

Malignant transformation of the epithelial component in Warthin's tumor

Transformarea malignă a componentei epiteliale în tumora Warthin

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Abstract

In 1929, pathologist Aldred Warthin described for the first time a tumor called "papillary cystadenoma lymphomatosum", which has been known since as "Warthin's tumor". Warthin's tumor is a benign salivary neoplasm occurring mainly in the parotid gland, and has an epithelial component and a lymphoid stroma. However, rarely, either the epithelial or the lymphoid component of Warthin's tumor can undergo malignant transformation. Malignant transformation of the lymphoid component is relatively common but the epithelial malignancy is very rare. Aim: We present a rare case with in situ carcinoma and squamous metaplasia arising in a Warthin's tumor of a parotid gland in a 79-year-old man and the differential diagnosis to be considered for this case. Method: Formalin-fixed paraffin-embedded tissue samples were cut at 4 microns and stained using hematoxylin and eosin (HE). For the immunohistochemical evaluation we have used monoclonal antibodies against cytokeratin (CK) (MNF 116, Dako) and epithelial membrane antigen (EMA) with an EnVision (K5007, Dako) visualization system. Results: In HE stain, Warthin's tumor was composed of papillary cystic structures lined by a bilayered oncocytic epithelium and lymphoid stroma. Areas with squamous metaplasia and in situ carcinoma were also present. Immunohistochemically, the benign oncocytic and squamous metaplastic areas and the in situ carcinoma were positive for EMA and CK. Conclusion: The epithelial malignancy was labeled with CK and EMA. The main differential diagnosis in this case must be made with an invasive squamous carcinoma and metastasis from another primary site of such carcinoma.

Keywords: Warthin's tumor, salivary gland, immunohistochemistry

Rezumat

Patologul Aldred Warthin descrie pentru prima dată în 1929, o formațiune tumorală, pe care o denuște papillary cystadenoma lymphomatosum-de atunci, această tumoră este cunoscută mai ales ca tumora Warthin. Tumora Warthin este o neoplazie benignă a glandelor salivare care interesează în principal glanda pa-

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rotidă, fiind alcătuită dintr-o componentă epitelială și o stromă limfoidă. Cazurile cu malignizare a componentei stromale limfoide sunt întâlnite mai frecvent, pe când tumorile Warthin malignizate pe linie epitelială sunt neobișnuite. Scop: Prezentăm cazul unui pacient de 79 de ani cu tumoră Warthin cu metaplazie scuamoasă, pe care s-a dezvoltat un carcinom in situ, discutând și diagnosticul diferențial care se impune în asemenea situații. Metodă: Fragmentele tisulare au fost fixate în formol, incluse la parafină, secționate la o grosime de 4μm și colorate cu hematoxilină și eozină. Pentru evaluarea imunohistochimică am utilizat anticorpii monoclonali: citokeratină (CK) (MNF 116, Dako) și antigenul membranelor epiteliale (EMA)(E29, Dako), folosind sistemul de vizualizare EnVision (K5007, Dako). Rezultate: pe colorația HE tumora Warthin este alcătuită din structuri chistice papilare marginite de un strat epitelial dublu de oncocyte și o stromă limfoidă. Asociat, sunt prezente zone de carcinom in situ și metaplazie scuamoasă. Imunohistochimic, ariile benigne cu metaplazie scuamoasă și cele de carcinom in situ au fost pozitive la CK și EMA. Concluzii: diagnosticul de neoplazie de origine epitelială a fost stabilit imunohistochimic prin pozitivarea la CK și EMA. Diagnosticul diferențial se impune cu un carcinom scuamocelular invaziv sau metastazele parotidiene ale unui asemenea carcinom.

Cuvinte cheie: tumora Warthin, glanda salivara, imunohistochimie

Introduction

Warthin's tumor, also known as adenolymphoma and cystadenolymphoma, was first described as papillary cystadenoma lymphomatosum in 1929 by Aldred Warthin (1). Warthin's tumor is a benign salivary gland neoplasm, commonly present in the sixth or seventh decade of life and has a definite male predominance. Histologically, it is a tumor composed of bilayered oncocyctic and basaloid epithelium forming cystic structures, papillae and glands accompanied by a dense lymphoid stroma. It is the second most common salivary gland tumor and accounts for 5.3% of all tumors of the parotid gland (2-4). Clinically, Warthin's tumors occur almost exclusively in the parotid gland, in its superficial lobe, and can rarely appear in the deeper lobe (10%). The tumor presents as a slow growing nodular, indolent mass, firm or fluctuant at palpation, multicentric (12-20%), and bilateral (5-14%). The patients can be asymptomatic or have facial pain (rarely); facial nerve palsy may be seen in tumors associated with inflammation and fibrosis, which can be mistaken for malignant tumors (2,5,6).

Malignant transformation of Warthin's tumor is uncommon, but the lymphoid component evolving into malignant lymphoma (7-13) and the epithelial component evolving into adenocarcinoma (14,15), mucoepidermoid car-

cinoma (16-19), squamous cell carcinoma (20-24), oncocyctic carcinoma (25) and Merckell cell carcinoma (26) have been documented.

Material and methods

Between 2002 and 2009, 204 salivary gland tumors were found, out of which 24 patients with Warthin's tumor were admitted in Timisoara City Hospital and treated at the Department of Maxillofacial Surgery. During this period, only one case of Warthin's tumor was diagnosed with malignant transformation and the epithelial component was involved.

A 79-year-old male presented with an asymptomatic right parotid mass that had been enlarging slowly over 5 years. Grossly the 4 cm mass was well-circumscribed, spherical, and partially cystic on the cut surface. The solid areas were gray and friable.

The resection samples were fixed in 10% neutral buffered formalin for 24 hours and embedded in paraffin. For conventional histology, 4μm thick paraffin wax embedded serial sections were stained with hematoxylin-eosin (HE).

In order to evaluate the epithelial origin of the tumor cells we investigated the immunoreactivity for cytokeratin (CK) (MNF 116, Dako) and epithelial membrane antigen (EMA)(E29, Dako) with EnVision (K5007, Dako) visualization

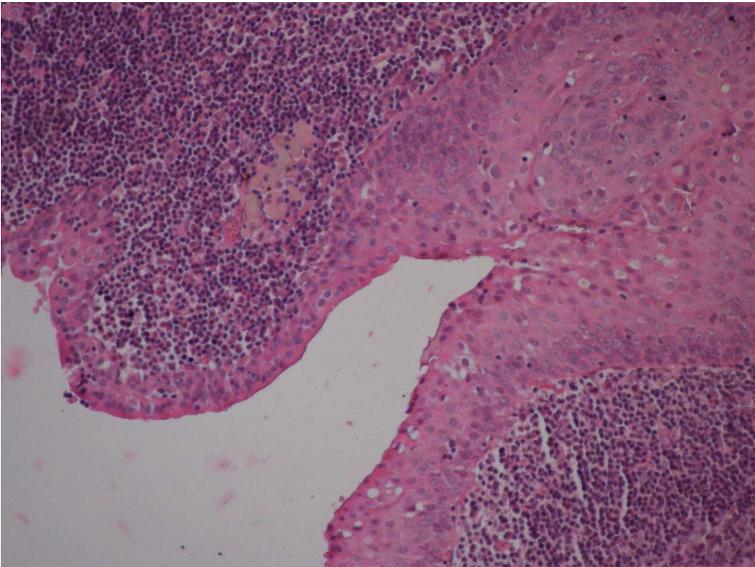


Figure 1. Bilayered oncocytic cells (left) and squamous metaplasia (right) and prominent lymphoid stroma (HE x 200).

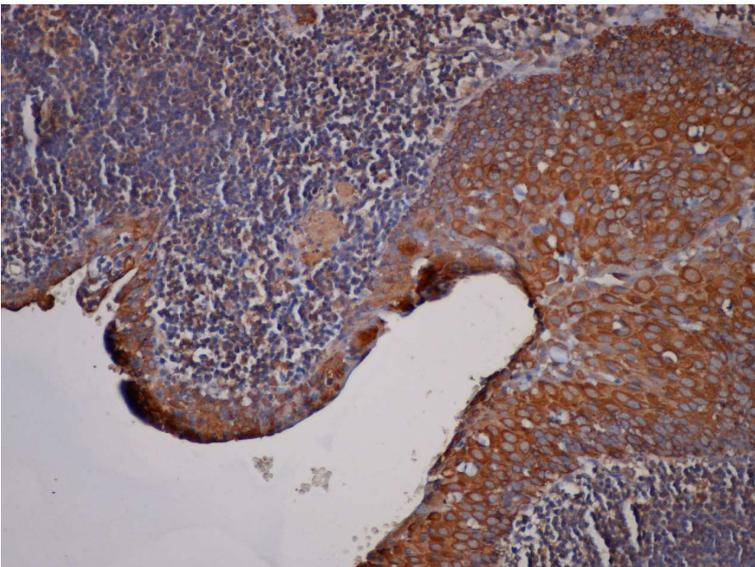


Figure 2. Epithelial component highlighted by antibody for cytokeratin (MNF116). Note the transformation from oncocytic columnar cells to squamous cells (x 200).

system. Paraffin wax embedded sections were deparaffined and rehydrated through a decreasingly concentrated alcohol series up to distilled water. Applied on formalin-fixed, paraffin-embedded tissue, the mouse anti-human cytokeratin (MNF

116) needed pretreatment of tissue with proteolytic enzyme Proteinase K, RTU (code S 3020) for 5 minutes and 10 minute incubation at room temperature with the primary antibody. For monoclonal mouse anti-human epithelial membrane antigen proteolytic enzyme tissue pretreatment was not required, but incubation time for this primary antibody was 20 minutes. For immunohistochemistry we used the Avidin-Biotin Complex method. For the visualization of the reaction diaminobenzidine-tetrahydrochloride (DAB) was used, followed by counterstaining with Mayer's hematoxylin. Negative external control staining was done by omitting primary antibodies and tonsil was used for positive external control.

Histological sections were reviewed independently by two pathologists, and then discussed for consensus. All specimens were examined and photographed on a Nikon Eclipse 600 microscope.

The particular nature of this case is the rarity of these tumors in our experience. From a total of 204 salivary gland tumors studied for a period of seven years we have diagnosed only one such case of Warthin's tumor with malignant transformation of the epithelial component.

Results

Histological examination of HE stained slides demonstrated benign areas - cystic lesions with papillary projections of epithelial cells with oncocytic features. These cells were arranged in two layers: the oncocytic luminal cells were tall and columnar and showed palisading on a discontinuous basal layer of small cells. The Warthin's tu-

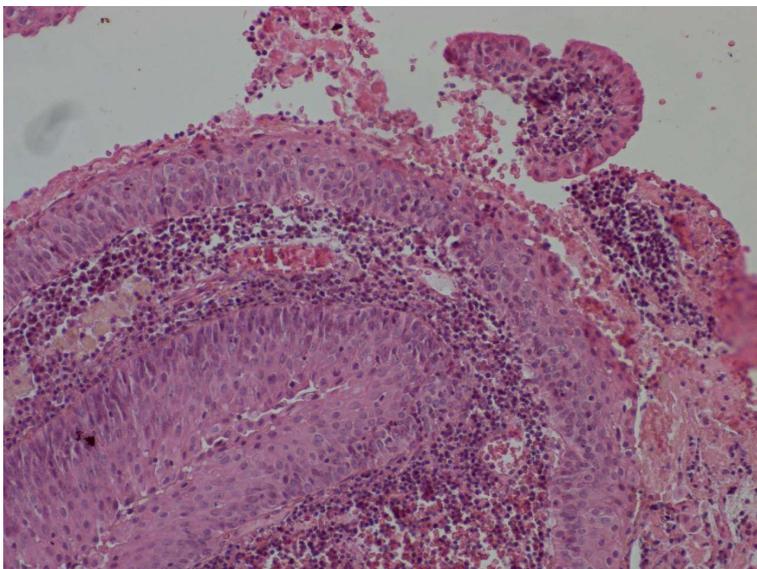


Figure 3. Benign Warthin's tumor (upper right) and areas with mild dysplasia (HE x200)

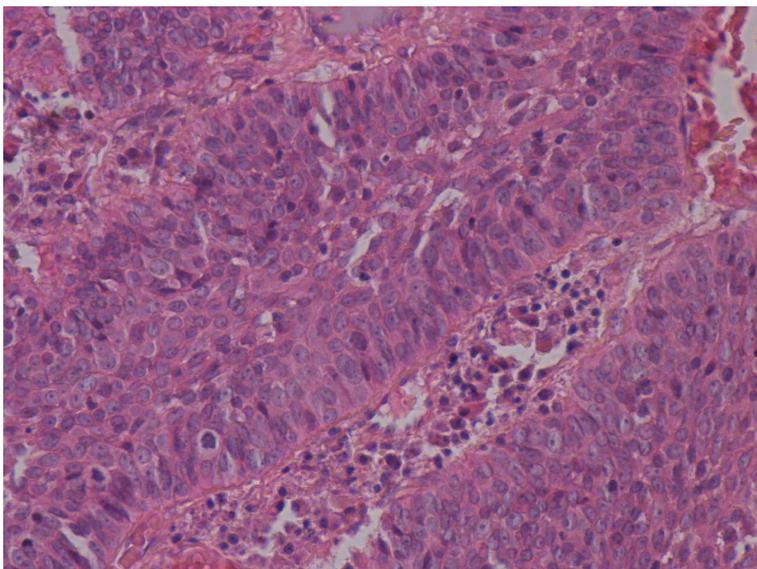


Figure 4. In situ carcinoma. Note the cytological atypia involving the full thickness of the epithelial component and the mitotic abnormal figures (HE x400).

mor stroma consisted of lymphoid tissue with germinal centers (*Figure 1*). The cystic spaces contained eosinophilic secretions, macrophages with foamy cytoplasm and plasma cells. Squamous metaplasia was present in many areas of this tu-

mor and often the squamous cells were extending into surrounding tissues in a pseudoinfiltrative pattern. A transformation from benign oncocytic columnar cells to squamous metaplasia and malignant squamous cells could be identified (*Figures 1, 2, 3*). Areas with necrosis and mild atypia were also encountered (*Figure 3*). Prominent cytological atypia and mitotic abnormal figures were present in the areas of in situ carcinoma (*Figure 4*).

Immunohistochemically, EMA and CK antibodies were positive both in benign oncocytic areas and malignant squamous cells, proving the epithelial origin of the tumor (*Figures 2, 5*). In CK stain the positivity of the cytoplasm in the epithelial component was obvious. For EMA the staining pattern was mostly cytoplasmic but we could also notice peripheral membrane staining. The diaminobenzidine-containing substrate working solution yielded a brown color at the site of the target antigen recognized by the primary antibody. Nuclei were stained blue by the hematoxylin counterstain. These markers were used to establish the epithelial origin of the malignant proliferation. Tumor cells with cytoplasmic staining were considered positive.

The rarity of a Warthin's tumor with malignant transformation of the epithelial component in our experience prompted us to report this case.

Discussion

Malignant tumors evolving from pre-existing Warthin's tumors are very rare and their incidence has been estimated to be 0.3% of all Warthin's tumor cases (24). In our study this incidence proved to be 0.49% of all 204

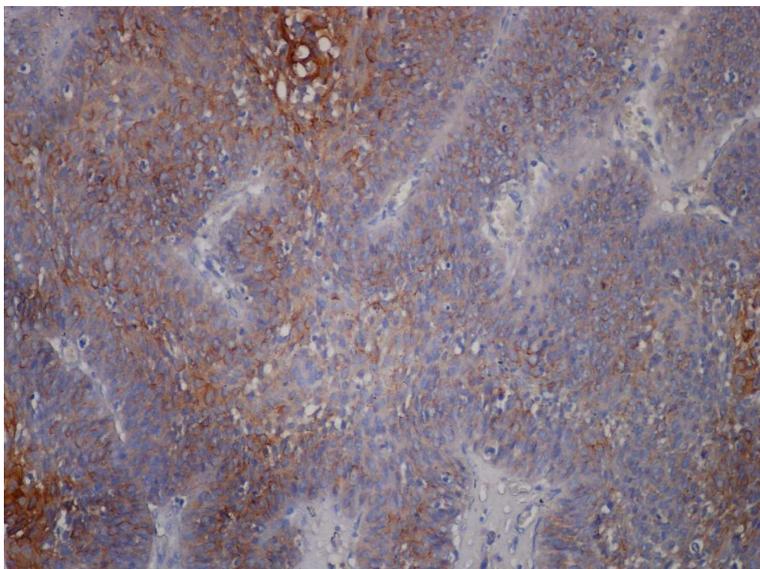


Figure 5. The in situ carcinoma areas positive for epithelial membrane antigen (x 200)

salivary glands studied. Cases of carcinoma, e.g. pleomorphic adenoma, are well-known entities, but carcinomas arising in Warthin's tumors are rare (14). Damjanov I et al reported only nine cases with epithelial malignancy arising within a Warthin's tumor (20). Bolat F et al, Gunduz M et al, Skalova A et al - all reported cases of squamous cell carcinoma arising in Warthin's tumor also (21,23, 24). Ferrero S et al reported a case of poorly differentiated carcinoma arising from adenolymphoma of the parotid gland and Perroti et al found an adenocarcinoma arising in a Warthin's tumor (14, 15). Nagao T et al studied two cases of mucoepidermoid carcinoma arising in Warthin's tumor of the parotid gland, Yamada SI et al one case and Williamson JD et al reported five such cases (17-19). Seifert G described bilateral mucoepidermoid carcinomas arising in bilateral pre-existing Warthin's tumor of the parotid gland (22). Fornelli A et al reported two cases of Merckel cell carcinoma of the parotid gland associated with Warthin's tumor (26). Bengoechea O et al found a case of oncocytic adenocarcinoma arising in Warthin's tumor

(25). Several studies reported the malignant transformation of the lymphoid stroma showing that it is relatively common (7-13).

The case we presented was diagnosed as in situ squamous cell carcinoma arising in a Warthin's tumor based upon the microscopic features and the absence of invasion of surrounding tissue. On the slides a pre-existing benign Warthin's tumor was found and we also identified transitional zones from benign oncocytic to frankly malignant epithelium (*Figure 3*). Due to the rarity of this type of carcinoma arising in a Warthin's tumor, a metastatic carcinoma from another site must be excluded. We distinguished metastasis from another primary carcinoma of a different site

by the presence of dysplastic pleomorphic squamous cells of in situ carcinoma extending into the normal oncocytic epithelial component. And also, in our case, there was no epidermoid carcinoma located elsewhere at the time of diagnosis, and the cervical lymph nodes were not involved. The clinical stage was classified as Stage II with T₂N₀M₀ according to World Health Organization TNM classification system (2).

The pathogenesis of the development of malignant tumors in pre-existing Warthin's tumor is uncertain. Damjanov I et al and Gunduz M et al postulated that epidermoid carcinoma arises from squamous metaplastic areas and stated that the transformation of cylindrical cells into squamous cells suggests a carcinoma arising from the foci of squamous metaplasia (20, 23). It is known that cases of Warthin's tumor associated with infarct-like necrosis often exhibit squamous metaplasia (27, 28). In our case we noted areas with necrosis and squamous metaplasia extending into surrounding tissues in a pseudoinfiltrative pattern (*Figures 1, 2*) which might be suggestive for the etiology of the in situ epidermoid carcinoma seen here.

The epithelial malignancy was labeled with CK and EMA. Cytokeratins and epithelial membrane antigen are the two most widely used markers for epithelial origin cells, and they usually do not show up in the mesenchyme-derived ones (29). The in situ epidermoid carcinoma was defined by the atypia involving the full thickness of the oncocytic epithelial component, without invasion of surrounding tissues. Based upon these immunohistochemical findings and the histopathological aspect - with atypical squamous cells and areas of classic benign Warthin's tumor, the in situ squamous cell carcinoma arising in Warthin's tumor was diagnosed.

The treatment for this tumor was surgical, without radical neck dissection. Five months follow-up period in this patient revealed no evidence of recurrence and metastasis.

There is no consensus as to the optimal treatment, as there is a paucity of data regarding the treatment of Warthin's tumors that undergo carcinomatous transformation. The mainstay of treatment for these rare tumors has been surgical resection. Superficial or total parotidectomy and tumor-free margins, with or without neck dissection, has been reported with excellent outcomes (30).

The long-term prognosis of these patients is unclear. Most cases of Warthin's tumor with malignant transformation did not show distant metastasis and one year disease-free survival has been reported in the literature (24).

Acknowledgements

This work was partially supported by grant for scientific research from CNCSIS-PNII-Resurse Umane, type: TD, code CNCSIS: 314, contract no. 138/15.09.08.

Abbreviations

CK – cytokeratin
EMA - epithelial membrane antigen
HE - hematoxylin and eosin

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