### Original Article Primary clear cell carcinoma of nasal cavity: report of six cases and review of literature

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Abstract: Aims: This study is to retrospectively analyze the clinical and pathological data of six cases of primary nasal clear cell carcinoma in our hospital since 1992 and to review literatures on the clinical manifestations, pathological features, immunohistochemistry, diagnosis and treatment of the disease. Methods: The pathological archives that were diagnosed as salivary gland nasal tumors in Shandong Cancer Hospital during 1992-2013 were reviewed. Careful review of sections was performed by two experienced pathologists. The samples were labeled using EnVision method. Immunostaining was performed using 3, 3'-diaminobenzidine reagent followed by counterstaining with hematoxylin. The immunohistochemical results were classified according to positive cells: no positive staining cells (-); positive cells <30% (+); positive cells between 30% and 50% (++); and positive cells >50% (+++). Results: Among the 6 cases of primary nasal clear cell carcinoma in our hospital since 1992, 4 cases were diagnosed as clear cell carcinoma of nasal cavity after exclusion of other nasal cavity tumors with clear cells, and 2 cases were directly diagnosed as clear cell carcinoma of nasal cavity. Hyalinizing clear cell carcinoma of salivary gland (HCCC) tissues were mainly composed of polygonal epithelioid tumor cells arranged into the shapes of beehives, and separated by fibrous tissues containing rich thin-wall capillaries. The cytoplasm of HCCC cells was rich and translucent with some cells having multiple vacuoles. Reticular fiber staining showed that the tumor cells were arranged in shapes of beehives and separated by rich reticular fibers. HCCC tumor reacted differently on S-100 protein, glial fibrillary acidic protein, actin and vimentin. The ultrastructure of HCCC cells showed characteristics of ducts but no myoepithelial differentiation. Conclusions: This study demonstrates that correct diagnosis, timely surgical resection and postoperative radiotherapy are effective in treating nasal clear cell carcinoma.

Keywords: Primary clear cell carcinoma, nasal cavity, hyalinizing clear cell carcinoma of salivary gland

#### Introduction

Clear cell carcinoma, not otherwise specified (CCC-NOS), is a malignant epithelial tumor composed of transparent single cells with conventional dyeing. Clear cell carcinoma is commonly found in kidney, lungs, ovary and jaw odontogen, etc. Primary nasal clear cell carcinoma is derived from the small seromucous gland, having similar histology with malignant salivary gland tumor. Salivary gland tumors contain varying amounts of clear cell components, such as mixed tumor, epithelial-myoepithelial cell carcinoma, mucus, epidermoid carcinoma, acinic cell carcinoma, myoepithelial carcinoma, and sebaceous adenoma. Hyalinizing clear cell carcinoma (HCCC) of salivary gland is a rare type of salivary gland tumor. It lacks the characteristics of salivary gland tumor and is composed of single clear cells. Ultrastructural and immunohistochemical studies demonstrated that HCCC had ducts but not myoepithelial differentiation [1]. In 1988, Peison and colleagues reported one case of nasal clear cell mucoepidermoid carcinoma [2], which was first described in details and named as HCCC by Milchgrub et al. [3], but was called clear cell adenocarcinoma by Air Force Information Program et al. [4]. In 2003, HCCC was included into the new classification of salivary gland tumors by the World Health Organization. In 2005, CCC-NOS was listed as a new type of salivary gland tumor in the classification of head and neck tumors by the World Health Organization [5]. Clear cell carcinoma of salivary glands has obvious site specificity. Previous reports indicated that CCC mainly occurs in the mouth of minor salivary glands, especially at the palate. In addition, CCC may also appear in buccal cavity, tongue, floor of mouth, and retromolar area, but is rarely found in the nasal cavity [6-15]. Seven cases of CCC in nasal cavity were listed by Pubmed [16-22], and ten cases were found in China Hospital Knowledge Database (CHKD) [23-31]. By now, few clinical data on HCCC have been reported in the literature, and reports on nasal clear cell carcinoma are even rare. In this study, we perform a retrospective analysis of clinical and pathological data of six cases of primary nasal clear cell carcinoma in our hospitals since 1992 (23 cases in total including the 6 cases). A literature review about clinical manifestations, pathological features, immunohistochemistry, diagnosis and treatment was listed [23].

### Materials and methods

### Patients

The pathological archives of salivary gland nasal tumors in Shandong Cancer Hospital during 1992-2013 were reviewed. According to the new classification standards of the World Health Organization, 23 suspected cases were selected for immunohistochemical and special staining. After careful inspection of the images by two experienced pathologists, 4 cases were diagnosed as clear cell carcinoma of nasal cavity after exclusion of other nasal cavity tumors with clear cells, and 2 cases were directly diagnosed as clear cell carcinoma of nasal cavity.

### Ethical considerations

All procedures were approved by the Ethics Committee of Shandong Cancer Hospital. Informed consents were obtained from all patients or their families.

### Immunohistochemistry

The samples were labeled using EnVision (Dako, USA) method. Antibodies against HMB-

45, S-100, vimentin, keratin, actin, A-SMA, CGA, and Syn were purchased from Dako (USA). The samples were pretreated with 3% hydrogen peroxide for 20 min and then incubated with 1% calf serum albumin for 30 min. After incubation with primary antibody at 4°C overnight, EnVision reagent (Dako, USA) was added for incubation at 37°C for 30 min. Next, immunoblotting was performed using 3, 3'-diaminobenzidine reagent followed by counterstaining with hematoxylin. Positive and negative controls were set for each staining. The stained tumor sections were inspected by two pathologists for the exclusion of other tumors containing clear cells. The immunohistochemical results were classified according to positive cells: no positive staining cells (-); positive cells <30% (+); positive cells between 30% and 50% (++); and positive cells >50% (+++).

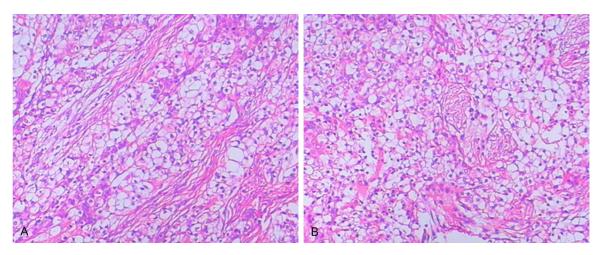
### Results

Clinicopathological data, treatment and followup results of patients with CCC in nasal cavity

Patient history, ages, names, clinical manifestations, diagnosis and treatments for the six cases were recorded (Table 1). In addition, the 4 cases mentioned above underwent conventional serial sections (4 µm) for further immunohistochemical investigation. Six cases were treated by surgical operation. Because the tumors were friable and easy bleeding, postoperative bleeding from the nose and watery nasal discharge was paid close attention to. None of the 6 cases had cerebrospinal fluid leakage. In addition, all 6 patients received postoperative radiotherapy (a dose of 60-70 Gy). Using computed tomography (CT)-simulation localization system, six cases underwent double neck prophylactic irradiation (target area in 4 cases was from prevention area to the bilateral supraclavicular area, and that in 2 cases was from prevention area to the bilateral upper neck). Normal organs were outlined in CT images, including the optic nerve, crystal, eyeballs, brainstem, spinal cord, and parotid gland. A safe boundary of 0.3 cm was set outside the brainstem, forming planning target volumes of risk. Target volume, maximal dose, minimal dose and average dose were identified in the dose-volume histograms. A volume for 95% of prescription dose was used as the reference point for evaluating the target coverage. Planning target volume was 0.3 cm outside the

Cases	Gender	Age (year)	Pathogenesis	Clinical manifestation	Tumor diameter (cm)	Treatment	
1	Female	28	5 months	Congestion for five months with occasionally bleeding	0.2×0.22×0.5	Postoperative radiotherapy	
2	Female	60	1 year	Blood in nasal discharge for one year and congestion in left nasal for two months	0.6×0.4×0.3	Postoperative radiotherapy	
3	Male	33	2 months	Blood in nasal discharge for two months	1.2×1.1×2.15	Postoperative radiotherapy and chemotherapy	
4	Male	60	5 months	Congestion for five months and blood in nasal discharge for three months	0.3×0.2×0.4	Postoperative radiotherapy	
5	Male	58	1 year	Congestion for one year and blood in nasal discharge for one months	1.0×0.4×0.6	Postoperative radiotherapy and chemotherapy	
6	Female	49	6 months	Congestion for six months and blood in nasal discharge for four months	1.3×0.7×1.1	Postoperative radiotherapy	

Table 1. Clinical data of 6 cases of clear cell carcinoma of nasal cavity



**Figure 1.** Microscopic observation of HCCC tissues and cells after carmine staining. The tissues were obtained form a 28-year-old female nasal clear cell carcinoma patient who underwent radiotherapy after surgery with no recurrence during 5 years of follow-up. A. HCCC tumor cells that were arranged in the shape of beehives (×100). B. Tumor interstitial fibers that demonstrated hyaline degeneration (×100).

clinical target volume and 0.3 cm inside the skin. The dose limits of normal organs were  $D_{max} \leq 50$  Gy for brain and optic nerve,  $D_{max} \leq 9$  Gy for crystal, and  $D_{max} \leq 25$  Gy for 50% volume of bilateral parotid. The follow-up time ranged from 4 to 22 years, with an average of 11 years. Six patients were tumor-free, and no recurrence or metastasis was observed in the follow-up period. The main adverse effect of radiotherapy was skin effect. No long-term adverse effect was observed.

# HCCC tissues and cells appear to be abnormal under microscope

To observe the microscopic presentations of HCCC, carmine staining was performed before microscopy. HCCC tissues were mainly composed of polygonal epithelioid tumor cells arranged into the shapes of beehives, and separated by fibrous tissues containing rich thinwall capillaries. HCCC cells were polygonal with different cell sizes. The nuclei of the cells were small and located in the center or on one side of the cells, with rare mitosis. In addition, the cytoplasm was rich and translucent with some cells having multiple vacuoles (Figure 1A). Reticular fiber staining showed that the tumor cells were arranged in shapes of beehives and separated by rich reticular fibers (Figure 1B). Glycogen staining showed that the tumor cells contained abundant glycogen (data not shown). Alcian blue staining showed negative results (data not shown). These data demonstrated

that tissues and cells in HCCC appeared to be abnormal under the microscope, which needed further confirmation by immunohistochemical investigation.

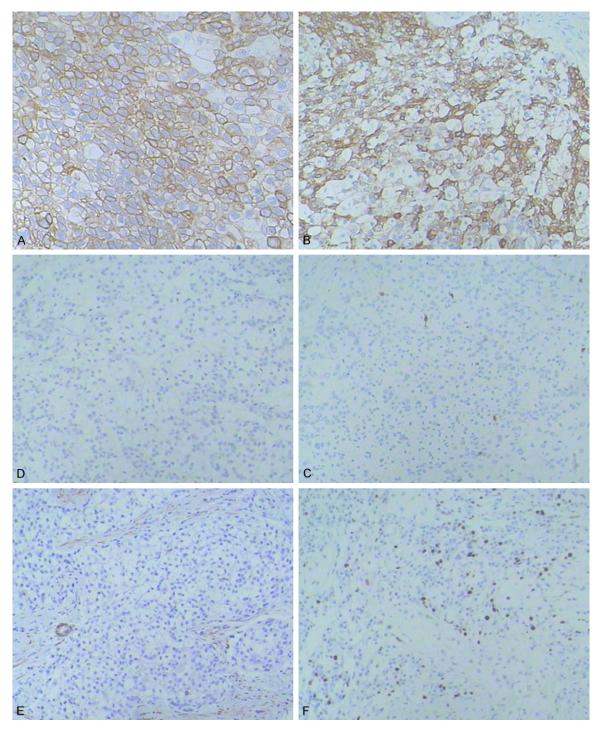
## Immunohistochemistry shows that HCCC is abnormally differentiated

To identify positive HCCC cells, immunohistochemical analysis was performed. Focal expression of creatine kinase was detected. The data showed that the tumor reacted differently in S-100 protein, glial fibrillary acidic protein (GFAP), actin and vimentin. In addition, myoepithelial differentiation was best characterized by myoepithelial carcinoma clear cell variants. The ultrastructure of HCCC cells showed characteristics of ducts but no myoepithelial differentiation (**Figure 2; Table 2**). These data indicated that tissues with focal expression of creatine kinase and myoepithelial carcinoma clear cell variants could be diagnosed as HCCC.

### Discussion

Their clinical features were described as follows.

Clinical manifestation. More common symptoms of recurrent epistaxis, nasal, and runny nose were observed, with no significant differences being observed between men and women. If the orbit and skull were seen, the corresponding symptoms may also appear. Anterior rhinoscopy was commonly found in



**Figure 2.** Immunohistochemical staining of HCCC cells with (A) CK (+) (magnification  $\times 100$ ), (B) CK56 (+) (magnification  $\times 200$ ), (C) GFAP (-) (magnification  $\times 100$ ), (D) S-100 (-) (magnification  $\times 100$ ), (E) Calponin (-) (magnification  $\times 100$ ) and (F) Ki-67 (+) (magnification  $\times 100$ ). The tissues were obtained form a 28-year-old female nasal clear cell carcinoma patient who underwent radiotherapy after surgery with no recurrence during 5 years of follow-up.

nasal cavity, nasal middle meatus, or olfactory cleft, accompanied by red color. CT showed soft tissue density and partial bone destruction. Literature review demonstrated three cases of bone destruction, which were found in the anterior wall, the medial wall and the inside wall of maxillary sinus. Primary clear cell carcinoma of nasal cavity is characterized by local

Cases	СК	CK5/6	EMA	S-100	Calponin	Ki-67	GFAP				
1	+	+	-	-	-	+	-				
2	++	++	-	-	-	+	+				
3	+	+	-	-	-	+	-				
4	+	+	+	-	-	+	-				
5	++	+	-	-	-	+	-				
6	+		-	-	-	+	-				

**Table 2.** Immunohistochemistry for the 6 cases ofclear cell carcinoma of nasal cavity

invasive growth. Therefore, for patients with nasal bleeding or blood in the nose, detailed patient history should be inquired and careful physical and various auxiliary examinations are necessary.

Pathological characteristics. Primary nasal clear cell carcinoma originates from the small seromucous gland and belongs to malignant tumors of salivary gland. Among salivary gland tumors, mixed tumor, epithelial myoepithelial cell carcinoma, mucinous carcinoma, epidermoid carcinoma, acinic cell carcinoma, myoepithelial carcinoma, and sebaceous adenoma contain varying amounts of clear cell components. By immunohistochemical analysis, clear cell carcinoma can be differentiated from salivary gland tumors with clear cells, including epithelial myoepithelial carcinoma, mucoepidermoid carcinoma, myoepithelioma, myoepithelial carcinoma, and clear cell type squamous cell carcinoma. Immunohistochemical and histological studies demonstrated that myoepithelial differentiation was best characterized by variant myoepithelial carcinoma clear cells [1, 3, 32]. However, the disease is easy to be misdiagnosed as renal clear cell carcinoma of nasal cavity metastasis, clear cell metastasis of thyroid cancer, malignant melanoma, epithelial myoepithelial carcinoma, and malignant myoepithelioma [29, 33]. Therefore, CT of chest and abdomen and B ultrasound examination of pelvis confirmed that there was no substantive metastasis in abdominal organs and bilateral thyroid. According to the tumor pathological data, one case was diagnosed as adenocacinoma, two cases were diagnosed as squamous cancer, and one case was diagnosed as metastatic clear cell carcinoma of unknown origin. With increasing awareness of the disease, more and more cases will be diagnosed in the future.

Treatment and prognosis. Primary clear cell carcinoma of nasal cavity is characterized by local invasive growth and may result in lymphatic and blood metastasis. In addition, metastasis to cervical lymph nodes, lungs, and bone may appear, but are rarely seen. According to literatures, among 12 patients with primary clear cell carcinoma of nasal cavity, simple operation was performed in 3 cases (all lateral rhinotomy), surgical operation combined with radiother-

apy and chemotherapy was conducted in 7 cases (2 cases of lateral rhinotomy, and 5 cases of nasal endoscopic surgery), and radiotherapy and chemotherapy were performed in 2 cases. After treatment, the follow-up lasted for 6 months to 10 years. Ereno et al. reported one case of hypopharyngeal clear cell carcinoma metastasis to the lungs but no recurrence was found in other cases [34]. Six patients in our hospital received postoperative radiotherapy and two patients received chemotherapy, without further recurrence afterwards. At present, HCCC is considered as a low-grade malignant tumor. The tumor size, cell atypia, mitoses and P53 expression may be closely related to prognosis [3, 32]. In addition, the recurrence of tumor is correlated with the tumor edge infiltration [35], and thorough resection of the tumor is necessary. Because the tumor contains abundant capillaries and sinusoids, it is easily bleeding during operation. Therefore, before operation, careful preparation for timely hemostasis must be performed. Because of the invasive growth of HCCC as well as local restrictions of nasal anatomy, complete surgical removal is not easy. If postoperative margin is not clear or the security boundary is not enough after the operation, postoperative radiotherapy must be conducted. Radiotherapy is suggested to be conducted on the basis of the nasal cavity tumor radiotherapy principle. Gross tumor volume should include the primary focal region and positive neck lymph node that are confirmed by preoperative tumor area and imaging examination. Clinical target volume should be determined according to the primary site and tumor staging. Tumor is limited to one side of the nasal cavity, without infringing adjacent organs or tissues. The target areas include bilateral nasal cavity, bilateral anterior ethmoid sinus, hard palate and ipsilateral maxillary sinus. If the tumor transcends the nasal cavity, target area expands to the adjacent organs or

structures. If the anterior ethmoid sinus is infringed, clinical target volume should include the ipsilateral ethmoid sinus. If the tumor infringes adjacent choana or nasopharynx, radiation field should include nasopharynx. For the early well-differentiated tumors with smaller lesions and clean resection, the irradiation of cervical lymph nodes is not necessary. For patients at T3-T4 stage, cervical lymph node preventive irradiation should be conducted, with the radiation covering only II lymph drainage area. When pathological changes invade 1/3 of posterior nasal cavity, preventive irradiation should be performed on retropharyngeal lymph nodes and lymph nodes in areas II and III of the double neck. When nasopharynx is invaded, retropharyngeal lymph node and double neck zone II-V prophylactic lymph nodes should be irradiated. For patients with lymph node metastasis, therapeutic irradiation for corresponding metastases and prophylactic irradiation for the supraclavicular lymph node drainage area should be conducted. Usually, the radiation amount is 60-66 Gy for the primary foci and invaded lymph node region, and 44-64 Gy for the lymph node region without infiltration. For patients with relative large lesions and resection difficulty, preoperative radiotherapy should be performed. However, a large number of cases are still needed for developing specific treatment principles.

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### Disclosure of conflict of interest

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