



Primary oncocytic carcinoma of the salivary glands: A clinicopathologic and immunohistochemical study of 12 cases

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SUMMARY

Oncocytic carcinoma (OC) of salivary gland origin is an extremely rare proliferation of malignant oncocytes with adenocarcinomatous architectural phenotypes, including infiltrative qualities. To help clarify the clinicopathologic and prognostic features of this tumor group, herein, we report 12 OC cases arising from the salivary glands, together with follow-up data and immunohistochemical observations. There were 10 males and 2 females with an age range of 41 to 86 years (median age: 61.3 years). Most occurred in the parotid gland (10/12) with one in the palate and one in the retromolar gland. The tumors were unencapsulated and often invaded into the nearby gland, lymphatic tissues and nerves. The neoplastic cells had eosinophilic granular cytoplasm and round vesicular nuclei with prominent red nucleoli. Ultrastructural study, PTAH, and immunohistochemistry staining confirmed the presence of numerous mitochondria in the cytoplasm of oncocytes. Cellular atypia and pleomorphism varied in the current series. Double nuclei and mitoses were observed in some cases, while one case that showed mild cellular pleomorphism but had local invasion following local recurrence was also identified as an OC. Of the 11 cases with follow-up information, 7 cases had local recurrence. Regional or distant metastases were found in 6 and 4 cases, respectively. Five-year disease-specific survivals were 54.9%. In summary, OC of salivary gland origin is a high-grade tumor, often with local recurrence, regional or distant metastasis, diagnosis of which based on a combination of clinical and histopathological features. Immunohistochemistry for mitochondria is considered as a practical and helpful adjuvant diagnosis. Complete surgical excision is the treatment of choice while the role of radiotherapy or chemotherapy is controversial, and careful follow-up is necessary.

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Introduction

Oncocytes are typically large epithelial cells with a low nuclear-to-cytoplasmic ratio, a centrally situated round nucleus with a prominent nucleolus, and abundant bright eosinophilic granular cytoplasm, characterized ultrastructurally by numerous mitochondria.¹ The term “oncocyte” derives from the Greek root word “onkousthai”, meaning “increase in bulk”, swollen or tumor. This type of cells were originally referred to as “onkocytes” by Hamperl at 1931,² which are found in many organs including the salivary gland, thyroid, parathyroid, and kidney, under various pathological conditions including neoplasia (such as oncocytomas).³

Oncocytic lesions of the salivary glands are usually benign, including oncocytosis and oncocytic tumors. Normal oncocytes are also observed in the salivary glands of the aged patients, and are considered to represent an age-related metaplasia or

degenerative process.⁴ Oncocytic carcinoma (OC) of salivary gland origin, however, is an extremely rare tumor composed of malignant oncocytes with adenocarcinomatous architectural phenotypes and infiltrative qualities, including local invasion, regional or distant metastases.¹ To help clarify the clinicopathologic and prognostic features of this tumor group, herein, we report 12 OC of salivary gland origin, together with follow-up data and immunohistochemical observations.

Materials and methods

Cases diagnosed as OC or malignant oncocytomas were retrieved from the files of the Department of Oral Pathology, Peking University School and Hospital of Stomatology, during the period 1985–2009. Standard hematoxylin and eosin stained slides were reviewed in all cases, of which 12 OC cases were confirmed according to the WHO Histological Typing of Salivary Gland Tumors.¹

Clinical and gross features were obtained from the surgical and pathology records. Paraffin embedded tumor tissues were available in all cases. Four-micrometer-thick serial sections were cut and

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used for phosphotungstic acid-hematoxylin (PTAH) and immunophenotypic analysis. Residual specimens were re-fixed with 2% glutaraldehyde and 2% OsO₄. Ultra-thin sections were cut, and observed by transmission electron microscopy. Immunohistochemical staining was performed using a standard streptavidin-biotin-peroxidase complex method (LAB-SA kits, Zymed Laboratories, South San Francisco). Details of primary antibodies used are listed in Table 1.

Follow-up information was obtained by clinical interviews or reviewing medical records of the patients.

Results

Clinical features

The clinical data for 12 identified cases are summarized in Table 2. The patient age at first presentation ranged from 41 to 86 years (median age: 61.3 years). There were 10 males and 2 females with a male to female ratio of 5:1. Most cases in this series originated from the parotid gland (10/12) with 1 in the palate and 1 in the retromolar gland. The tumors ranged in size from 1.0 to 8.0 cm (median size: 3.8 cm).

The duration of symptoms ranged from 2 to 18 months with a median of 10.3 months. Seven patients presented with a painless, firm mass, while intermittent radioactive pain was complained in 5 patients, of whom three also with facial numbness or paralysis. Four patients were initially treated with superficial parotidectomy, of which two also performed with postoperative radiotherapy. Two patients were treated with parotidectomy, of which one also included neck dissection. One patient presented with a painless, multinodular mass in the parotid gland with quick growth, involving almost the whole neck at the time of examination, and was treated with radiotherapy and chemotherapy after biopsy. Tumor

excision was performed in 4 patients, of whom two also treated with ¹²⁵I seed implant brachytherapy. One patient was treated with extended tumor excision together with neck dissection.

Follow-up data were available on 11 patients ranging from 6 to 171 months with a median of 56.2 months. All the cases were found primary and local recurrence after initial treatment occurred in 7 cases. Cervical lymph node metastases and distant metastases were found in 6 and 4 patients, respectively. Four patients died of disease 13, 14, 47 and 54 months after treatment in our hospital. Four patients are alive with disease after a follow-up of 6, 42, 74 and 171 months; 3 patients are alive with no evidence of disease at 29, 77, and 85 months. Five-year disease-specific survivals (DSS) were 54.9% (Fig. 1).

Pathologic features

Grossly, oncocytic carcinomas were usually unencapsulated and irregular-shaped, such as dumbbell-like or cord-like, tan to grey masses. The cut surface was pale yellow or light brown, solid, and sometimes non-homogeneous with cystic degeneration, necrosis or hemorrhage.

Microscopically, the tumors were composed of large, round to polyhedral cells arranging in solid sheets, duct-like structures and cords or as scattered individual cells (Fig. 2A). The tumors were unencapsulated, often found to invade muscle, lymphatic tissues and nerves (Fig. 2B). Extensive necrosis was presented in 3 cases. Hemorrhage foci or microcyst formation was also found in area and sometimes there were eosinophilic, amorphous cellular debris in the cystic structures. The tumor cells had abundant eosinophilic cytoplasm that appeared to be finely granular, and round centrally placed vesicular nuclei, often with prominent, big and red nucleoli. Cellular atypia and pleomorphism varied in 12 cases. Marked pleomorphism was seen in most cases, however, one case (case 9)

Table 1
Antibodies used for immunohistochemistry.

Antibody	Company	Clone	Pretreatment	Dilution
CK5/6	Zymed, Carlsbad, CA	ZM-0313	Citrate HIER	Ready to use
CK10/13	Zymed, Carlsbad, CA	ZM-0314	Citrate HIER	Ready to use
CK8/18	Zymed, Carlsbad, CA	ZM-0315	Citrate HIER	Ready to use
CK19	Zymed, Carlsbad, CA	ZM-0074	Trypsin (20')	Ready to use
S-100	Zymed, Carlsbad, CA	ZM-0024	None	Ready to use
SMA	Zymed, Carlsbad, CA	ZM-0003	None	Ready to use
EMA	Zymed, Carlsbad, CA	ZM-0095	Citrate HIER	Ready to use
Ki-67	Dako, Carpinteria, CA	MIB-1	Citrate HIER	1:100
Mitochondria	Millipore, Temecula, CA	MAB1273	EDTA pH8.0	1:100

SMA, smooth muscle actin; EMA, epithelial membrane antigen.

Table 2
Clinical features of 12 oncocytic carcinomas.

Case	Age	Sex	Site	Size (cm)	Dura (mo)	LM	DM	Initial treatment	Course (mo)
1	60	M	Pa	3.5	02	+	Lung	Tumor excision	Rec/09 DOD/47
2	57	M	P	7.0	18	+	–	Parotidectomy/neck dissection	N/A
3	48	M	P	3.0	10	–	Lung	S-parotidectomy	AWD/171
4	59	M	P	8.0	12	+	Brain	Radiotherapy/and chemotherapy	DOD/13
5	75	M	P	3.0	07	+	Lung	S-parotidectomy	Rec/30/44 DOD/54
6	68	M	P	4.0	12	–	–	Parotidectomy	Rec/65 AWD/74
7	41	M	P	3.0	12	–	–	S-parotidectomy/radiotherapy	NED/77
8	55	M	P	2.5	06	+	–	S-parotidectomy/radiotherapy	DOD/14
9	67	F	P	3.5	05	–	–	Tumor excision/ ¹²⁵ I seed plant	Rec/31 AWD/42
10	86	M	P	1.0	13	–	–	Tumor excision	Rec/60/71 NED/85
11	51	F	Rm	4.0	18	+	–	E-tumor excision/neck dissection	Rec/14 NED/29
12	68	M	P	3.0	08	–	–	Tumor excision/ ¹²⁵ I seed plant	Rec/05 AWD/06

M, male; F, female; Pa, palate; P, parotid; Rm, retromolar gland; mo, months; S, superficial; E, extended; Rec, recurrence; DOD, dead of disease; N/A, no available; NED, no evidence of disease; AWD, alive with disease.

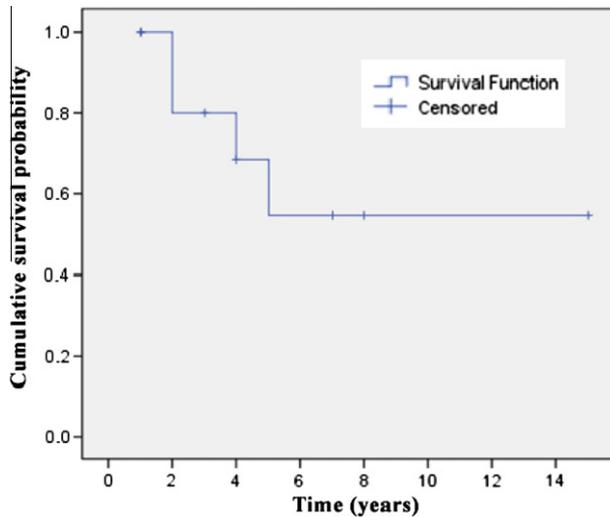


Figure 1 Kaplan–Meier survival curves of the patients with oncocytic carcinoma (OC) of salivary gland origin.

showed an oncocytoma appearing in that the tumor cells arranged in solid sheets without obvious pleomorphism but were uncircumscribed, invading into the nearby salivary gland tissues, lymph nodes and perineural tissues (Fig. 2C and D). Some tumors showed cells with double nuclei occasionally and multinucleated cells rarely (Fig. 2E). Mitoses were observed and pathologic mitoses were also seen sometimes.

Histochemistry and immunophenotype

Histochemically, PTAH staining distinctly illustrated positive, small, dark-blue cytoplasmic granules, which represented mitochondria (Fig. 3A).

All the tumors showed intense immunoreactivity for mitochondria, CK5/6, CK8/18, CK10/13, CK19, and EMA, whereas there was no reactivity documented for SMA or S-100. Frequency of Ki-67 positive cells with nuclear staining was higher in oncocytic carcinomas comparing to that in the control of oncocytomas. (Fig. 3B–F)

Electron microscopy

Electron microscopy demonstrated numerous mitochondria closely packed within the cytoplasm of the tumor cells. The mitochondria were enlarged, variably shaped, and had an increased number of parallel aligned, fine, tubular, lamellar cristae. Glygranules were evident but not markedly increased (Fig. 4).

Statistical analysis

The SPSS 16.0 software was used to perform statistical analysis and create diagram. Five-year DSS were calculated by the Kaplan–Meier method.

Discussion

Oncocytic carcinoma of salivary gland origin is an extremely rare tumor, defined by WHO as a proliferation of cytologically malignant oncocytes with adenocarcinomatous architectural phenotypes including infiltrative qualities, which may arise de novo and also can be seen in association with a pre-existing oncocytoma.¹ This tumor group is also known as malignant oncocytoma or malignant oxyphilic adenoma, and accounts for much less than 1% of all the salivary gland tumors. To our knowledge, since the first case described by Bauer and Bauer at 1953,⁵ only 59 cases have been reported in the English-language literature by the end of 2008, most of which are individual case report with unequal information.^{6–14} Herein, we report 12 OC cases arising from the

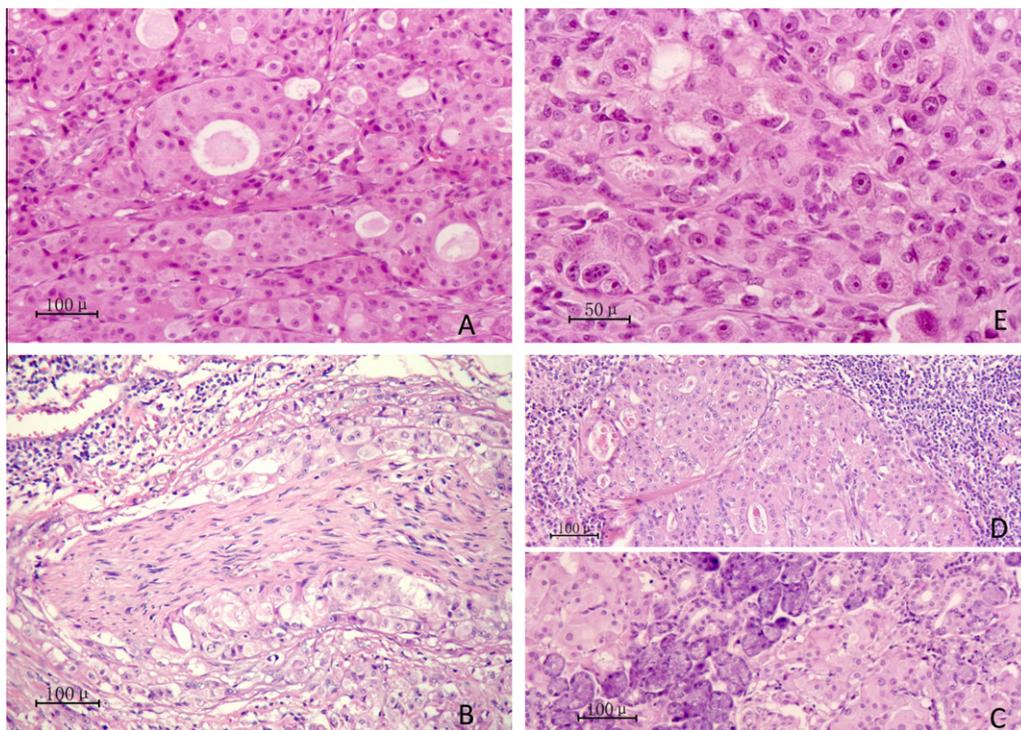


Figure 2 Hematoxylin and eosin stain. (A) The solid sheets and duct-like structures in OC. (B) The tumor cells invading into surrounding tissues with perineural invasion observed. (C) The tumor cells invading into the salivary gland tissues. (D) The tumor cells invading into the lymph nodes. (E) The tumor cells having eosinophilic granular cytoplasm and round vesicular nuclei (sometimes double nuclei), often with big and red nucleoli.

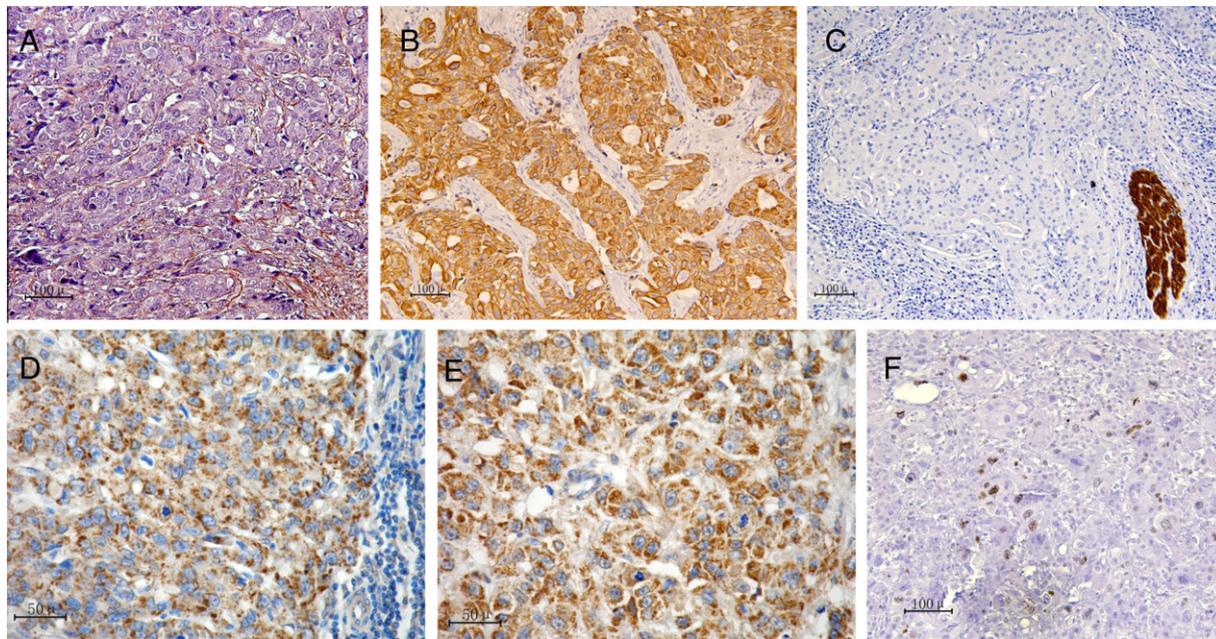


Figure 3 (A) Phosphotungstic acid-hematoxylin stain with positive cytoplasmic granules (mitochondria). (B) Immunohistochemical reactivity of the tumor cells for CK8/18. There is also reactivity for CK5/6, CK10/13, CK19, and EMA (data not shown). (C) No S-100 reactivity in tumor cells. Oncocytes in both lymph nodes with metastasis (D) and primary foci (E) had intense granular cytoplasmic immunoreactivity for anti-mitochondria immunostaining. (F) Ki-67 positive nuclear staining in malignant oncocytes.

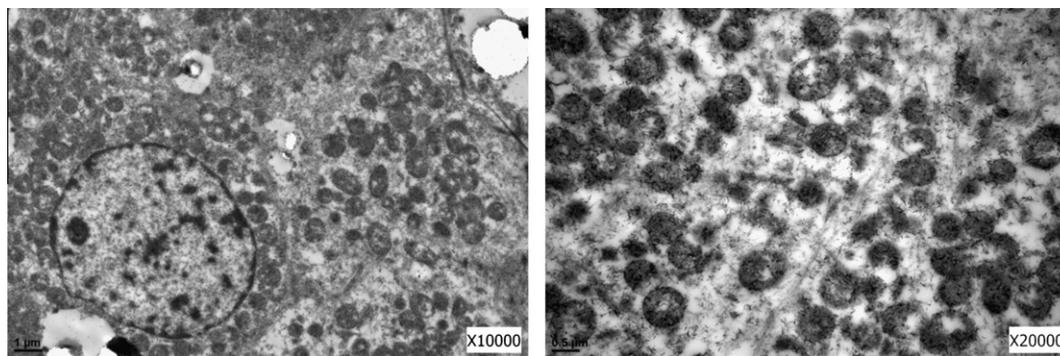


Figure 4 Electron microscopy demonstrating numerous mitochondria in the cytoplasm of the tumor cells. (left, $\times 10,000$; right, $\times 20,000$).

salivary glands, together with follow-up data and immunohistochemical observations.

It has been reported that OC occurs predominately in the parotid gland of older adults with a mean age of 62.5 years, and men are affected in two-thirds of cases.¹ In the current series, patient age (ranged from 41 to 86 years with a median age of 61.3 years) and tumor location (83.3% in the parotid gland) were in general agreement with those of the previous reports, whereas the male predilection in our patients was much more apparent with a male to female ratio of 5:1. Of the 2 cases occurring in the minor salivary glands, one was in the palate. To our knowledge, only 15 OC have been reported in the minor salivary glands and this is the second case occurring in the palate.^{11,12} Briggs and Evans reported the first case of malignant oncocytoma involving the palate, but the follow-up data was not available. In this series, the patient with OC of the palate was treated with tumor excision initially. The tumor recurred 9 months later, and the patient received surgery including neck dissection and died of distant metastasis 47 months after the initial treatment. Another OC of minor salivary gland origin was found in the retromolar gland and was treated with extended

tumor excision including neck dissection. The tumor recurred 14 months later and the patient is alive without evidence of disease 15 months after the second surgery.

Since the cellular phenotype does not vary dramatically between oncocytomas and oncocytic carcinomas and oncocytomas occasionally share the diagnostic features of oncocytic carcinomas, the diagnosis of malignancy should be based on a combination of clinical and histopathological features. It has been suggested that the malignant nature should be identified by the criteria as follows: (1) lack of encapsulation; (2) frequent mitoses and cellular pleomorphism; (3) perineural, intravascular, or lymphatic invasion; (4) regional or distant metastases.¹⁵ Local recurrence was also considered as one of the characteristics of OC. According to the WHO Histological Typing of Salivary Gland Tumors (2005),¹ two criteria are necessary to establish the diagnosis of OC. Firstly, the tumor cells must be identified as oncocytes. Secondly, the diagnosis of malignancy should be based not only on cellular and nuclear pleomorphism, but also on local infiltration and metastasis. In the current series, case 9 presented oncocytes without obvious cellular pleomorphism, arranging in solid sheets. However, it was

considered malignant based on the findings of lack of encapsulation and infiltration of the tumor cells into adjacent gland tissues, lymph nodes and perineural tissues. The tumor recurred 31 months after treatment and is alive with disease 42 months after treatment.

Ki-67 immunostaining also has been suggested in separating OC from oncocytomas.¹⁶ In the current series, frequency of Ki-67 positive cells with nuclear staining was higher in OC comparing to that in the control of oncocytomas.¹⁷ Besides oncocytomas, other tumors are also considered to distinguish from OC. Acinic cell adenocarcinoma can be differentiated from OC since the cytoplasmic granules in acinic cell adenocarcinoma are amphophilic or basophilic and the patterns of growth can be microcystic or papillary. Salivary duct carcinoma, in contrast to OC, usually forms duct-like spaces with papillary and cribriform growth and often shows comedo-like necrosis. In the meantime, the presence of numerous mitochondria in the cytoplasm of the oncocytes that is confirmed on ultrastructural examination is not found in the neoplastic cells from other malignancies mentioned above, which can be considered for adjuvant diagnosis. However, the processes of fixation or embedding of specimens for light microscopy often destroy the fine structure of organelle in the cytoplasm so that it is difficult to observe mitochondria clearly.

PTAH staining has also been utilized to identify oncocytes. Brandwein and Huvos especially recommended the use of prolonged (48 h) PTAH staining.¹⁸ It has also been reported that immunohistochemistry using an anti-mitochondrial antibody is a highly sensitive and specific method for identifying the mitochondria by light microscopy.¹⁹ This procedure is easy to perform and readily available for the specimens embedded in paraffin which would otherwise not be appropriate for analysis by electron microscopy. In the current series, 5 cases were examined under electron microscopy in view of its practical limitation, whereas PTAH and immunohistochemical staining for mitochondrial were performed in all cases. PTAH staining illustrated positive dark-blue cytoplasmic granules and anti-mitochondria immunostaining showed an intense, finely granular immunoreactivity in the cytoplasm of oncocytes. Both results implied the presence of mitochondria, whereas immunohistochemistry was considered as a more practical and helpful method for the diagnosis of OC.

There is no definitive theory about the pathogenesis of this tumor group. In the present study, immunohistochemical staining showed positive reactivity for CK5/6, CK8/18, CK19, and EMA, but negative for S-100 or SMA in oncocytes, which suggested its origin from the intercalated duct cells.

In view of its rarity, the prognosis of OC is not well known, while it was considered as a high-grade tumor based on the follow-up data in the current series. Briefly, the 5-year disease-specific survivals were 54.9%, and the local recurrences were found in 7 cases. Regional lymph node metastases and distant metastases were found in 6 and 4 patients, respectively. Goode and Corio reported that the tumors smaller than 2 cm in diameter appeared to have a better prognosis than those that were larger.²⁰ The size of the tumors in this series ranged from 1.0 to 8.0 cm with a median of 3.8 cm in diameter. Only one tumor was found no larger than 2 cm in diameter, and the patient is alive without evidence of disease at 85 months in spite of the recurrences found in 60 and 71 months after the initial treatment.

Surgery especially radical resection is the widely accepted treatment for OC. When the tumor invades the facial nerve, the nerve should be sacrificed in principle. Immediate nerve grafting for reconstruction of facial nerve defect could be performed. Goode et al. reported 4 cases treated only by conservative surgery all recurred after the operation, three of which metastasized.²⁰ Furthermore, many cases described in the literature were treated with surgery including neck dissection. Nakada et al. reported 23 cases

of OC with cervical lymph node metastasis in a review of 42 cases.⁶ The current series also showed a high incidence (6/11) of cervical lymph node metastasis. It has been suggested that elected neck dissection be indicated when the tumor size is larger than 2 cm or the histopathologic features suggest the tumor spreads to the cervical lymph nodes.²⁰ Adjuvant radiotherapy has also been used for the treatment of OC.^{6,8,12,13,16,20,21} In the current series, 5 patients received radiotherapy, of whom two had multiple metastases and died of disease about 1 year after initial treatment, 2 patients are alive with disease 6 and 42 months after treatment but both with recurrence occurred, and only one is alive without evidence of disease 77 months after treatment. Altogether, for the extensive tumors, radical resection combined with elective neck dissection may be the first choice for treatment, while the role of radiotherapy or chemotherapy is still controversial.

In summary, oncocytic carcinomas of salivary gland origin are high-grade tumors with local recurrences, regional or distant metastases, diagnosis of which based on a combination of clinical and histopathological features. Immunohistochemistry for mitochondria is considered helpful for the adjuvant diagnosis. Complete surgical excision is the treatment of choice while the role of radiotherapy or chemotherapy is still controversial, and careful long-term follow-up is necessary.

Conflict of interest statement

None declared.

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