Tumori, 93: 499-503, 2007

NEUROENDOCRINE CARCINOMAS OF THE UPPER AIRWAYS: A SMALL CASE SERIES WITH HISTOPATHOLOGICAL CONSIDERATIONS

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Neuroendocrine carcinomas are rare tumors. In the head and neck region they are most common in the larynx, where they represent 0.5-1% of epithelial cancers. Diagnosis requires the recognition of the typical neuroendocrine architecture and morphology and the immunohistochemical confirmation of neuroendocrine differentiation. In the 1991 WHO classification laryngeal neuroendocrine carcinomas have been divided into carcinoids, atypical carcinoids, small cell carcinomas and paragangliomas. Atypical carcinoids in the head and neck region usually show an aggressive behavior analogous to poorly differentiated carcinomas, and are resistant to chemo- and radiotherapy. For this reason, it was recently proposed to change their designation to "moderately differentiated neuroendocrine carcinomas".

We present the clinical and histopathological features of 2 moderately differentiated neuroendocrine carcinomas of the larynx, one large cell poorly differentiated neuroendocrine carcinoma of the oropharynx, and one small cell carcinoma of the minor salivary glands of the tongue. The patient with small cell carcinoma was free from disease 26 months after radical surgery, while the other patients showed liver, lung and bone metastases 18, 26 and 24 months after the diagnosis despite radical surgery or concomitant intra-arterial chemotherapy and radiotherapy.

Key words: atypical carcinoid, head and neck tumor, immunohistochemistry, neuroendocrine carcinoma.

Introduction

Neuroendocrine tumors are rare in the head and neck region. Carcinomas with neuroendocrine differentiation have been described in the ear, nose, paranasal sinuses, tongue, salivary glands, larynx and trachea[1-3]. They are most common in the larynx, where they represent 0.5-1% of epithelial cancers and are the most frequent non-epidermoid carcinoma type[4]. Within the larynx, they occur more often in the epiglottis and in the supraglottic region[5]. The diagnosis of neuroendocrine carcinoma (NEC) of the head and neck region is based on the recognition of the typical neuroendocrine architecture and morphology and on the immunohistochemical confirmation of neuroendocrine differentiation, while auxiliary techniques such as electron microscopy are not required for the diagnostic routine[6,7].

According to the 1991 WHO classification, laryngeal NECs are divided into carcinoids, atypical carcinoids, small cell carcinomas and paragangliomas, by analogy with the classification of pulmonary neuroendocrine tumors[8]. The atypical carcinoid category includes tumors defined as moderately differentiated NEC, large cell NEC, and medullary carcinoma-like carcinoid[9]. Since the latter often show an aggressive clinical behavior, with 43%, 22% and 44% of patients affected by lymph node, skin and systemic metastases, respectively[10,11], it has recently been proposed to apply to the larynx the modified classification accepted for lung neuroendocrine tumors, which distinguishes the following categories: well differentiated, moderately differentiated and poorly differentiated neuroendocrine tumor of small or large cell type. Interestingly, in the salivary glands small cell NECs are the most common type of NECs, representing 2% of all tumors, while more differentiated neoplasms are exceedingly rare[9].

Unfortunately, no specific treatment exists at present for neuroendocrine tumors of the head and neck region, and despite the improved histological classification they are mostly treated as conventional squamous cell carcinomas or, less often, as small cell carcinomas of the lung[8,10,12-14].

In this paper we present the clinical and histopathological features of 2 cases of NEC of the larynx, one of the oropharynx, and one of the minor salivary glands of the tongue. These cases represent 1.4% of the head and neck tumors diagnosed in our center in the last 3 years.

Patients and methods

Laboratory methods

Biopsy samples were fixed in formalin and routinely processed by paraffin inclusion and hematoxylin and eosin staining of 4-µm-thick sections. Fine-needle aspiration samples were smeared on slides, fixed in alcohol and stained with Papanicolaou. Immunohistochemical reactions were performed on unstained paraffin sections with a panel of antibodies including cytokeratin 8-18 (CAM 5.2, Becton Dickinson, working dilution 1:200), chromogranin A (DakoCytomation, working dilution 1:5000); neuron-specific enolase (NSE, Biogenex, working dilution 1:2), synaptophysin (DakoCytomation, working dilution 1:1000), n-CAM/CD56 (Zymed, working dilution 1:100), calcitonin (DakoCytomation, working dilution 1:800), thyroid transcription factor (TTF-1, DakoCytomation,

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Received September 26, 2006; accepted January 18, 2007.

working dilution 1:100), cytokeratin 20 (DakoCytomation, working dilution 1:300), and high molecular weight cytokeratin 34betaE12 (DakoCytomation, working dilution 1:300). The following antigen retrieval procedures were required for the immunostains: 20 minutes trypsin digestion at 36°C (CAM 5.2); 15 minutes microwave 600W in pH 6 citrate buffer (cytokeratin 20, n-CAM, chromogranin A, synaptophysin, TTF-1, calcitonin); 5 minutes pepsin digestion at 36°C followed by 15 minutes microwave 600W in pH 6 citrate buffer (cytokeratin 34betaE12). Reactions were revealed with peroxidase-conjugated streptavidin-biotin complex (LSAB+, DakoCytomation) using diaminobenzidine tetrahydrochloride (Dako liquid DAB, DakoCytomation) as chromogen.

Case histories

Case 1

P.P, a 59-year-old man, was referred to our center in April 2003 complaining of hoarseness and progressive dysphagia. Under laryngoscopic examination, an endolaryngeal mass of 1.5 cm involving the epiglottis and the left false vocal cord was discovered. Laryngeal motility was preserved. A laryngoscopic biopsy was performed and the neoplasm was diagnosed as a basaloid squamous cell carcinoma. After CT scan the tumor was clinically staged as T2N0M0. The patient underwent partial supracricoid laryngectomy and bilateral selective neck dissection. Based on the surgical sample a diagnosis of moderately differentiated NEC was made, and confirmed after revision of the previous biopsy. The tumor infiltrated the submucosal stroma of the anterior portion of the left false vocal cord and of the base of the epiglottis. Cervical lymph nodes were negative. Pathological stage was pT2N0M0. Twenty-six months after the diagnosis, a follow-up CT scan showed a mass in the lower lobe of the left lung. Cytological examination of a fine-needle aspiration biopsy specimen documented a pulmonary metastasis of moderately differentiated NEC of the larynx, with a high mitotic index and negative pulmonary markers (TTF-1). Review of the original slides and of the pulmonary lesion confirmed the metastatic nature of the latter. The lesion was resected and systemic chemotherapy was started at another hospital, so we had no information about the type of drugs employed and the control of the disease. The patient was lost to follow-up.

Case 2

B.L., a 68-year-old man, was referred to the ENT Department of our hospital in January 2004 complaining of a sore throat, odynophagia, pyrosis and bilateral otalgia of a few months' duration. Endoscopy revealed a polypoid non-ulcerated mass with a diameter of 1 cm involving the laryngeal surface of the epiglottis; laryngeal motility was preserved and the hypopharynx was free of disease.

A biopsy was performed and the lesion was diagnosed as moderately differentiated NEC. Tumor stage (T2N2aM0) was determined after clinical and instrumental evaluation including CT scan. The patient underwent a total laryngectomy and bilateral selective neck dissection. Histological examination of the surgical specimen confirmed the neuroendocrine nature of the tumor, which infiltrated the epiglottis and its cartilage, the pre-epiglottic space, and the anterior portion of the left false vocal cord and of the aryepiglottic fold. Four homolateral and 1 contralateral lymph node metastases were identified. Pathological stage was pT3N2cM0. Eighteen months after the diagnosis, liver ultrasonography showed multiple metastases and systemic chemotherapy was started with cisplatin and etoposide, without any regression of the disease. The patient died 29 months after the initial diagnosis.

Case 3

F.P., a 68-year-old man, was admitted to our department in April 2004 complaining of pain in the tongue that had lasted several months. Examination of the mouth showed a painful, oval mass of hard consistency with a diameter of 2 cm, located on the left margin of the tongue. No regional lymph nodes were involved.

Analysis of the biopsy sample showed a small cell carcinoma. TNM staging after CT scan was T1N0M0. The patient underwent a hemiglossectomy and left selective neck dissection. No metastases were found. The patient is currently free of disease after a 26-month follow-up.

Case 4

B.F, a 60-year-old man, came to our attention in June 2004 with a sore throat, foreign body sensation and cervical pain of a few months' duration. Endoscopy of the upper airways revealed a 4-cm enlarging, ulcerated mass of the tongue base. The tumor invaded the valleculae and the pharyngeal-epiglottic folds bilaterally as well as the tongue side of the epiglottis, while there was no evidence of tumor in the glottic, hypoglottic and hypopharyngeal regions. Motility of the tongue and larynx was preserved. Physical examination of the neck showed a 2-cm left adenopathy at level II. A biopsy was performed and the tumor was diagnosed as a poorly differentiated large cell carcinoma with neuroendocrine differentiation. Clinical stage was T3N1M0.

Because of the advanced stage of the neoplasm, the patient underwent concomitant intra-arterial chemotherapy and radiotherapy. A dose of 350 mg/m^2 of carboplatin was administered in the tumoral bed by means of superselective catheterization from the femoral artery up to the lingual artery. Four injections were given (1 every 2 weeks) for a total amount of 1.4 g/m^2 of carboplatin. At the same time 3D conformal radiotherapy was performed with a dose of 60-70 Gy to the primary site and the postive nodes (PTV I) and 50-55 Gy to the regional lymph nodes at risk of metastasis (PTV II). Radiographic examination at 24 months of follow-up showed 2 nodular opacities in the upper lobe of the left lung and a hylar mass in the right lung. A biopsy performed under bronchoscopic guidance confirmed the

metastatic nature of the lesions. Systemic CT scan documented the presence of multiple vertebral and costal metastases. The patient is currently alive with disease.

Histological and immunohistochemical study

In case 1, histological examination showed a neoplasm composed of intermediate-sized cells with eosinophilic cytoplasm, granular chromatin and scant nucleoli with an organoid structure. The cells were immunoreactive for the neuroendocrine markers chromogranin A, synaptophysin, n-CAM, and calcitonin. TTF-1 was not expressed. Focal reactivity for high-molecular-weight cytokeratin was present. The mitotic index was highly variable, and in some areas more than 10 mitoses per 10 high-power fields (HPF) were counted (Figure 1). The same characteristics were observed in the metastatic lesion in the lung. The definitive diagnosis was moderately differentiated NEC of the larynx, with histologically proven pulmonary metastasis.

Case 2 was microscopically characterized by intermediate-sized neoplastic cells with eosinophilic cytoplasm, vesicular nuclei and occasional nucleoli. The cells were organized in large nests and were immunoreactive for the neuroendocrine markers chromogranin A, synaptophysin, n-CAM, and calcitonin. TTF-1 was not expressed. The epithelial nature was confirmed by cytokeratin 8-18 immunoreactivity, while high-molecular-weight cytokeratins were not expressed. The mitotic index was >10 mitoses per 10 HPF (Figure 2). The definitive diagnosis was moderately differentiated NEC of the larynx.

In case 3 the tumor was immunoreactive for the neuroendocrine markers chromogranin A and n-CAM; CK20 showed dot-like paranuclear expression, while TTF-1 was negative (Figure 3). After a thorough clinical investigation aimed at excluding a metastatic lesion from a cutaneous Merkel cell carcinoma, a diagnosis of primary small cell NEC of the minor salivary glands was made.

In case 4 the biopsy showed a neoplasm composed of intermediate-sized cells with large vesicular nuclei, prominent nucleoli and frequent mitoses. Cells were organized in nests and trabeculae, with areas of necrosis. Immunoreactivity for PGP 9.5 and chromogranin A but not for NSE, synaptophysin or n-CAM was observed (Figure 4). Marked reactivity for high-molecular-weight cytokeratin was present. The final diagnosis was poorly differentiated large cell NEC.

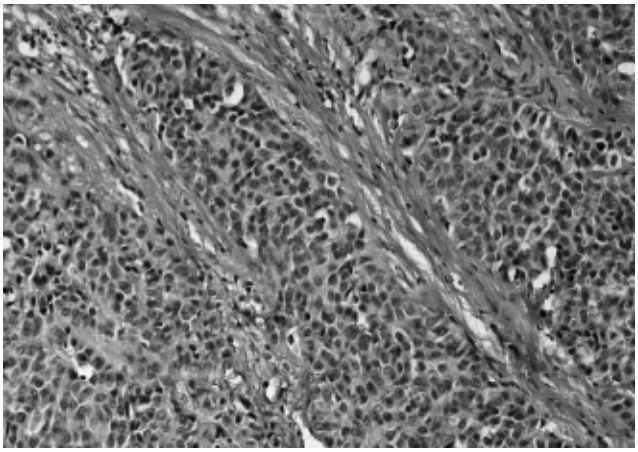

Figure 1 - Atypical carcinoid of the larynx in patient 1. The tumor is composed of intermediate-sized cells with eosinophilic cytoplasm, granular chromatin and scant nucleoli with an organoid structure (H&E, ×20).

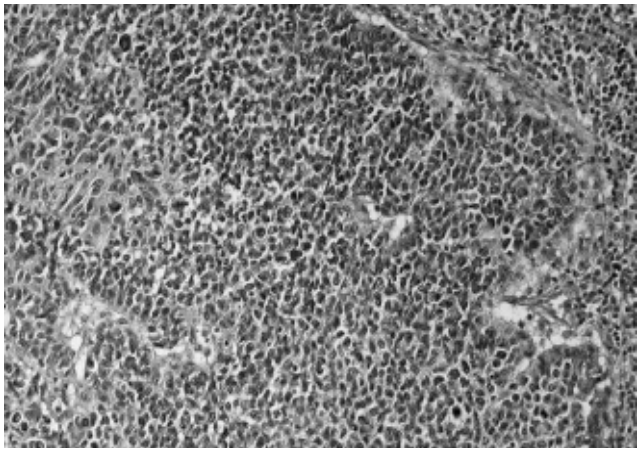

Figure 2 - Atypical carcinoid of the larynx in patient 2, characterized by intermediate-sized neoplastic cells with eosinophilic cytoplasm, vesicular nuclei and occasional nucleoli, organized in large nests (H&E, ×20).

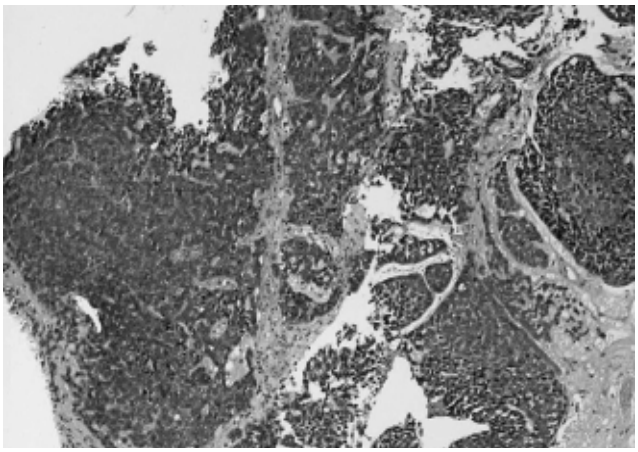

Figure 3 - Small cell neuroendocrine carcinoma of the minor salivary glands in patient 3, showing the typical oat cells, nuclear molding and a high mitotic index (H&E, ×10).

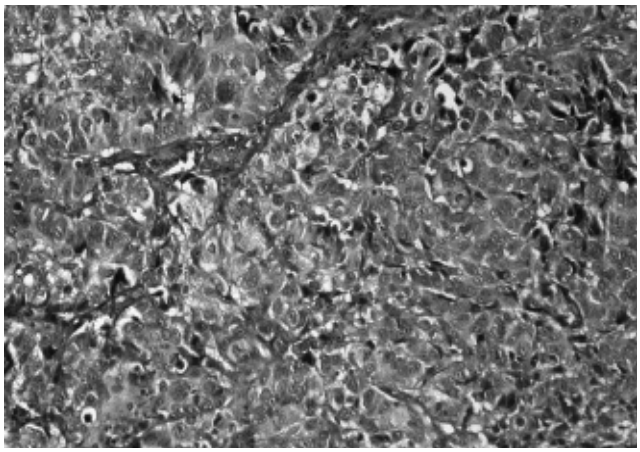

Figure 4 - Poorly differentiated neuroendocrine carcinoma of the oropharynx in patient 4, composed of markedly atypic cells of intermediate size with large vesicular nuclei, prominent nucleoli and frequent mitoses, with areas of necrosis (H&E, ×40).

Discussion

The present case series documents that aggressive neuroendocrine tumors can be encountered rather frequently in the head and neck region; they account for 1.3% of the head and neck tumors observed in 3 years at our center, while the well differentiated counterpart, also called typical carcinoid, is exceedingly rare. The diagnosis of a neuroendocrine tumor is based on the recognition of the characteristic morphology and architecture, which may be overlooked or misdiagnosed especially in small biopsy samples; immunohistochemical confirmation of the neuroendocrine nature of neoplastic cells is always required. The morphological features that can orient the pathologist towards a correct diagnosis are the organoid pattern of growth, the absence of keratinization, the finely granular chromatin, and, in small cell tumors, a high mitotic index. To rule out a metastatic tumor from sites where neuroendocrine neoplasms arise more frequently (thyroid, lung, skin), thorough clinical and instrumental screening can be supported by a few immunohistochemical reactions: the frequent expression of calcitonin in laryngeal tumors (2 of 2 cases in our series, 6 of 7 cases in Woodruff's series of "large cell neuroendocrine carcinomas"[15]), brings metastatic medullary carcinoma of the thyroid into the differential diagnosis, which can be excluded by a negative immunohistochemical reaction for TTF-1. Furthermore, the absence of TTF-1 nuclear reactivity in tumor cells is of value to exclude a metastatic NEC of the lung, particularly of the small cell type. Small cell NECs of the lung are positive for TTF-1 in up to 90% of cases[16], while small cell carcinoma of the Merkel type, the most frequent tumor in the salivary glands, is negative for TTF-1 and shows typical paranuclear reactivity for cytokeratin 20[17]. The expression of high-molecular-weight cytokeratin has been suggested to be useful in the differential diagnosis of basaloid carcinomas and NECs in the lung[18]; however, a few pulmonary neuroendocrine carcinomas reactive to 34beta-E12 have been described[18,19]. Since the issue of its expression in head and neck NECs has never been addressed, it cannot be excluded that the frequent expression observed in our small series of cases (1 moderately differentiated and 1 poorly differentated NEC) depends on the cellular origin from pluripotent undifferentiated cells in the seromucous glands rather than from cells of the dispersed neuroendocrine system, as in the lung[15].

It was recently proposed to define as large cell NECs a subgroup of laryngeal NECs with more aggressive histopathological characteristics, which would meet the criteria for pulmonary large cell NECs[20]. The histological criteria of cell size, nuclear characteristics and amount of necrosis[21] were only fulfilled in case 4 of the present series, but not in cases 1 and 2 despite the high mitotic index. The tumor of case 4 was composed of large atypical cells with an organoid pattern of growth, necrosis, high mitotic index and reactivity for at least 1 neuroendocrine marker and could be classified as a large cell NEC of the oropharynx. The tumors observed in patients 1 and 2 fitted best in the category of atypical carcinoid according to the 2003 WHO classification of head and neck tumors[9]. However, we believe that the definition of moderately differentiated NEC of the larynx would be preferable to atypical carcinoid, and agree with the classification of head and neck neuroendocrine tumors proposed by Mills *et al.*[21], which includes well and moderately differentiated NECs, and poorly differentiated NECs of either small or large cell type[21]. The term moderately differentiated NEC stresses more clearly that the so-called atypical carcinoid in the larynx is an overtly malignant tumor deserving an aggressive therapeutic approach. In fact, despite histopathological features consistent with those reported for atypical carcinoids, both laryngeal tumors in the present series showed an aggressive course after radical surgery, with liver and lung metastases 13 and 26 months after diagnosis even in the absence of nodal involvement (case 1), and behaved analogously to the case with the more aggressive histological tumor type. A similar behavior has been reported for published series of atypical carcinoids of the larynx[9], which showed 48% and 30% 5- and 10-year cumulative survival rates, respectively, which is much lower than those observed in atypical carcinoids of the lung (61-73% and 35-59%)[22].

As far as treatment options are concerned, radical surgery of moderately differentiated NEC does not prevent the development of metastatic disease, and radiotherapy and/or chemotherapy do not influence survival because these tumors are usually chemo- and radioresistant[12]. By contrast, chemoradiotherapy is indicated as the treatment of choice for poorly differentiated NEC[14]. This was confirmed in our study, where case 4 showed complete regression of the tumor after an integrated protocol of intra-arterial chemotherapy with carboplatin and concurrent RT. Molecular markers, such as somatostatin receptor subunits, could be useful to select tumors that could benefit from somatostatin analogs[14]; however, a classification of neuroendocrine tumors of the head and neck adequate to predict the clinical behavior is required to support therapeutic decision-making. Clinical-morphological correlations in large series of cases are necessary to provide clear diagnostic categories and to define the best treatment options.

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