases showed a background of a classically hyalinized stroma (Fig. 1A–C), while the rest 1 (case 4) had a myxoid stroma (Fig. 1D). No evidence of necrosis, or vascular or perineural invasion was found.

1 tumor (case 3) was an expansile mass composed of cells in cords to solid architectures. The majority of cells revealed similar morphological features, with abundant clear, variably eosinophilic cytoplasm embedded in a hyalinized stroma. However, unusual morphology was present in < 10% of the tumor, showing large polygonal cells with high-grade nuclei (Fig. 1E).

1 tumor (case 5) was an ill-defined mass composed of tumor cells predominantly arranged in cords to trabeculae patterns with occasional nests structures, demonstrating clear to slightly eosinophilic cytoplasm. Besides moderately irregular nuclei with mildly enlarged nucleoli, explicit perineural invasion was identified in this tumor (Fig. 1F).

3.3. Immunohistochemistry

100% HCCC cases (5/5) were moderately (2+) to strongly (3+) immunoreactive for CKpan. Additionally, 80% HCCC cases (4/5) displayed moderate (2+) to strong (3+) positivity for P63, whereas 80% (4/5) were weakly (1+) to strongly (3+) reactive for CK14 (Fig. 2A–C). However, none (0/5) expressed immunoreactivity for S-100, Calponin, or GFAP. As for Ki-67 in case 3, the positive rate was