Myoepithelial carcinoma of submaxillary gland: A case report

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Abstract. Myoepithelial carcinoma (MEC) is a malignant tumour composed almost entirely of cells differentiated from the myoepithelium. It is rare, most commonly occurring in the parotid gland. In the current study, a rare case of MEC in the submandibular gland was presented. An 82-year-old male patient was admitted with a swelling in the upper left neck that had been present for 60 years. Magnetic resonance imaging indicated a tumour in the region of the left submandibular gland, which was not easy to clearly distinguish from the gland itself and an enhancement scan was recommended but not performed. Furthermore, multiple lymph nodes of varying sizes were present in the submandibular region of the neck on both sides. These enlarged lymph nodes had signs of involvement. Accordingly, a surgery was performed. The pathological diagnosis indicated the presence of a malignant tumour in the left upper neck. The tumour was most likely an MEC with pleomorphic adenoma, based on the results of immunohistochemistry. The size of the tumour mass was 5x5x4 cm. Histology indicated that the tumour cells exhibited infiltrative growth, with a sparse arrangement and a predominance of hyaline and spindle-shaped cells. In certain regions, the tumour tissue displayed characteristics similar to pleomorphic adenoma. The results of the immunohistochemical analysis were as follows: CD31(-), CD34(-), cytokeratin (CK)7 (+), vimentin (+), Ki-67 (+, 70%), erythroid growth factor receptor (-), desmin (-), anaplastic lymphoma kinase (-), P40 (+), CK pan-antibody (+) and epithelial membrane antigen (-). The patient underwent a surgical procedure to remove the lesion in the neck under sedation with complex anaesthesia. The sample excised from the surgery was sent to the pathology department for diagnosis. Given the high recurrence frequency of MEC, monitoring patients closely after surgery is crucial.

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Introduction

Myoepithelial carcinoma (MEC) of the salivary gland is a rare malignant tumour that primarily affects the parotid gland, accounting for ~1% of all salivary gland tumours (1). Its intricate and varied morphology, derived from the epithelial cells of the excretory ducts, often leads to confusion with similar tumours (2). Factors such as cell type, growth pattern and treatment approach may contribute to a lower disease-free survival rate (3). Clinically, MEC typically presents as a slowly growing, asymptomatic mass. Early symptoms are subtle and the clinical manifestations are non-specific, making it easy for both patients and clinicians to overlook the condition. MEC is characterised by the cellular, uniform growth of myoepithelial cells and a multinodular, expansile, invasive pattern with zonal cellular distribution (4). Myoepithelial tumours, including myoepithelioma and MED, demonstrate myoepithelial differentiation and lack a ductal component (5). These neoplasms can be particularly challenging to diagnose, as they are often mistaken for other cutaneous or soft tissue neoplasms (6). The primary criteria for diagnosis are histopathology and immunophenotype. MEC, a highly malignant salivary gland tumour, has a higher propensity to involve cervical or distal lymph nodes than epithelial-MEC, carcinoma ex pleomorphic adenoma or mucinous adenocarcinoma. In addition, MEC has a high risk of recurrence or multiple recurrences following surgery and the prognosis is poor (7). A retrospective analysis was conducted on a single case of MEC of the submandibular gland who was admitted to our hospital. The following aspects were examined: Clinical features, pathological characteristics, differential diagnosis and patient prognosis.

Case report

An 82-year-old man was admitted to the Affiliated Hospital of Jining Medical University (Jining, China) in May 2024 with a 60-year history of a mass on the upper left side of the neck. Approximately 60 years previously, the patient had noticed a lesion on the left side of the neck, initially about the size of a pea. The swelling was asymptomatic, with no itching, numbness or discomfort, and no treatment was sought. However, the swelling gradually increased in size over time. At one month prior, the patient had undergone an ultrasound examination at Juancheng Friendship Hospital (Heze, China), which revealed a homogeneous paramedian gland mass on the left side of the neck. The nature of the irregular lesion adjacent to the left

submandibular gland was unclear. The patient was advised to monitor the condition and no treatment was administered at that time. Five days later, a follow-up ultrasound at the same hospital showed an inhomogeneous mass adjacent to the left submandibular gland, distinct from the mass within the gland itself. Referral to a higher-level hospital for further evaluation and treatment was recommended. Subsequently, the patient was admitted to the stomatology department of the Affiliated Hospital of Jining Medical University (Jining, China) for further management. Surgical treatment was advised and the patient was admitted to the outpatient clinic with the diagnosis of a 'left cervical mass'. Throughout this period, the patient's level of consciousness remained clear and the patient's general condition was stable, with a consistent diet, normal sleep patterns, regular bowel movements and no significant changes in body weight or physical strength since the onset of the swelling. The patient had a five-year history of 'cerebral infarction' but had not been on any medication for this condition, which had remained stable. The patient's past medical history was otherwise unremarkable. Although the patient did not smoke, there was a history of occasional alcohol consumption, drinking ~250 ml 40-degree alcohol per occasion for the past 50 years. There was no family history of hereditary or infectious diseases and no similar ailments had been previously reported.

Stomatology specialist examination indicated the following: The face of the patient showed no sores or carbuncles and maxillofacial symmetry was intact. There was a noticeable swelling in the left upper part of the neck, measuring ~6x5 cm. The skin over the lesion had a medium texture, with a distinct boundary and poor mobility. The local skin temperature, colour and tension were normal and no numbness or paralysis were observed on the left side of the neck. The patient's bite was satisfactory and the mouth opening and shape were acceptable. The secretion from the salivary glands was clear and the entrance to the parotid ducts showed no redness or swelling on either side. The patient was admitted to the hospital for further necessary examinations. On admission in May 2024, neck MRI (Fig. 1) indicated the following: i) An occupying lesion in the left submandibular gland area, not clearly confined to the gland itself; enhancement scanning was recommended, but enhancement was not performed; ii) numerous lymph node shadows of varying sizes were present in the bilateral submandibular region. The patient was then subjected to surgery. Pathological examination (macroscopy) indicated a single grey-red mass measuring 5x5x4 cm with a rough texture and a cut surface showing grey-white and grey-yellow areas. The report of intraoperative frozen pathology described the presence of a malignant tumour in the left upper neck, confirmed by paraffin section-based analysis. The tissue was paraffin-embedded or was rapidly frozen. For the paraffin-embedding samples, 10% formalin was used to fix the tissue for ~18 h at 36˚C. The sections were ~4 μm-thick, and were placed in melted paraffin, and soaked at ~58˚C. The wax dipping time was determined according to the size and type of the tissue, and was typically 2-4 h. The wax-impregnated tissue sample was placed in an embedding mould. For the intraoperative frozen samples, the fresh tissue was quickly placed in a frozen microtome at -20˚C for rapid freezing for 7-15 min. The thickness of ordinary tissue sections was 7 μm. The section thickness of lymph nodes or small cell tumours was 4-5 μm. The adipose tissue sections were 10 μm-thick, and the pure adipose tissue sections were 12-15 μm-thick. A paraffin slicer was used to cut the paraffin embedding block into thin slices. The samples were fixed with 95% ethanol and ice acetone for 20 min. After which, the sample was washed with PBS twice for 1 min each time. The sample was incubated for 2-3 min with haematoxylin dye solution to nucleate the sample at 26˚C and then rinsed with tap water. After which, the sample was immersed in eosin dye solution for 1 min for cytoplasmic staining at 26˚C and then rinsed with tap water. The diagnosis was made by observing the sections with an optical microscope and combining the immunohistochemical result(s).

Malignancy was defined by the presence of severe nuclear atypia with easily discerned nucleoli with or without high mitotic count and necrosis (8). Microscopic observation indicated that the cells of the tissue were closely arranged, the nuclei were large and deeply stained and the tissue had atypia; accordingly, it was not a benign tumour. Pathological microscopic observations showed that tumour cells varied in size, with deeply stained nuclei and distinct nucleolus (Fig. 2). The immunohistochemistry data showed that the myoepithelium expressed smooth muscle actin, calponin, vimentin and P40 (9). In the routine pathology report from the end of May 2024, the paraffin section was confirmed as a malignant tumour in the left upper neck, with features consistent with pleomorphic adenoma and MEC, based on immunohistochemistry. First, paraffin embedding of the tissue samples, dewaxing in xylene and rehydration in an ethanol series were performed. The samples were then soaked in PBS twice for 5 min each at room temperature. For antigen repair, samples were heated under 100 kpa in 0.01 mol/l citric acid buffer, at 99˚C for 10 min. After soaking in PBS twice for 5 min each at room temperature, samples were blocked with 100 ml non-immune normal goat serum (Beyotime Institute of Biotechnology) at room temperature for 60 min. Following the aspiration of the sealing liquid, antibody incubation was performed. A drop of the monoclonal antibody was added at a dilution ratio of 1:100 and incubated at 4˚C for 14 h. Slides were fully soaked in PBS with Tween-20 (PBST) 3 times for 5 min each. Subsequently, an immunohistochemical secondary antibody was applied with a dilution ratio of 1:200 and incubation at room temperature for 30 min. Samples were fully soaked in PBST 5 times for 5 min each. Subsequently, visualisation was performed with diaminobenzidine. Following counterstaining with hematoxylin, samples were dehydrated with ethanol, sealed with neutral gum and and observed under a microscope.

The following primary antibodies were used (all from Beyotime Institute of Biotechnology): CK pan-antibody (PanCK) rabbit antibody, cat. no. AG8362; P40 rabbit antibody, cat. no. AG8590; anaplastic lymphoma kinase (ALK) rabbit antibody, cat. no. AG8114; CD34 rabbit antibody, cat. no. AG8229; Desmin rabbit antibody, cat. no. AG8366. epithelial membrane antigen (EMA) rabbit antibody, cat. no. AF2191; CD31 rabbit antibody, cat. no. AG8226; vimentin rabbit antibody, cat. no. AG8772; Ki-67 rabbit antibody, cat. no. AG8471. Secondary antibodies were as follows: Biotin-labeled goat anti-rabbit IgG(H+L), cat. no. A0277;

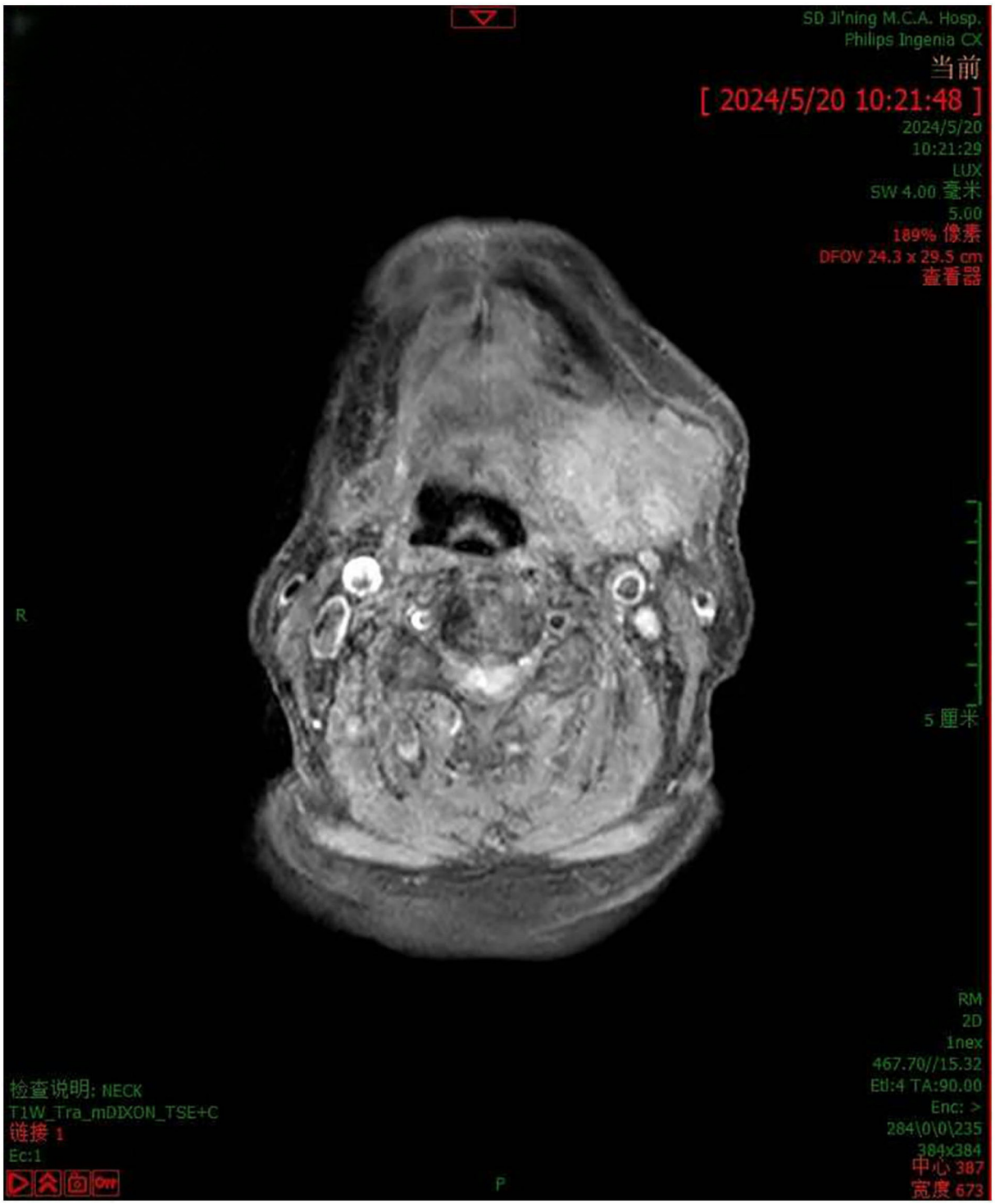

Figure 1. MRI shows a tumour in the region of the left submandibular gland.

biotin-labeled goat anti-mouse IgG(H+L), cat. no. A0288 (both from Beyotime Institute of Biotechnology).

Due to the high variability of salivary gland myoepithelial cells, a combination of immunohistochemical tests with a number of markers of myoepithelial origin, including SMA, vimentin, p40 and p63, are required to confirm the diagnosis (9). The tumour cells in the present study were positive for myoepithelial cells. S-100, p63 and CK5/6 can play a certain auxiliary role in the diagnosis of MEC, and in combination with other myoepithelial-specific antigenic markers, they have significance in the qualitative diagnosis of MEC (10). It is hypothesized that the use of Ki-67 antibody immunohistochemical staining to identify the proliferative activity of cells may be helpful in the differential diagnosis of benign and malignant myoepithelioma (11). The immunohistochemistry results were as follows: CD31 (-) (Fig. 3B), CD34 (-) (Fig. 3C), cytokeratin (CK)7 (+), vimentin (+) (Fig. 2D), Ki-67 (+, 70%), erythroid growth factor receptor (-), desmin (-) (Fig. 3D), ALK (-) (Fig. 3A), P40 (+) (Fig. 2E), PanCK (+) (Fig. 2F) and EMA (-) (Fig. 3E). The patient underwent neck

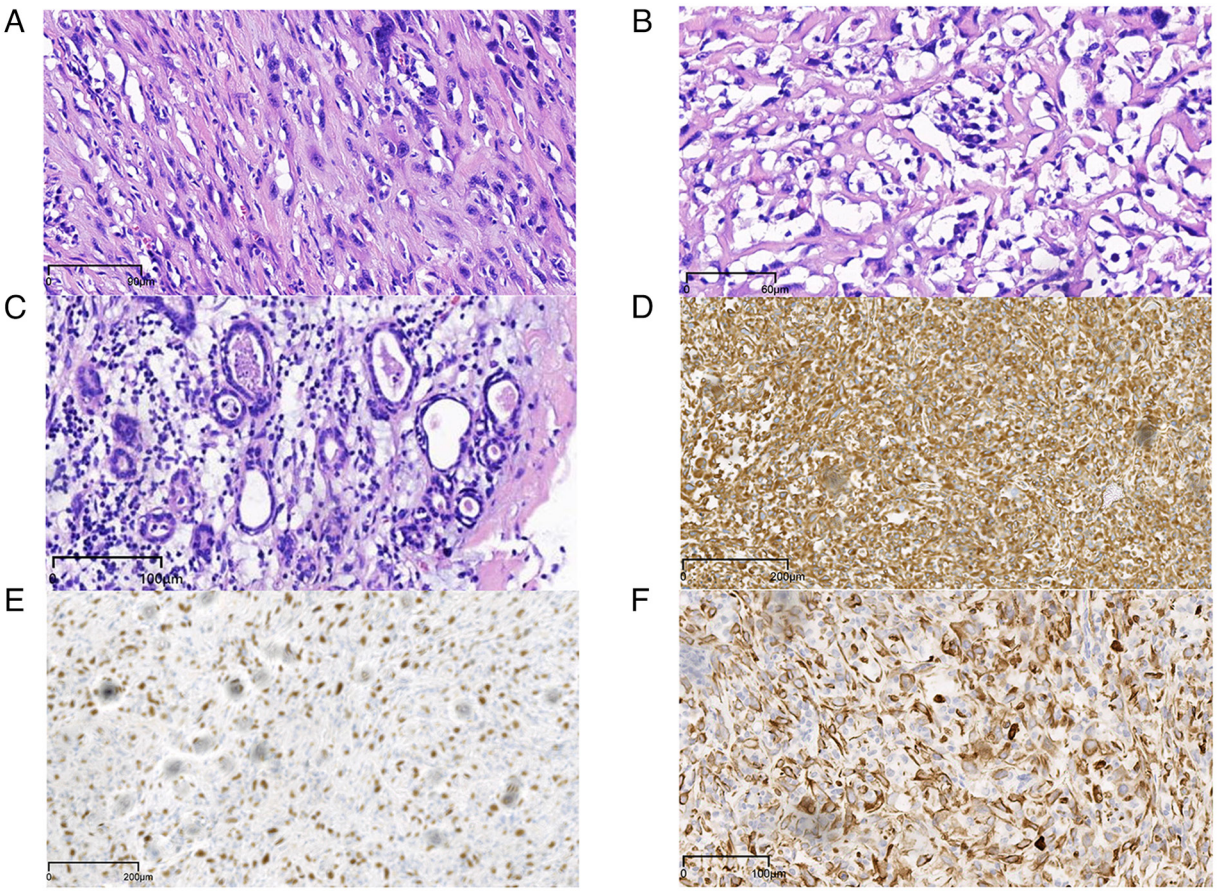

Figure 2. Microscopic analysis of the tumour. (A) Pathological microscopic observations showing spindle-shaped tumour cells in a fascicular arrangement (haematoxylin and eosin; scale bar, 90 μm). (B) Pathological microscopic observations showing tumour cells in the sparse area with abundant, clear cytoplasm (haematoxylin and eosin; scale bar, 60 μm). (C) Pathological microscopic observations showing focally pleomorphic adenoma with a double-layered structure, mucus interstitium and a peripheral membrane (haematoxylin and eosin; scale bar, 100 μm). (A-C) show different morphological characteristics of the same tumour sample. (D-F) Immunohistochemical staining of the tumour indicating (D) positive expression of vimentin (scale bar, 200 μm). (E) positive expression of P40 (scale bar, 200 μm) and (F) positive expression of cytokeratin (scale bar, 100 μm).

mass resection under sedation with complex anaesthesia 5 days after admission. No surgical contraindications were identified. The postoperative course was uneventful and no radiotherapy or chemotherapy was administered, as myoepithelial carcinoma is not sensitive to radiotherapy or chemotherapy (12). At 3 months after the surgery, patient visited the Affiliated Hospital to see the stomatology specialist and no evidence of recurrence or metastasis was found. The patient is being followed up by telephone calls at fortnightly intervals to enquire if the patient has any abnormalities, and the patient is coming for monthly visits to the hospital's Dental Department.

Histological analysis performed according to standard procedures indicated that the tumour exhibited infiltrative growth with uneven cell density. Observation under the microscope revealed that the tumour had grown into the surrounding adipose tissue, showing invasive growth, which is consistent with the growth pattern of malignant tumours, and the density of different areas of the tumour was different. The tumour cells were primarily spindle-shaped, consistent with myoepithelial cell morphology. Furthermore, certain cells were polygonal or oval, with abundant translucent cytoplasm, centrally located rounded nuclei and clear nuclear pleomorphism, including nuclear schizophrenia, and these were all showing a high degree of antypia. Certain areas of the tumour displayed features of pleomorphic adenoma, characterised by a two-layered structure of inner adenoepithelium and outer myoepithelium, with a mucus-like mesenchymal stroma and a peritoneal membrane on the surface.

Discussion

MEC, also known as malignant myoepithelioma, is a morphologically diverse tumour type that can arise *de novo* or result from the malignant transformation of its benign counterpart (13). MEC was first recognised as a distinct entity in 1991, when it was included in the second edition of the World Health Organization's histological classification of salivary gland tumours (14). It is characterised by the absence of duct formation and typically exhibits only myoepithelial differentiation (15). The most common site for MEC is the parotid gland, followed by the minor salivary glands (16). Beyond the salivary glands, MEC can also occur in other sites, such as the epidermis, soft tissues, breast, nasal sinuses, nasopharynx, tongue, lacrimal gland, as well as in the lungs, heart, liver and stomach (16). Clinically, MEC frequently presents as a mass in the parotid gland, oral cavity or neck. These masses are typically slow-growing, painless and movable, with no apparent signs of malignancy during initial imaging (17). As a result, they

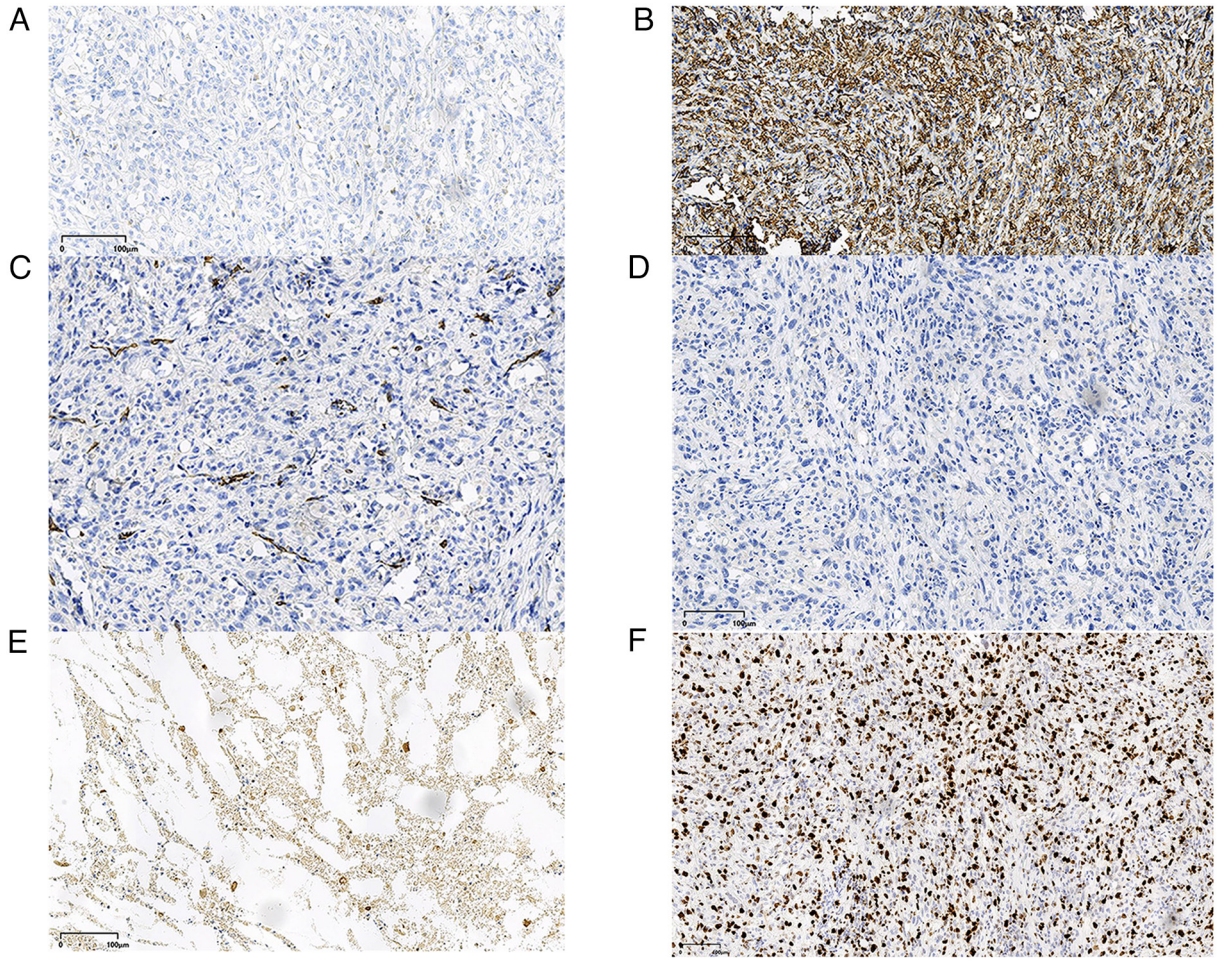

Figure 3. Immunohistochemical analysis of the tumour. (A) Negative expression of anaplastic lymphoma kinase (scale bar, 100 μm). (B) Negative expression of CD31 (scale bar, 200 μm). (C) Negative expression of CD34 (scale bar, 200 μm). (D) Negative expression of Desmin (scale bar, 100 μm). (E) Negative expression of epithelial membrane antigen (scale bar, 100 μm). (F) Positive expression of Ki-67 for 70% (scale bar, 100 μm).

are frequently misdiagnosed as benign tumours. In advanced stages, the tumour often invades surrounding tissues, leading to infiltrative growth and dysfunction of adjacent organs. As the tumour enlarges, patients may experience a variety of symptoms, including pain or numbness of the tongue if the lingual nerve is affected (18). MEC tumour cells exhibit a range of histological patterns, including spindle- and plasma-like, epithelial and hyaline cells. Different cell patterns may coexist within the same tumour (19). Current diagnostic criteria for MEC include both morphology and immunohistochemistry, which demonstrate significant myoepithelial differentiation and clear infiltration into adjacent salivary glands or other tissues (20). Malignancy is determined by assessing cellular heterogeneity, regional necrosis and infiltration (21). The presence or absence of tumour necrosis is critical in determining prognosis. Tumours without necrosis tend to have a lower incidence of distant metastases, while necrosis is associated with a lower disease-free survival rate (22). The Ki-67 proliferation index is a useful tool in distinguishing between benign and malignant myoepitheliomas (10). A Ki-67 proliferation index >10% supports a diagnosis of MEC (11), while an index >50% is linked to a poor prognosis (10). In the present case, the mass infiltrated the surrounding fatty tissue and the Ki-67 proliferation index exceeded 10%. The densely packed spindle cell area displayed significant heterogeneity. Immunohistochemistry results were consistent with the diagnosis of MEC.

MEC must be distinguished from the following tumours: i) Epithelial-MEC: This tumour is most commonly found in the parotid gland (57.7%) and the submandibular gland (20). It is more prevalent in females, with an average age of diagnosis at 60 years (23). Epithelial-MEC is characterised by a dual population of epithelial and myoepithelial cells, showing epithelial differentiation (24). Immunohistochemistry reveals that the inner layer of the glandular epithelium expresses CK, EMA and other specific markers, while the outer myoepithelial cells express vimentin, S-100 and P63 to varying degrees. P63 has the highest diagnostic accuracy (10). Both adenoepithelial and myoepithelial cells express PanCK (+). ii) yoepithelioma: This is a benign tumour with well-defined boundaries a peripheral membrane, low cellular heterogeneity, rare nuclear pleomorphism, a low cell proliferation index and a low cellular proliferation rate. Unlike MEC, myoepithelioma does not exhibit any infiltrative growth pattern, and MEC shows significant heterogeneity (25).

In summary, MEC is relatively rare but exhibits a high rate of metastasis and local recurrence (13). Therefore, early diagnosis is critical (26). Complete surgical excision remains the treatment of choice (27). Factors such as age at diagnosis, American Joint Committee on Cancer stage,

Tumour-Nodes-Metastasis stage and treatment type significantly impact survival (28). To reduce the risk of postoperative recurrence, the initial surgery should be comprehensive, with adequate safety margins. Early diagnosis and treatment can significantly reduce the local recurrence rate and improve both the survival rate and prognosis for patients.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

YL performed case data collection and manuscript drafting. QW modified the manuscript, analyzed the results of the HE sections and immunohistochemistry and made corresponding diagnosis, which provided guidance for the definition, diagnostic points and differential diagnosis and differentiation knowledge of myoepithelial carcinoma. YL and QW confirmed the authenticity of the raw data. Both authors agreed on the journal to which the article was submitted and agreed to be accountable for all aspects of the work. Both authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

The patient provided written informed consent for the case study to be published.

Competing interests

The authors declare that they have no competing interests.

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