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Research article

MAML2 gene rearrangement occurs in all Warthin-like mucoepidermoid carcinoma: A reappraisal in a series of 29 cases

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ABSTRACT

Background: Warthin-like Mucoepidermoid carcinoma (MEC) is a new and rare morphological variant of MEC, with only a few case reports in the literature. The clinicopathological, molecular features and bio-behaviors of Warthin-like MEC has not been studied extensively. We reappraisal all Warthin-like MEC patients diagnosed and treated at our hospital.
Methods: Patient characteristics including clinicopathological features, genetic aberrations, treatment, and prognostic information were assessed and evaluated.
Results: Twenty-nine Warthin-like MEC patients were identified, 19 patients were female (65.5 %), and 10 were male (34.5 %). The patients' age varied widely from 8 to 68 years (mean 42.3 years). Genetic aberrations of *MAML2* rearrangement were detected in all Warthin-like MEC patients, which suggesting this genetic event is the unique feature of Warthin-like MEC. Twenty-five patients (86.2 %) were assessed as having a low-stage disease (I/II), and four (13.8 %) as having high-clinical stage disease (III/IV). More than half of the patients (16/29) underwent only partial sialoadenectomy; 2 patients underwent extended sialoadenectomy, and 11 patients underwent extended sialoadenectomy with cervical lymph node dissection. After a median follow-up time of 73 months (5–128 months), Twenty-eight patients were alive without recurrence at the end of the follow-up period, one patient died 1 year after surgery due to lung metastasis.
Conclusion: Our data suggested that most Warthin-like MEC exhibited mild clinicopathological course and less aggressive bio-behavior, and an aggressive bio-behavior seemed to be very rare. In addition, in the salivary gland, MAML2 rearrangement seems to be a unique molecular feature of salivary Warthin-like MEC.

1. Introduction

Mucoepidermoid carcinoma (MEC) is a highly heterogeneous neoplasm arising mainly from salivary glands [1,2]. MEC shows a

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broad spectrum of morphologies [3,4]. Typical MEC mainly comprises mucous, intermedia, and epidermoid cells, although clear, oncocytic, mucoacinar and spindle cell-predominant variants of MEC also exist [5–7]. An unusual histologic appearance of the oncocytic epithelium with dense lymphoid infiltration has been identified in some MEC cases. The first report describing a multi-layered oncocytic epithelium and nests of squamous and mucous cells in MEC was published by Garcia et al. (García et al., 2011) Six cases were initially diagnosed as oncocytic MEC with a large number of Warthin-like lymphoid stroma. Subsequently, Ishibashi et al. formally proposed and named the variant of Warthin-like MEC in 2015 [8]. In practical diagnosis, it is difficult to differentiate Warthin-like MEC from metaplastic Warthin tumor (mWT), especially when the histologic image shows an atypical bi-layered oncocytic epithelium with dense lymphoid infiltration with or without a small focal group of mucous cells in MEC. The diagnostic criteria of Warthin-like MEC are vague. In addition, as a new subtype of MEC, there are few data on Warthin-like MEC. Therefore, investigation of its specific clinical, histological, and genomic features is needed to facilitate accurate diagnosis and treatment.

Recent studies have demonstrated that 33.7–85.5 % of MECs harbor the *CREB*-regulated transcriptional co-activator 1 (*CRTC1*)-mastermind-like gene family-2 (*MAML2*) translocation, which is associated with low-grade (LG) or intermediate-grade (IG) MEC [9,10]. The fusion product is formed by *CRTC1* (also known as *MECT1*, *TORC1*, and *WAMTP1*), a *CREB* transcriptional coactivator gene in chromosome band 19p13, and the transcriptional coactivator *MAML2* in the N-terminal Notch-binding domain of chromosome band 11q12. This translocation was discovered by Nordkvist et al. and Tonon et al. [11,12]. Subsequently, *CRTC3*, which has 32 % homology with *CRTC1*, was identified by Fehr [13] to form the *CRTC3-MAML2* fusion gene in MEC, which results from the translocation t (11 ~ 15) (q21; q26) [14]. *CRTC1*, as a member of the CREB family, regulates cellular metabolism and growth by co-activating CREB-mediated transcription. As a member of a transcriptional coactivation family, *MAML2* exerts its biological function mainly via the transcriptional activation of Notch target genes [15]. The fusion protein is a nuclear protein with no enzymatic activity. *MAML2* rearrangement may provide an auxiliary cue for Warthin-like MEC diagnosis.

In this study, we collected all patients with Warthin-like MEC of head and neck diagnosed and treated at our hospital between 2005 and 2020, and analyzed the clinicopathological and the presence of *MAML2* by FISH in Warthin-like MECs in order to increase the understanding of the diagnosis and guide treatment of Warthin-like MEC.

2. Material and methods

This retrospective study was approved by the Institutional Review Board of the Peking University School and Hospital of Stomatology (PKUSSIRB-201948111).

2.1. Case selection

Six hundred and eighteen cases of head-and-neck MEC were identified from the surgical pathology database between January 2005 and July 2020. All hematoxylin and eosin (H&E)-stained slides were reviewed and re-graded by two head-and-neck pathologists (Binbin Li and Huiying He). Inclusion criteria of Warthin-like MEC: i) MEC diagnosed according to the criteria of WHO Classifications of Head and Neck Tumors (2022). (MEC is composed of mucinous, intermediate (clear-cell) and squamoid tumor cells forming cystic and solid patterns). ii) Additionally, all cases histologically consisted of the diversity of cystic lining oncocytic epithelium and dense lymphoid stroma, with epithelial components arranged in a papillary cystic pattern. Each case was re-graded using the AFIP [16], Brandwein [17], Healey [18] and MSK [19] grading systems by two pathologists. Cases with metastatic MEC from other primary lesions and secondary tumors were excluded. Inclusion criteria of metaplastic Warthin tumor (mWT): i) WT diagnosed according to the criteria of WHO Classifications of Head and Neck Tumors (2022). (Warthin Tumor is composed of oncocytic epithelial cells lining ductal, papillary, and cystic structures in a lymphoid stroma); ii) metaplastic changes of mucinous and/or epidermoid. Furthermore the following criteria were used to exclude the malignant transformation of WT: i) the presence of a pre-existing benign WT; ii) the presence of transition zones from benign oncocytic to malignant epithelial; iii) infiltrating growth in the surrounding lymphoid tissue; iv) exclusion of metastases to the lymphoid stroma component of a primary extra salivary tumor [20]. Diagnostic H&E-stained slides and formalin-fixed paraffin-embedded (FFPE) blocks were retrieved from the surgical pathology archives.

Clinical characteristics were obtained from the electronic medical records, including the age at the time of diagnosis, sex, tumor site, symptoms, smoking history and signs, the interval between initial symptoms and histologic verification, surgical treatment (local resection, complete resection with or without neck lymph node dissection), and adjuvant treatment (radiation therapy, chemotherapy, and/or I^{125} seed implantation). The tumors were staged according to the American Joint Committee on Cancer (AJCC) Cancer Staging Manual, 8th edition [21]. Recurrence was determined based on clinical examination or imaging studies and confirmed via fine-needle aspiration or biopsy.

Patient status at the last follow-up was classified as follows: no evidence of disease, alive with disease, and died of disease. Clinical follow-up information was available for all chosen cases.

2.2. Histological evaluation

H&E staining was performed according to standard procedures. Briefly, sections of 4 μm thickness were generated from paraffin-embedded tissue and mounted on slides. After deparaffinization and rehydration, the sections were stained with Mayer's hematoxylin solution and 0.125 % eosin. Following staining, the sections were washed, dehydrated, decalcified, and mounted with neutral balsam mountant. After H&E staining, the tumor size, growth pattern (well-defined/peripheral growth in nests and islands), increase in mitosis (4+, 2–3, or 0–1/10 high-power field [HPF]), tumor necrosis status, the incidence of atypical mitosis, nuclear pleomorphism

status, the proportion of mucous cells, intracystic component (solid, <50 %, <25 %), and presence of perineural invasion, bone invasion, vascular invasion, muscle invasion, lymph node metastasis, and distant metastasis were evaluated.

2.3. Fluorescent in situ hybridization

All Paraffin-embedded sections were assessed by *MAML2* probes for fluorescence *in situ* hybridization (FISH). The probes were dual-color break-apart probes. One probe was a 680-kb fragment of the 5′ *MAML2* gene labeled with ZyGreen, and the other was a 370-kb fragment of the 3′ *MAML2* gene labeled with ZyOrange (Z-2014-200, Zytovision, Bremerhaven, Germany). Briefly, 3-μm sections on slides were baked at 60 °C for 2 h, deparaffinized in xylene twice for 10 min (Decon Laboratories Inc., King of Prussia, PA), and dehydrated twice with 100 % alcohol. They were pretreated using the Vysis Paraffin Pretreatment Kit (Abbott Park, IL). The slides were digested for 30 min in protease solution (0.5 mg/mL) at room temperature, followed by denaturation for 5 min in 70 % formamide (Chemicon, Billerica, MA) and dehydration in ethanol. The slides were incubated with the probes overnight in a humidified chamber at 37 °C. The probes were denatured for 5 min before hybridization at room temperature. Post-hybridization washes were carried out using 0.3 % NP40/2 × SSC at 73 °C for 2 min and subsequently, 0.3 % NP40/2 × SSC at room temperature for 1 min. The slides were air-dried in the dark and counterstained with 3 μL of 4,6-diamino-2-phenylindole (DAPI; Vysis). One hundred randomly selected nonoverlapping tumor cells were manually evaluated for the presence of orange and green or yellow fluorescent signals and the percent split signal was recorded. Positive cases were defined as more than 15 % break-apart signals [22].

2.4. Statistical analysis

Statistical analysis was performed using Student t-test and Fisher's exact tests. Survival analysis was performed using the Kaplan-Meier and Life Table method. Statistical programming was conducted in R (v. 4.0.0) and GraphPad Prism (v. 9.0), using the packages

Table 1
Clinicopathological characteristics of patients with Warthin-like MEC.

	Warthin-like MEC
Evaluable patients, N	29
Age, years, n (%)	
<50	16 (55.2)
≥50	13 (44.8)
Mean (range)	42.3 (8–68)
Gender, n (%)	
Male	10 (34.5)
Female	19 (65.5)
Smoking history, n (%)	2 (6.9)
Location, n (%)	
Parotid gland	18 (62.1)
Other sites	11 (37.9)
Tumor size (cm)	
Mean (range)	1.9 (0.4–4)
Well circumscribed	1 (3.4)
Cellular atypia (mild, moderate and severe)	26,1,2 (90.7,3.4,7.9)
Necrosis	3 (7.9)
Invasive growth	20 (69.0)
Nodal status	
Positive	3 (10.3)
Negative	26 (89.7)
Stage,n (%)	
I+II	25 (86.2)
III+IV	4 (13.8)
Histology Grade system (LG, IG, HG, %)	
AFIP	82.8,6.9,10.3
Brandwein	20.7,13.8,65.5
Modified Healy	69.0,20.7,10.3
Katabi	48.3,41.4,10.3
Treatment, n (%)	
Resection only	18 (62.1)
Extended resection & cervical Lymph node dissection	11 (37.9)
Adjuvant treatment, n (%)	
rRdiation	5 (17.2)
I^{125} seed implantation	4 (13.8)
Follow up	5-128 (73.3)
Prognosis, n (%)	
Live without recurrent	28 (96.6)
Recurrence, metastasis, and Death of disease	1 (3.4)

Note: N: number.

ggplot2. $P < 0$ 0.05 (2-tailed) was considered indicative of statistical significance.

3. Results

3.1. Clinical features

The clinicopathological features of 29 Warthin-like MECs are listed in Table 1. Among the patients diagnosed with Warthin-like MEC, 34.5 % (10/29) were males and 65.5 % (19/29) were females, giving a ratio of approximately 1:2. The patients' age varied widely from 8 to 68 years (mean 42.3 years). The mean tumor size was 1.9 cm (0.4–4.0 cm). The most frequent site was the parotid

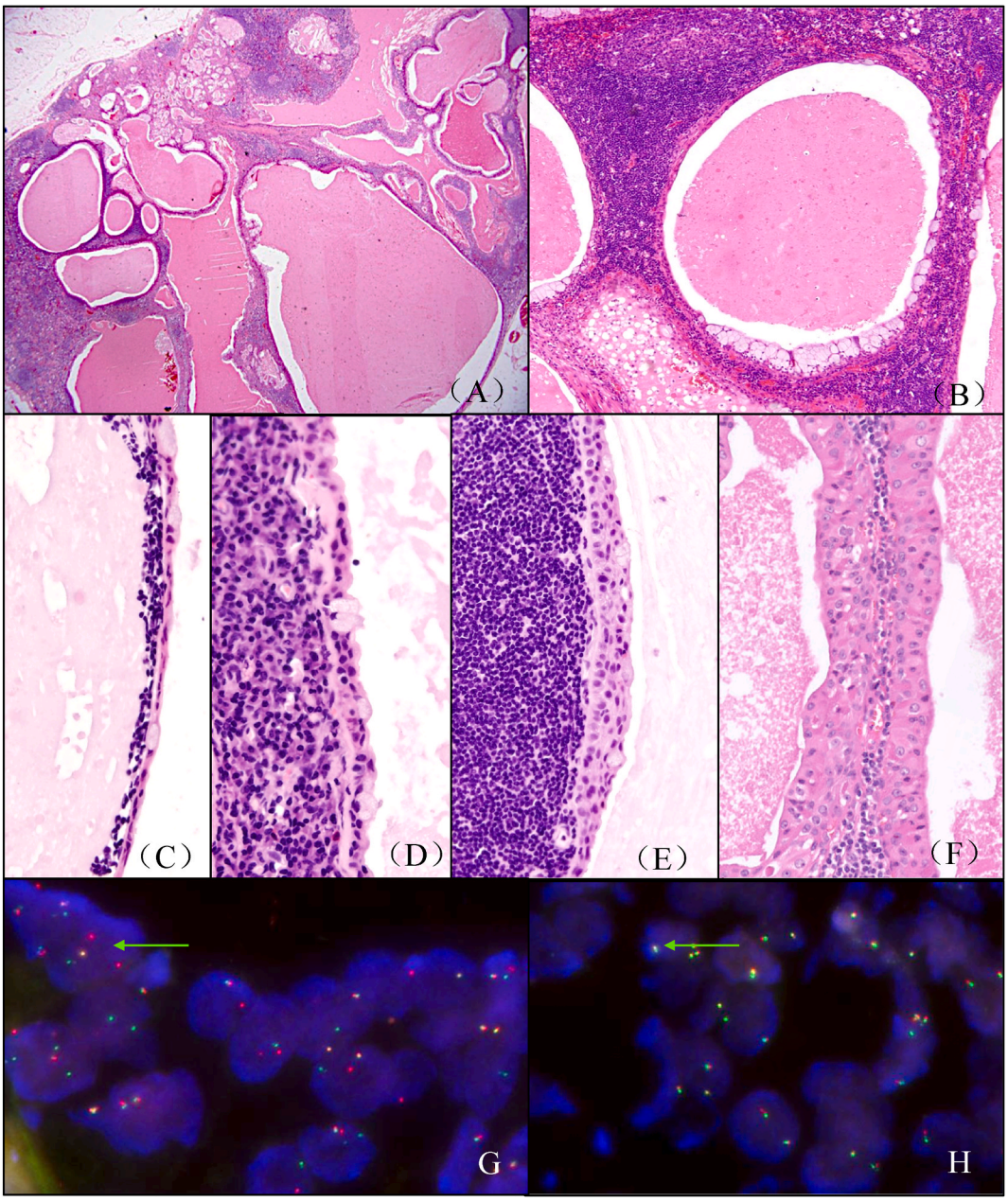

Fig. 1. Histopathological features of Warthin like-MEC (A-E) and mWT(F). A: Typical feature of Warthin-like MEC under low magnification (HEX100); B: Dense lymphocytes (HEX200); C–E: mono-layered, disorganized bi-layered and multilayered oncocytic epithelia in Warthin like-MEC (HEX400); F: Typical bi-layered tumor epithelium in mWTs (HEX400); *G: MAML2* fusion positive in Warthin-Like MEC (× 1000); H: *MAML2* negative in mWT (× 1000). Note: HE: hematoxylin and eosin; mWT: metaplastic Warthin Tumor; Warthin-Like MEC: Warthin-Like Mucoepidermoid Carcinoma. FISH: Fluorescence In situ hybridization.

gland, accounting for 62.1 % of all cases, followed by the sublingual gland, mandible, tongue, and infratemporal fossa. Two patients had a history of smoking (6.9 %); one of 2 years and the other of >30 years. There were three alcohol drinkers (10.3 %). Seven patients were symptomatic, of which the most common symptom was pain (13.8 %, 4/29), whereas swelling, numbness, and restricted mouth opening were presented in one patient each. The mean interval between the initial symptoms and diagnosis was 21 months (1–180 months). Twenty-five patients (86.2 %) were assessed as having a low-stage disease (I/II), and four (13.8 %) as having high-clinical stage disease (III/IV).

Seventeen mWT cases were included. The mean age of mWT patients was 62.23 years with a range of 33–80 years. The male: female ratio was approximately 4:1. A history of cigarette smoking was noted in eight patients (50 %). Alcohol intake was also documented (13 patients did not consume alcohol, and 4 patients consumed more than 30 g of alcohol daily). The mean tumor size was 3 cm (range 2–8 cm). All patients were treated with parotidectomy. None of the patients experienced recurrence during follow-up (mean 31 months, range 6–69 months). The patients with Warthin-like MEC were younger and had smaller tumors than did those with mWTs.

3.2. Histologic features

Microscopically, each Warthin-like MEC consisted of multiple cysts of various shapes and sizes under low magnification (Fig. 1A). The cysts were filled with eosinophilic substances and infiltrated by dense lymphocytes (Fig. 1B). Cystic spaces were lined by mono-layered, disorganized bi-layered and multilayered oncocytic cells (Fig. 1C–E). The cells were flattened with vacuolar nuclei. These oncocytes cells are more likely the squamoid cells. A transition zone between the disorganized bi-layered and mono-/multilayered oncocytic epithelia was observed in most Warthin-like MEC patients. The forms of oncocytic epithelium are diverse. In addition, the cords of squamoid cells were usually accompanied by areas of mucous cells. The germinal center was found frequently in the stroma. Extensive hyalinization and mucus extravasation were also observed. Peripheral growth in nests and islands was observed in 20 (69.0 %) patients. Lymphovascular invasion was noted in 3 patients (10.3 %), perineural invasion in 1 patient (3.4 %), and bone invasion, tumor necrosis and moderate or severe cellular atypia were present in 3 patients (10.3 %)(Table S1).

The 29 cases of Warthin-like MEC were graded according to the four grading systems as follows: AFIP, 24 (82.8 %) LG, 2 (6.9 %) IG, 3 (10.3 %) HG; Brandwein, 6 (20.7 %) LG, 4 (13.8 %) IG, 19 (65.5 %) HG; Katabi, 14 (48.3 %) LG, 12 (41.4 %) IG, 3 (10.3 %) HG; and modified Healy grading, 20 (69.0 %) LG, 6 (20.7 %) IG, 3 (10.3 %) HG. Only the grades of 31.0 % (9/29) of the patients achieved consensus among all four grading systems. Twenty LG tumors according to AFIP grading were upgraded to IG or HG tumors according to the Brandwein grading system. Modified Healy and Katabi grading were inconsistent for 27.6 % of the patients (8/29). One case met the criteria for an HG tumor for all four grading systems. The grades of 29 Warthin-like MECs based on the four grading systems are shown in Table 1.

Of the 17 mWTs, squamous metaplasia was encountered in 41.2 % (7/17) and was often associated with ischemic necrosis. Mucinous metaplasia appeared in 58.8 % (10/17) of the patients. The mucous cells were often large, resembling goblet cells, and interspersed within the oncocytic epithelium as single cells or cell clusters. The typical bi-layered tumor epithelium was always found in the mWTs (Fig. 1F). The key features for differential diagnosis of salivary Warthin-like MEC and mWT were listed in Table 2.

Table 2
Summary of key points in differential diagnosis of salivary Warthin-like MEC, MEC with TALP and mWT.

	Tumor genesis	Tumor parenchyma				Tumor mesenchymal	Molecular signature	
		structure	Oncocytic Cell	Epidermoid cell	Mucus cell	Lymphocytes	Mucus extravasation	
Warthin-like MEC MEC	excretory ducts	glandular architecture with cystic structures	The diversity of cystic lining epithelial and the transition zone between the disorganized bi-layered oncocytic epithelium and mono-/multi-layered oncocytic epithelium appeared	Epidermoid	Mucus- producing cells	Dense lymphocytic infiltrate with lymphoid follicle	occur	CRTC1/3-MAML2 rearrangement
MEC with TALP		Solid or cystic structures	Lack the diversity of cystic lining oncocytic epithelium					
mWT	excretory ducts	Papillary architecture	Typical bi-layered oncocytic cells.	Focal Nonkeratinized squamoid cells	mucoid metaplasia accompanied with necrosis, haemorrhages and fibrosis.	Dense lymphocytic infiltrate with lymphoid follicle	absent	Non-specific

Note MEC: mucoepidermoid carcinoma, TALP: Tumor-associated lymphoid proliferation (TALP), mWT: Metaplastic Warthin tumor.

3.3. MAML2 rearrangement tested by FISH

The *MAML2* rearrangement was positively detected in all 29 Warthin-like MEC cases. Signals indicating translocation were detected in 23.0–95 % (median 61 %) of the fusion-positive cases. 17 mWT cases were included, and all of them lack *MAML2* rearrangement. Representative FISH images are shown in Fig. 1G and H.

3.4. Treatment and follow-up information

More than half of the patients (18/29, 62 %) underwent extended resection of the mass, and 11 (38 %) patients underwent extended resection of the mass and cervical lymph node dissection. Five patients underwent postoperative radiotherapy, and four patients underwent I^{125} seed implantation. However, there is no relationship between excision modality and prognosis in the Warthin-like MEC patients ($P = 0.448$). Follow-up information was available for all patients, and the mean follow-up time was 73 months (5–128 months). The detailed outcomes of the cohort are listed in Table 1. At the end of follow-up, one patient died. The time between the surgery and the first recurrence was 12 months, and lung metastases were observed at the same time. Twenty-eight patients were alive without recurrence at the end of the follow-up period (5–128 months). The overall survival rate of 29 Warthin-like MEC patients was 97.0 %. Fig. 2 displays the OS curve for Warthin-like MEC patients.

4. Discussion

Owing to its relative scarcity and novelty, few case reports of Warthin-like MEC have been reported in the English-language literature [23–26]; García et al., 2011 [27–29]; from 2015 to now, all occurred in the parotid gland, and most were painless masses with progressive enlargement. Few patients reported pain or a history of numbness, suggesting indolent malignancy. The overall prognosis of the patients was favorable; only one case recurred 4 years after the operation [28].

In this study, we analyzed the clinicopathological and genetical features of the 29 cases of Warthin-like MEC in head and neck, which is the largest single-center retrospective study of this kind reported in English literature to date.

Patients with salivary gland MEC have a wide age range, from 7 to 86 years old, with a similar mean and median age of 47 years [30]. In Andrew et al. study [9], the average age of the MEC with MAML2 rearrangement patients was 49.3 years. The ratio of male: female was almost 1:1 (26:27). The most frequent primary site was the parotid gland. The histological grading of LG, IntG and HG was 34(64 %),18(32 %) and 1 (2 %). And TNM stage I and II accounted for 73 %, III and IV accounted for 26 %. In the present study, the ages of the Warthin-like MEC patients ranged from 8 to 68 years old, with a mean age of 42.3 years and a median of 45 years. They were just slightly younger. In Nishimura et al.'s review of 238 cases of Warthin tumor, the age distribution ranged from 20 to 85 years with a mean age of 63 years [31]. However, in comparison to Warthin tumor, Warthin-like MEC patients were significantly younger. The male-to-female ratio of Warthin-like MEC in the present study was 1:2, showing a predilection towards females, which is consistent with the results of MEC reported by Taniuchi [32]. On the contrary, Warthin tumor was seen predominantly in male patients (Male/Female: 7/1) in previous study [33].

The clinical presentation of most MEC usually is nonspecific. Its characteristic feature is spontaneous pain/tenderness or adhesion to the surrounding tissue [32]. In the present study, the disease duration after first detection of a mass ranged from 2 month to 15 years.

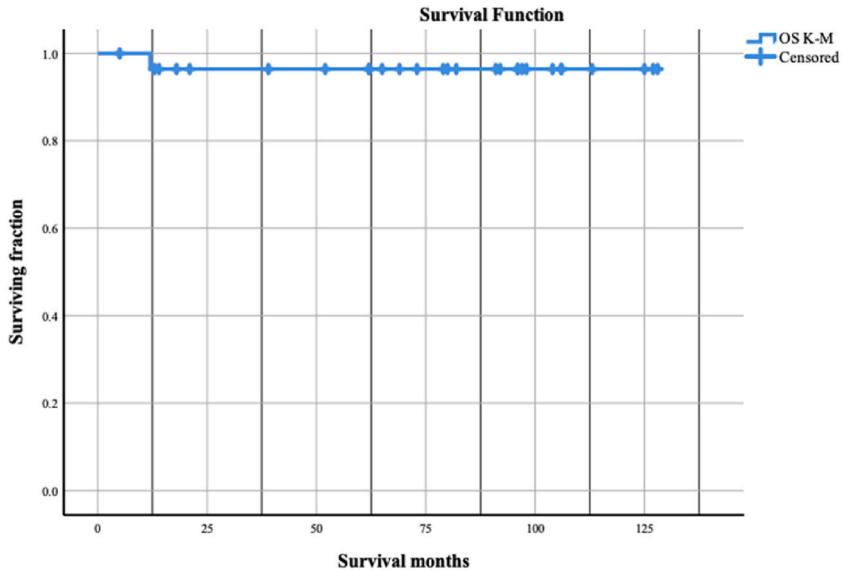

Fig. 2. Kaplan-Meier overall survival curve of Warthin-Like Mucoepidermoid Carcinoma patients.

A painless mass is the most common presenting sign. Pain, swelling, numbness, and restricted mouth opening, were unusual symptoms in our data. Obvious cervical lymph node involvement is detected clinically about 26 % of patients with salivary gland MEC. In our study, regional lymph nodes metastasis was only observed in 10.3 % (3/29) of patients of salivary Warthin-like MEC, which was significantly lower than MEC with MAML2 rearrangement patients (17 %) [9].

The histological features of Warthin-like MEC are characteristic and identical to disorganized bilayer oncocytic epithelial and abundant lymphoid stroma. At least partially, classic histologic cell types of MEC (mucinous cells, intermediate cells, and epidermoid cells) can be observed. In some Warthin-like MEC, extensive hyalinization and mucus extravasation could be observed. In salivary gland tumor, an mWT should be considered for distinction from Warthin-like MEC. Commonly, smoking was likely to trigger the development of an mWT. And the male was the most suspicious population. Histologically, only 1.8 % WT presented significant squamous or mucinous metaplasia [34]. Acute, chronic infection, injury, FNAB may be the cause of metaplasia in WT. Squamous metaplastic lesion is commonly accompanied by extensive hemorrhage, necrosis, and infarction and it is often related to the previous FNAB procedures. In the Mucinous metaplasia area, cells with clear and polarized nuclei resembling goblet cells interspersed with the layers of the oncocytic epithelium. And the oncocytes changes possibly following the damage to the mitochondrial DNA induced by smoking. The accurate differential diagnosis of mWT is as following: the epithelial structure is the typical glandular epithelium dominated by high columnar cells; multiple foci of classic bi-layered oncocytic epithelium in an mWT; lymphocytes and a germinal center in the stroma. Key points in the differential diagnosis of Warthin-like MEC, mWT and MEC with tumor-associated lymphoid proliferation were shown in Table 2. Caution is needed to avoid misidentifying an mWT with multilayered oncocytic cells as Warthin-like MEC in cases lacking a solid nest of epidermoid cells. By contrast, a glandular epithelial structure points to a diagnosis of mWT.

In the present study, most Warthin-like MEC were low-grade and have a good prognosis, but rarely, the high-grade Warthin-like MEC still show the ability for distant metastasis. Except the aggressive histological phenotypes，the intermediate/high-grade Warthin-like MEC cases fulfill both morphological and molecular criteria for the diagnosis of Warthin-like MEC. Salivary MEC is characterized by a high degree of tumoral heterogeneity. And in clinical, the most widely used grading systems are the AFIP and Brandwein grading system, Healey grade, and the MSK grading system. However, histology grading is subjective and varies significantly among grading systems. Our data suggests that the heterogeneity of Warthin-like MEC is a factor that makes some limitations in accurate prognosis prediction and individualized precision therapy. Evaluation of detailed histological characteristics and molecular spectrums of the masses of Warthin-like MEC has pivotal importance to create comprehensive, clinically useful, and precise diagnoses. Owing to the smaller number of high-grade Warthin-like MEC cases in the present study, the clinicopathological characteristic and bio-behaviors of high-grade Warthin-like MEC need to be investigated further.

In the present study, all Warthin-like MEC patients had *MAML2* rearrangement, a finding consistent with the data on existing literatures. The results indicated that *MAML2* rearrangement might be a critical biological process contributing to the development of Warthin-like MEC.

In the study by Fehr et al., the 5-year overall survival rates of patients with MEC were 79.7 % [35]. In the present study, the overall 5-year survival rate of patients with salivary Warthin-like MEC was 97.0 %, which is higher than those of previous studies of patients with salivary MEC. Radiotherapy is used as first-line therapy for advanced or relapsed MEC patients who are not candidate for surgery. In the present study, although most patients are low-grade, 9 out of 29 patients still underwent postoperative radiotherapy. The exact importance of postoperative radiotherapy in salivary Warthin-like MEC is still inconclusive. A prospective study of salivary Warthin-like MEC who are undergoing a uniform treatment regimen and who have follow-up information will reflect patient outcomes more accurately.

As a group of rare and heterogeneous tumors, Warthin-like Mucoepidermoid carcinoma represents a challenge for precise diagnosis. The main strength of this study is the relatively larger sample size compared to studies currently reported. Another strength is the long follow-up period which increases the clinical relevance of outcomes.

In conclusion, this study indicated that Warthin-like MEC, as a rarely and uncommon subtype of MEC, most exhibited mild clinicopathological course and less aggressive bio-behavior, and an aggressive bio-behavior seems to be very rare. In addition, in the salivary gland, MAML2 rearrangement seems to be a unique molecular feature of salivary Warthin-like MEC. Surgery can yield a satisfactory prognosis for most Warthin-like MEC patients. Considering the broad spectrum of MEC and the sparse research on Warthin-like MEC, further studies focus on targeted therapy to benefit patients with poor prognoses is still required.

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Data availability statement

The data that support the present findings will be made available from the corresponding author upon reasonable request.

Patient consent

All pathology micrographs provided do not contain any identifying marks and are not accompanied by text that might identify the individual concerned.

CRediT authorship contribution statement

Xi Wang: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Formal analysis, Data curation. **Lingchao Liu:** Visualization, Methodology. **Huiying He:** Writing – review & editing, Validation, Supervision, Methodology, Funding acquisition, Formal analysis, Conceptualization. **Binbin Li:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:Binbin Li reports financial support was provided by Chinese Academy of Medical Sciences Innovation Fund for Medical Sciences. Huiying He reports financial support was provided by Peking University. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e24873.

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