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ORIGINAL PAPER

Large Cell Neuroendocrine Carcinoma of the Larynx: Definition of an Entity

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Abstract Laryngeal atypical carcinoids (AC/moderately-differentiated neuroendocrine carcinoma) are associated with moderately aggressive clinical behavior; however, a subset of tumors classified as AC have much greater aggressive potential. These tumors fulfill the proposed diagnostic criteria for pulmonary large cell neuroendocrine carcinoma, albeit in the larynx. In the current WHO classification, laryngeal large cell neuroendocrine carcinomas (LCNEC) are classified as *variants* of AC, whereas pulmonary LCNEC are classified as poorly-differentiated neuroendocrine carcinomas. Reported outcomes for pulmonary tumors support the separate classification of LCNEC. Five and ten year survival rates for pulmonary AC are 61–73, and 35–59%, respectively, while the 5-year survival rate for pulmonary LCNEC is as low as 30%. By extension, we postulate that the biologic potential of laryngeal LCNEC is similar to that of small-cell carcinoma (poorly-differentiated neuroendocrine carcinoma), and as such, warrants reclassification. The files of Barnes Jewish Hospital/Washington University were searched for the term "neuroendocrine" and the anatomic subsite larynx. Neuroendocrine carcinoma cases were evaluated using the WHO definitions for pulmonary AC and LCNEC; small cell carcinoma was excluded. Cases were also solicited from the larger head and neck pathology community. A literature search was also performed for cases of laryngeal neuroendocrine carcinoma, and cases which could be clearly classified as LCNEC by this scheme were captured as well. Six new cases plus four reported cases were identified which fulfill the WHO criteria for pulmonary LCNEC (eight men and two women). Nine patients presented at stage IV and 88% died of disease (DOD), 75 and 100% of these at 2 and 3 years, respectively. Laryngeal LCNEC is a rare entity, distinct from AC. We recommend that laryngeal tumors fulfilling WHO criteria for pulmonary LCNEC not be classified as variants of AC, but as variants of small cell carcinoma (poorly-differentiated neuroendocrine carcinoma) as they are associated with poorer outcome.

Keywords Atypical carcinoid · Larynx · Large cell · WHO classification · Neuroendocrine carcinoma

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Introduction

Laryngeal neuroendocrine carcinomas (LNEC) are uncommon tumors, particularly when compared to squamous cell carcinoma. However, they are the subject of considerable discussion and controversy among pathologists. The WHO classification of LNEC [1] generally

follows that for pulmonary neuroendocrine carcinoma [2]: typical carcinoid (TC), atypical carcinoid (AC), and small cell carcinoma, corresponding to well-, moderately-, and poorly-differentiated neuroendocrine carcinoma, respectively. According to WHO classification of head and neck tumors and several other proposed classification systems [1, 3], laryngeal tumors termed AC or moderately differentiated neuroendocrine carcinoma (MDNEC) would include neoplasms that, if lung diagnostic criteria were applied, would fulfill the diagnostic criteria of large cell neuroendocrine carcinoma (LCNEC). To our knowledge, differences in biologic behavior and patient outcome between these two distinct morphologic entities, MDNEC and LCNEC, have not been investigated in the larynx. Previous studies on patient outcome in cases of AC of the larynx did not separate LCNEC from MDNEC in their analysis [4–6]. In an attempt to define LCNEC of the larynx as a distinct entity, we report six new cases, review four previously published ones that fulfill the proposed diagnostic criteria of LCNEC, and compare patient outcomes to those of previously published series of AC.

Materials and Methods

The surgical pathology files of Barnes-Jewish Hospital and Washington University in St. Louis were searched for the terms "neuroendocrine", "atypical carcinoid", "neuroendocrine carcinoma", and "poorly differentiated carcinoma." The results were further limited to laryngeal cases fulfilling the WHO criteria for pulmonary LCNEC (Table 1) as follows: (1) tumor cells that are generally large, with moderate to abundant cytoplasm and round to oval nuclei with frequent prominent nucleoli (2) histological features such as organoid nesting, trabecular growth, rosettes and perilobular palisading patterns suggesting neuroendocrine differentiation (3) mitotic activity $>$ 10/10 high power fields (HPF)(an area of 2.0 mm^2) (4) confirmation of neuroendocrine differentiation by immunohistochemistry [chromogranin-A, synaptophysin, neuron specific enolase, and/or neural cell adhesion molecule (CD56)]. All four criteria were required. Cellular pleomorphism and large areas of necrosis were frequently seen, but these were not required for the diagnosis [7].

Cases were also solicited via e-mail from other head and neck pathologists. Lastly, a Medline literature search was also performed for cases of laryngeal neuroendocrine carcinoma. Cases that fulfilled the criteria for LCNEC were identified and included.

Immunohistochemistry was performed at the central location (Washington University) on the six new cases on representative 4 μm sections cut from formalin-fixed, paraffin-embedded tissue blocks using Ventana's Benchmark XT automated slide stainer using Ultra View Universal DAB detection kits. Immunohistochemistry was performed for Ki-67 (Ventana; clone 30-9; rabbit monoclonal; prediluted; antigen retrieval using Ventana CC1 consisting of Tris–EDTA at pH 8.0 with antibody incubated for 16 min at 37°C), for cytokeratin 5/6 (Ventana; clones D5 and 16B4; mouse monoclonal; prediluted; antigen retrieval using Ventana CC1 consisting of Tris–EDTA at pH 8.0 with antibody), and for cytokeratin 34betaE12 (Ventana; clone 34betaE12; mouse monoclonal; prediluted; antigen retrieval using Ventana CC1 consisting of Tris–EDTA at pH 8.0 with antibody).

Results

A total of ten cases were identified (Table 2). Six of these are newly reported: four from Barnes-Jewish Hospital, one from Montefiore/Albert Einstein Medical College, and one from University of Pittsburgh Medical Center. The remainder were identified from the existing literature [8–10].

Case 1

This 40-year-old Caucasian man presented with hoarseness and dysphagia. He denied alcohol or tobacco use. Laryngoscopy revealed a right-sided epiglottic mass that extended to the right false cord. A biopsy was interpreted as

Table 1 Criteria for large cell neuroendocrine carcinoma of the larynx—all four requisite criteria must be present

Requisite criteria	Other typical features
Tumor cells with moderate to abundant cytoplasm	Nuclei with prominent nucleoli
Features of neuroendocrine differentiation (organoid nesting, trabecular growth, rosettes, and peripheral palisading)	Cellular pleomorphism
Mitotic activity $>$ 10/10 hpf (2 mm^2)	Large areas of necrosis
Confirmation of neuroendocrine differentiation using immunohistochemical staining for chromogranin-A, synaptophysin, neuron specific enolase and/or neural cell adhesion molecule (CD56)	

Table 2 Cases of large cell neuroendocrine carcinoma

Case	Age/sex	Site of occurrence	Mitosis/10 hpf	Ki-67 positive (%)	Stage at presentation	Follow up
1	40 M	Supraglottic: epiglottis	65	80	T4 N0 M0	DOD 19 m
2	62 M	Supraglottic	92	60	T4 N3 M0	DOD 12 m
3	31 F	Supraglottic: epiglottis	71	10	T3 N2 M0	Alive NED 14 y
4	59 M	Supraglottic: epiglottis, AE fold	105	100	T2 N2 M1	DOD 11 m
5	60 F	Supraglottic: epiglottis, AE fold	158	45	T2 N2 M0	Dead NED 6 m
6	74 M	Supraglottic: arytenoid	48	90	T3 N2 M0	Alive NED 1 m
7	74 M [8][a]	Supraglottic: false cord	>10	N/A	1.5 cm 4/21 LN	DOD 9 m
8	67 M [9][a]	Glottic: true cord	>10	N/A	T3 N2 M0	DOD "weeks"
9	59 M [10][a]	Supraglottic: epiglottis, false cord	>10	N/A	T2 N0 M0	Lost to follow-up with lung metastases at 26 m
10	68 M [10][a]	Supraglottic: epiglottis	>10	N/A	T2 N2 M0	DOD 29 m

[a] Cases taken from existing literature; *M* male; *F* female; *DOD* dead of disease; *NED* no evidence of disease; *LN* lymph node; *y* years; *m* months; *N/A* not applicable

"neuroendocrine carcinoma, large cell type". Radiologic studies showed no evidence of metastatic disease. Laryngectomy and left neck dissection revealed a 2.5 × 2.2 × 0.6 cm supraglottic mass with pre-epiglottic extension and invasion of the thyroid cartilage. All 67 lymph nodes were free of metastases. The tumor stage was T4/N0/M0 (Stage IVA).

Histopathology

The tumor was composed of sheets, nests, and anastomosing cords of large cells with moderate amount of cytoplasm and large pleomorphic nuclei containing speckled chromatin, and infrequent small nucleoli (Fig. 1a, b) Mitotic figures and apoptotic cells were numerous. Necrosis was confined to the larger tumor islands comprising approximately 20% of the tumor volume. The mitotic rate was 65/10 hpf. Focal lymphvascular invasion was identified. Immunohistochemistry was diffusely positive for cytokeratin AE1/AE3, diffusely positive for chromogranin-A, and was positive for synaptophysin in approximately 30% of the tumor cells. Immunohistochemistry for cytokeratins 5/6 and 34betaE12 was negative. Immunohistochemistry for Ki-67 showed 80% of the cells to be positive (Fig. 2).

Outcome

Four months after laryngectomy the patient developed numerous pulmonary and liver metastases. He received chemotherapy (cisplatin, adriamycin, and vincristine) and radiotherapy, but died of disease 19 months after presentation.

Case 2

This 62-year-old Caucasian man presented with dysphagia, a large neck mass, and weight loss of 20–25 lb over 4 months. He smoked two packs of cigarettes per day for 45 years and drank an unstated quantity of alcohol. His past medical history was significant for chronic obstructive pulmonary disease. Endoscopically, a large supraglottic mass was found extending to the superior tongue base, left and right pyriform sinuses, and anterior commissure. A biopsy was interpreted as "poorly-differentiated squamous cell carcinoma with extensive necrosis". No immunohistochemical stains were performed on this initial biopsy. He initially refused laryngectomy. The tumor was stage T4/N3/M0 (Stage IVA). A tracheostomy was placed, and he was treated with 5-fluorouracil, cisplatin, and radiation; there was partial response to therapy. He agreed to laryngectomy and right radical neck dissection 6 months later, which revealed an ulcerated mass at the epiglottic base (1.2 × 1.1 × 0.9 cm) and metastatic carcinoma in three of 13 lymph nodes (largest 2.7 cm).

Histopathology

The tumor was poorly-differentiated with infiltrating borders consisting of islands of large cells separated by necrosis. Necrosis comprised approximately 50% of the tumor volume. The tumor cells had a moderate amount of cytoplasm and large pleomorphic nuclei with prominent nucleoli and granular chromatin (Fig. 1c, d). Apoptosis was abundant and mitotic activity was up to 92/10 hpf. Immunohistochemistry performed on the resection specimen showed diffuse positive staining for cytokeratin AE1/AE3 and neuron specific enolase (NSE) and focal staining

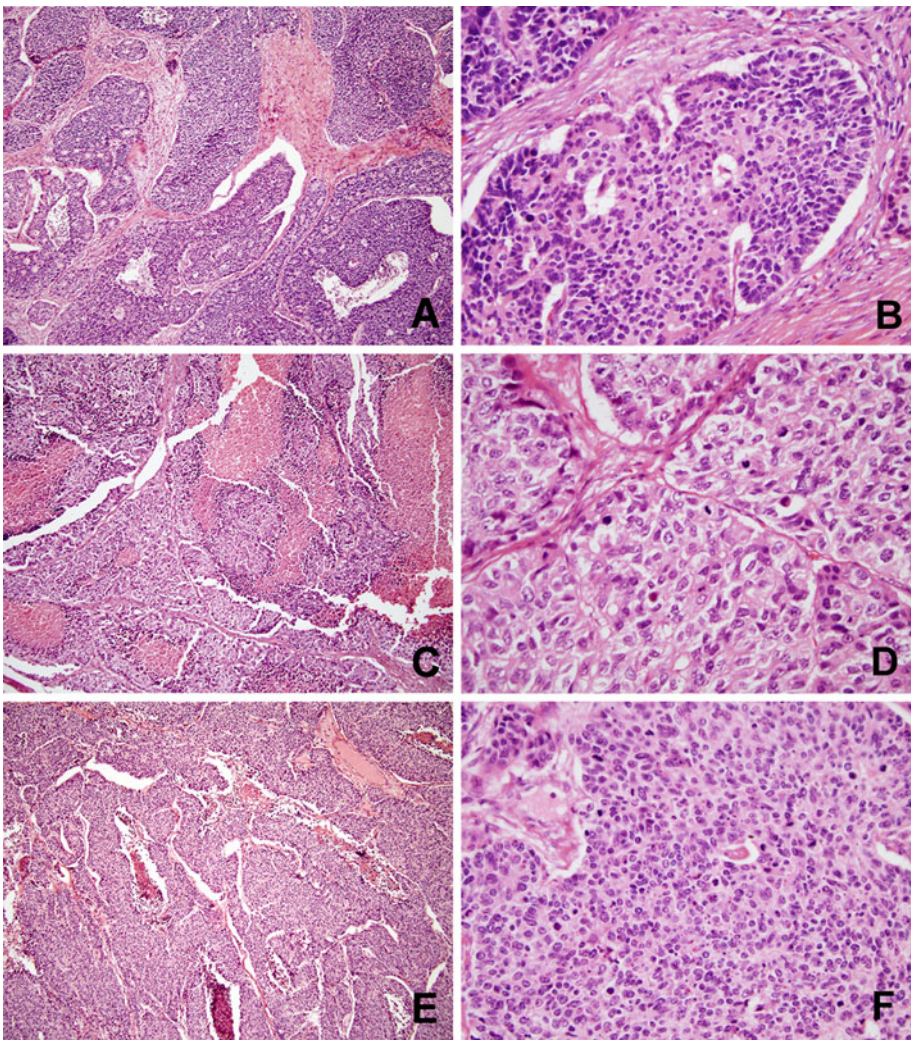

Fig. 1 H&E images of newly reported cases 1, 2, and 3. The images show nested tumors composed of cells with moderate eosinophilic cytoplasm and large, round nuclei, some with prominent nucleoli. There is some peripheral palisading in the nests along with brisk mitotic activity and necrosis (*left side images* **a**, **c**, **e** 100× magnification; *right side images* **b**, **d**, **f** 400× magnification)

for synaptophysin and chromogranin-A. Immunohistochemistry for cytokeratins 5/6 and 34betaE12 was negative. Immunohistochemistry for Ki-67 showed 60% of the cells to be positive.

Outcome

His postoperative course was complicated by a stroke 4 months after surgery. New pretracheal lymphadenopathy and bilateral pleural effusions were found, consistent with pulmonary metastases. The patient was discharged to hospice care and lost to follow up 12 months after diagnosis.

Case 3

A 31-year-old African American woman presented with a 4 month history of odynophagia, hoarseness, bilateral cervical adenopathy, cough, and 5 pound weight loss. She smoked ½ to 1 pack of cigarettes/per day for 14 years, and alcohol consumption was stated as one case of beer per week. Laryngoscopy showed an exophytic mass at the epiglottic base extending to the left true vocal cord. Biopsy of the mass was interpreted as "small cell undifferentiated carcinoma". A cervical lymph node biopsy showed metastatic carcinoma. No distant metastases were identified by radiology. The tumor was stage T3/N2/M0 (Stage IVA).

The patient underwent two courses of neoadjuvant chemotherapy with radiographic evidence of treatment response. Total laryngectomy and modified bilateral neck dissection revealed a 1.2 × 1.0 × 0.5 cm exophytic tumor originating in the anterior commissure and extending to the glottis. Metastatic carcinoma was present in three of 46 lymph nodes.

Histopathology

The tumor formed large islands with central patchy necrosis and abundant rosettes; necrosis comprised approximately 20% of the tumor volume. The tumor cells

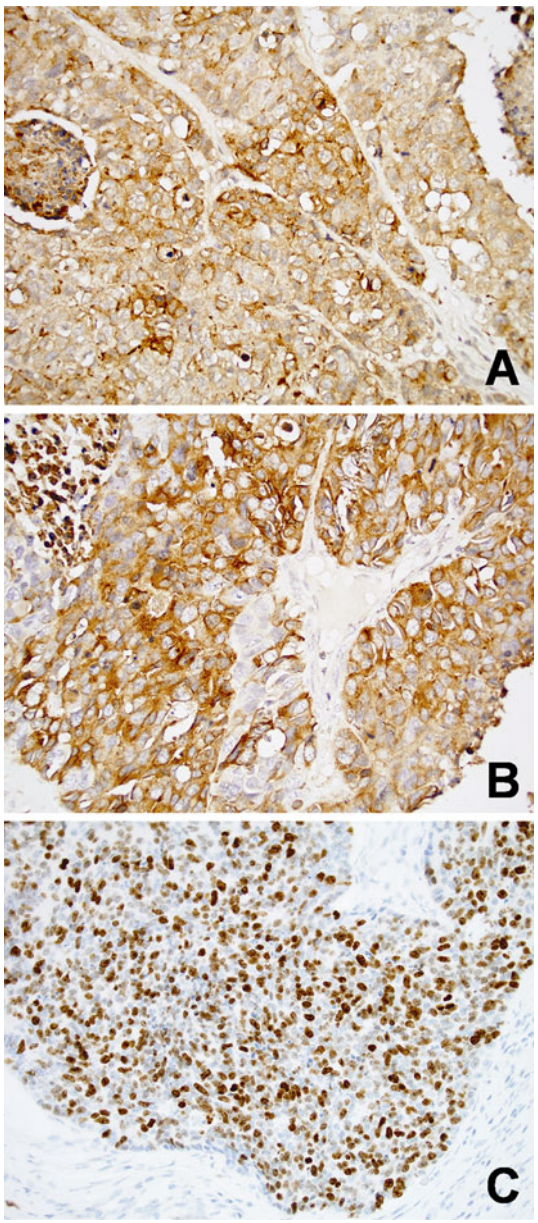

Fig. 2 Representative immunohistochemistry of LCNEC showing the tumor to be strongly positive for synaptophysin (**a** 200×) and chromogranin-A (**b** 200×). This tumor showed a very high percentage of Ki-67 positive cells (**c** 200×)

were large with moderate amounts of eosinophilic cytoplasm and large, oval, pleomorphic nuclei with coarse granular chromatin and infrequent nucleoli. Mitotic figures and apoptotic cells were numerous (Fig. 1e, f). Mitotic activity was as high as 71/10 hpf. Immunohistochemistry for cytokeratin AE1/AE3 and synaptophysin was strongly and diffusely positive and for chromogranin-A was strongly positive in approximately 40% of tumor cells. Immunohistochemistry for cytokeratins 5/6 and 34betaE12 was negative. Immunohistochemistry for Ki-67 showed 10% of the cells to be positive.

Outcome

The patient was disease free after 14 years of follow up.

Case 4

This 59-year-old man, who was a smoker and drinker, presented with a left neck mass of 1 month duration with occasional bloody discharge. A fine needle aspiration was positive for malignant cells. CT scan revealed a nodular mass in the left pyriform sinus, left aryepiglottic fold, and anterior supraglottic region with bilateral cervical lymphadenopathy; the largest lymph node measured 2.7 cm. Laryngoscopy confirmed a supraglottic mass. PET scan revealed liver and lung metastases. The tumor was clinical stage T2/N2c/M1 (IVC).

Histopathology

Biopsy revealed predominantly submucosal nodules of solid tumor nests, with scattered central necrosis and focal peripheral palisading. The tumor cells had abundant eosinophilic cytoplasm, large round to oval nuclei with open chromatin, prominent nucleoli, and moderate to severe pleomorphism (Fig. 3a, b). Apoptosis was abundant and mitotic activity was up to 105/10 hpf. There was also focal surface squamous dysplasia with a small component (less than 5%) of typical invasive moderately differentiated squamous cell carcinoma. Immunohistochemistry was positive for cytokeratin Cam 5.2, cytokeratin AE1/AE3, synaptophysin, neuron specific enolase, and CD 56 (neural cell adhesion molecule) in the neuroendocrine component. It was negative for S-100, chromogranin-A, and calcitonin. Immunohistochemistry for cytokeratins 5/6 and 34betaE12 showed strong positive staining in the squamous dysplasia and in the small component of invasive squamous cell carcinoma. The neuroendocrine component was also positive for each of these cytokeratins in approximately 50% of the cells. Immunohistochemistry for Ki-67 showed 100% of the cells to be positive.

Outcome

He was treated with primary radiochemotherapy. After initial resolution of his liver and lung nodules, new ones subsequently developed in the lung within 2 months which were histologically confirmed as metastatic neuroendocrine carcinoma. He died of disease 11 months after diagnosis.

Case 5

This 60 year old woman presented with dysphagia for 1 month. She was a smoker who denied alcohol use. On

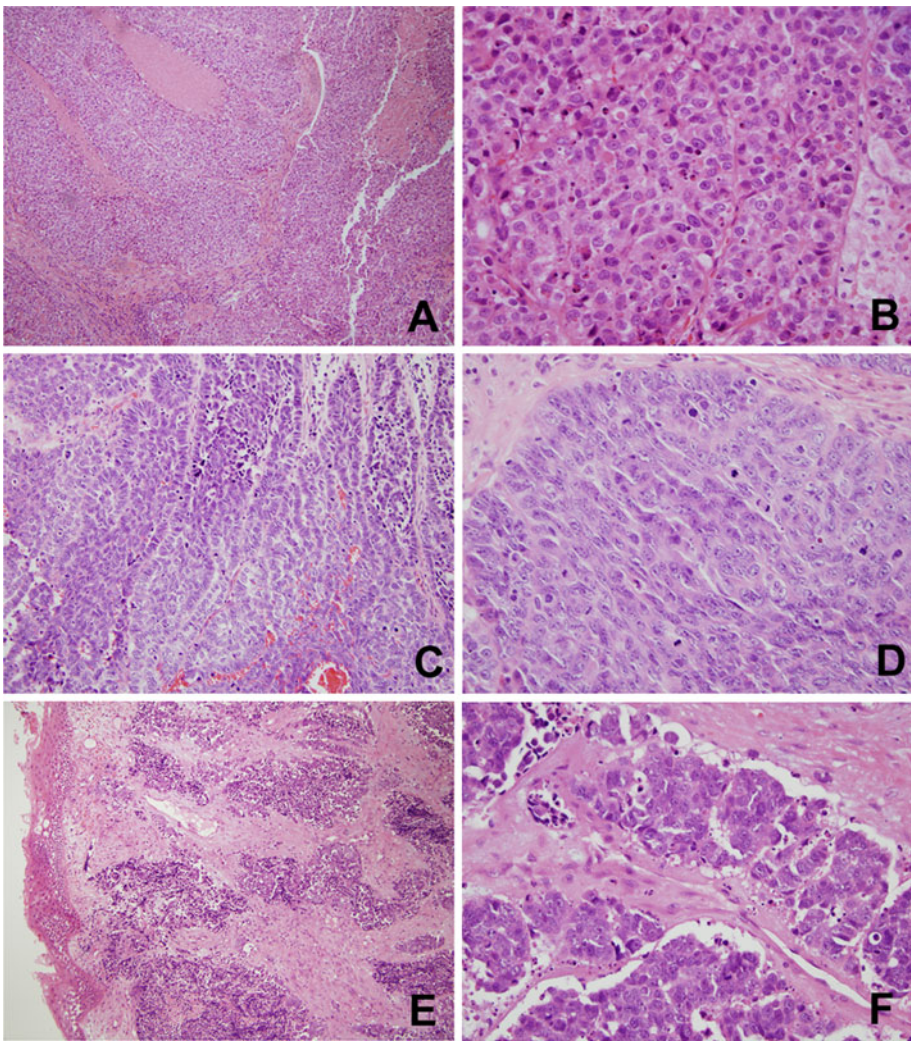

Fig. 3 H&E images of newly reported cases 4, 5, and 6. The images show nested tumors composed of cells with moderate eosinophilic cytoplasm and large, round nuclei, frequently with prominent nucleoli. There is brisk mitotic activity and necrosis (*left side images* **a**, **c**, **e** 100× magnification; *right side images* **b**, **d**, **f** 400× magnification)

examination she had a 3.0 cm left neck mass. She underwent laryngoscopy and biopsy of the left aryepiglottic fold which was diagnosed as "atypical epithelial cells suspicious for malignancy." Supraglottic laryngectomy revealed a 3.0 cm ulcerated tumor of the epiglottis. Metastatic carcinoma was present bilaterally and in three of 38 lymph nodes. The tumor was stage T2/N2c/M0 (IVA).

Histopathology

Sections showed well-circumscribed submucosal nodules of solid tumor in large nests and with patchy necrosis. The tumor cells had moderate amounts of eosinophilic cytoplasm with large, round to oval nuclei with finely granular chromatin and occasional prominent nucleoli. There was a moderate amount of nuclear pleomorphism (Fig. 3c, d). There was scattered apoptosis and brisk mitotic activity, up to 158/10 hpf. Immunohistochemistry was strongly positive for pan-cytokeratin AE1/AE3 and neuron specific enolase and negative for synaptophysin, chromogranin-A, and CD 56. Immunohistochemistry for cytokeratin 34betaE12 was focally positive with staining in approximately 10% of the tumor cells. Immunohistochemistry for cytokeratin 5/6 was negative and for Ki-67 showed 45% of the cells to be positive.

Outcome

She was treated with postoperative chemotherapy and radiation, completing the full course. However, she died 7 months after diagnosis with neutropenia and aspiration pneumonia, but no evidence of disease recurrence.

Case 6

This 74 year old man presented with hoarseness and dysphagia. On physical examination, he had palpable right sided cervical lymphadenopathy. Office endoscopy showed a large, pedunculated tumor on the right arytenoid with soft tissue infiltration. CT scan showed a 3.8 cm predominantly

supraglottic mass with transglottic extension and bilateral cervical lymphadenopathy suspicious for metastases. There was no evidence of distant metastatic disease in the chest or abdomen. Surgical debulking of the lesion was performed. The patient was stage T3/N2/M0 (Stage IVA).

Histopathology

The biopsy/debulking specimen showed a tumor composed of nests of cells in the submucosa with an intact mucosal surface without associated dysplasia. The tumor cells were in large, solid nests with prominent necrosis comprising up to 25% of the mass. The tumor cells had moderate amounts of eosinophilic cytoplasm and round nuclei with partially vesicular and partly stippled chromatin with scattered prominent nucleoli (Fig. 3e, f). Apoptotic bodies were extensive and mitotic activity was 48/10 hpf. Immunohistochemistry was positive for cytokeratin AE1/AE3 and synaptophysin and negative for chromogranin-A, serotonin, p63, p16, TTF-1, calcitonin, and thyroglobulin. Immunohistochemistry for cytokeratins 5/6 and 34betaE12 was negative. Immunohistochemistry for Ki-67 showed 90% of the cells to be positive.

Outcome

The patient began radiation and chemotherapy shortly after diagnosis and is a few months into this course of therapy.

Case 7 (Previously Published) [8]

This 74-year-old man presented with otalgia. Past medical history was significant for a 65 year smoking history and prior colon cancer. Endoscopic biopsy was interpreted as "invasive poorly differentiated carcinoma." Laryngectomy bilateral modified neck dissection revealed an exophytic, supraglottic mass that involved the left false cord. Metastatic disease was identified in bilateral cervical lymph nodes. The tumor was stage T3/N2c/M0 (Stage IVA). Shortly thereafter, metastatic carcinoma in the liver was diagnosed by ultrasound-guided biopsy.

Histopathology

The tumor was composed of large cells with eosinophilic cytoplasm, vesicular chromatin, and prominent nucleoli. The overall growth pattern was organoid and trabecular. Extensive necrosis and numerous mitotic figures were identified (reported rate > 10/10 hpf). Immunohistochemistry revealed expression of synaptophysin and chromogranin-A.

Outcome

The patient died of an unspecified cause within weeks of surgery.

Case 8 (Previously Published) [9]

This 67-year-old man presented with a 1 month history of hoarseness. Past medical history was significant for a 45 pack year history of smoking. Hemilaryngectomy and neck dissection revealed a 1.5 cm vocal cord mass. Four of twenty-one lymph nodes were positive for metastatic tumor. Although more specific data was not provided, the tumor was Stage IVA on the basis of the lymph node metastases.

Histopathology

The tumor was composed of large cells forming a nested pattern with central necrosis, separated by fibrous stroma. The mitotic rate was reportedly >10/10 hpf. Immunohistochemistry revealed expression of chromogranin-A and synaptophysin.

Outcome

Despite resection and radiotherapy, the patient died of metastatic disease at 9 months.

Case 9 (Previously Published) [10]

This 59-year-old man presented with hoarseness and progressive dysphagia. Laryngoscopy revealed a 1.5 cm mass the epiglottis and left false vocal cord. Supracricoid laryngectomy and bilateral neck dissections were performed. The tumor was stage T2/N0/M0 (Stage II).

Histopathology

The tumor was composed of intermediate-sized cells with granular chromatin and scant nucleoli. The mitotic index was variable, but had regions reported as >10/10 hpf. Immunohistochemistry was positive for chromogranin-A and synaptophysin.

Outcome

The patient developed pulmonary metastases after 26 months and was treated with a partial lung resection and chemotherapy. The patient was subsequently lost to follow up.

Case 10 (Previously Published) [10]

This 68-year-old man presented with sore throat, odynophagia, pyrosis, and bilateral otalgia. Endoscopy revealed a

1 cm mass of the laryngeal aspect of the epiglottis. Total laryngectomy and bilateral neck dissection revealed that the tumor involved the epiglottis, pre-epiglottic space, left false vocal cord and aryepiglottic fold. Metastases were present in five lymph nodes. The tumor was stage T3/N2c/M0 (Stage IVA).

Histopathology

The tumor formed a nested pattern with intermediate-sized cells with vesicular nuclei and occasional nucleoli. The mitotic count was reported as >10/10 hpf. Immunohistochemistry was positive for synaptophysin and chromogranin-A.

Outcome

He developed hepatic metastases after 18 months and died of disease at 29 months.

Summary

There were eight men and two women, and the average age was 59.4 years (range 31–74). Most patients were smokers (6 definitive, 1 denied, 2 unstated, and 1 unknown). Nine patients presented with stage IV disease, eight with cervical lymph node metastases, and one with distant metastatic disease. All tumors arose in the supraglottis or extensively involved it. Mitotic counts on the new cases (one through six) ranged from 65 to 158 per 10 high power fields. In all, seven of ten patients developed distant metastatic disease, with two of the three remaining patients having only brief follow up, dying from treatment-related or other causes shortly after diagnosis. Six of the patients died of disease (at 1, 9, 11, 12, 19, and 29 months) with an additional literature-derived patient being lost to follow-up at 26 months but with a pulmonary metastasis at this time. Only a single patient remained alive and disease-free at last follow up. Considering the nine patients with at least 6 months of follow up, the overall disease-related mortality was 89% with all deaths occurring within 3 years. The average time from diagnosis to death from metastatic disease was 1.2 years.

Discussion

The WHO schema stratifies laryngeal neuroendocrine carcinomas into three categories: typical carcinoid (3%), atypical carcinoid (54%), and small cell carcinoma (34%). All of these tumors are uncommon and have a predilection for the supraglottis [1]. In the current classification, laryngeal LCNEC is considered synonymous with atypical carcinoid/moderately-differentiated (Grade II) neuroendocrine carcinoma. On the other hand, the WHO classification for pulmonary neuroendocrine carcinomas separates LCNEC from atypical carcinoid. The prognosis of pulmonary LCNEC is significantly worse than for AC. The reported 5- and 10-year survival rates for pulmonary AC are 61–73 and 35–59%, respectively [2, 11]. By contrast, the 5-year survival rates for pulmonary LCNEC are 30–57% [12–14].

Historical survival rates for laryngeal AC patients can be found from two publications. The first, from Wenig et al. in 1988, is the largest series of AC. For patients with at least 3 years of follow up, disease specific survival was 68% with 25 patients alive and 12 dead of disease. Three year overall survival (death from any cause) was 55% [4]. In 1991, Woodruff et al. reviewed the entire existing literature on AC. Excluding those cases that were previously reported by Wenig, disease specific survival in the Woodruff series for patients followed at least 3 years was 52% with 26 alive and 24 dead of disease. Three year overall survival (death from any cause) was 48% [5]. By separating the two entities in our series, we found that survival in laryngeal LCNEC is substantially worse than that reported for AC. Three year overall survival for LCNEC of the larynx was only 11% as compared to 55 and 48% for AC as reported by Wenig and Woodruff, respectively. These observations support the concept that LCNEC of the larynx is a distinct and separate entity from AC. Similar to the pulmonary classification, we believe that LCNEC of the larynx should be classified as a high grade, or poorly differentiated, neuroendocrine carcinoma and considered, at least prognostically, as comparable to small cell carcinoma.

Although it has been alluded to from time to time, presently, there is no consistent definition for *laryngeal* LCNEC [1, 8, 10]. While there are scattered single case reports of head and neck mucosal LCNEC, there has been no series of such tumors, nor has there been a clear definition of specifically what constitutes LCNEC. And otherwise, most authors simply have regarded laryngeal neuroendocrine tumors with large cell size, necrosis, and mitotic activity higher than 10 per 10 high power fields as AC. However, as we have shown, *the major feature that distinguishes between LCNEC and AC is mitotic activity.* Typically, though, LCNEC also has much more extensive necrosis than AC and has more pleomorphic cells with prominent nucleoli. AC can also sometimes show mucin production. We did not find this in any of our cases of LCNEC.

Ours is the only series that defines laryngeal LCNEC as an entity distinct from AC. These are extremely rare tumors. We propose separating them as a subtype of laryngeal neuroendocrine carcinoma by their behavior and histology, but certainly their clinical features are very much

consistent with those of other laryngeal neuroendocrine carcinomas. They are almost always found in middle aged to older men, in smokers, and have a distinct predilection for the supraglottic larynx. The major limitation to our identifying more cases of LCNEC from the literature was lack of documentation of the mitotic activity in series of laryngeal AC/MDNEC. Most case reports and series did not report mitotic activity per high power field for individual cases. Thus, there are undoubtedly more cases of LCNEC that have been previously reported, but how many is certainly not clear.

The differential diagnosis for LCNEC most importantly includes basaloid squamous cell carcinoma (BSCC). This tumor also has a predilection for the supraglottic larynx, is strongly associated with smoking, and is defined by two major histopathologic features. The first is a basaloid component consisting of a solid, lobular growth of small, crowded cells with scant cytoplasm, hyperchromatic, round nuclei and small cystic spaces containing PAS or Alcian blue positive myxoid material. The second feature is an intimate association with SCC or dysplasia or the presence of focal squamous differentiation. Other accessory findings, including small or large central foci of coagulative necrosis and stromal hyalinosis are also described [15, 16]. BSCC typically grows as rounded nests in the submucosa, just as LCNEC. LCNEC lacks stromal hyalinosis, lacks the myxoid/mucoid stromal material, and has slightly different nuclear features, with more speckled chromatin and/or prominent nucleoli, both features that are lacking in BSCC, which has markedly hyperchromatic nuclei [7]. Immunohistochemistry in BSCC shows strong and diffuse staining for p63 and for the high molecular weight cytokeratins 34betaE12 and 5/6 and lacks staining for neuroendocrine markers, except in isolated cases. These rare neuroendocrine marker positive cases have been positive for synaptophysin or NSE, but not for chromogranin-A [17, 18]. This brings up a major issue—namely, how to further distinguish BSCC from high grade neuroendocrine carcinoma using markers other than the standard synaptophysin, chromogranin-A, NSE, and CD56. We previously showed that the high molecular weight cytokeratins 34betaE12 and 5/6 can be useful. They are almost always strongly expressed by BSCC but are rarely expressed by high grade neuroendocrine carcinoma [18]. In order to further characterize the LCNEC cases in the current study, we performed immunohistochemistry for these two cytokeratins on all six new cases. Four of them were negative, one showed focal staining (10%) for cytokeratin 34betaE12 and no staining for cytokeratin 5/6, and one showed strong staining of its squamous component and 50% positive staining for both cytokeratins in the neuroendocrine component. If one has a tumor, then, that is difficult to discern as LCNEC or BSCC and the neuroendocrine immunohistochemistry is borderline, we recommend using these high molecular weight cytokeratins. If they are negative, or even just focally positive, then this supports the tumor being neuroendocrine rather than squamous. One last marker that one might think useful to distinguish these two tumors is p63. BSCC, like all other SCC, strongly and consistently expresses p63. However, in the few cases previously studied, LCNEC is frequently positive for p63 as well, with diffuse, but with much weaker, expression than the overlying/adjacent squamous mucosa or the basal cells of adjacent minor salivary glands [18].

Solid adenoid cystic carcinoma (ACC) also enters the differential diagnosis for LCNEC, although it is rare in the larynx. It presents as well circumscribed tumor nests in the submucosa which have hyperchromatic nuclei and scattered abortive tubular structures or occasionally a minor component of tubular or cribriform tumor. The nuclei lack nucleoli and are generally regular, but can have some pleomorphism and sometimes have significant apoptosis and mitotic activity [19, 20]. Immunohistochemistry is positive for p63, but only in a patchy distribution which occurs characteristically at the periphery of the nests, presumably in cells with myoepithelial differentiation [21]. Immunohistochemistry in ACC is negative for neuroendocrine markers [18].

Finally, one must consider metastasis from another primary site, although this is rare [22, 23]. The most common tumors are melanoma and renal cell carcinoma, although rarely lung carcinomas can metastasize to the larynx (9% of all metastases to the larynx), so it is possible to tender metastatic pulmonary LCNEC as a possibility. While thyroid transcription factor-1 (TTF-1) seems logical to use for this differential diagnosis [24], TTF-1 has been shown to be positive in a significant number of non-pulmonary high grade neuroendocrine carcinomas [25]. In unpublished data, we have found that 5 of 14 (35.7%) mucosal high grade neuroendocrine carcinomas of the head and neck were positive for TTF-1. Clinical history would be critical for this distinction. Isolated laryngeal metastasis from pulmonary LCNEC would be extraordinary; rather, it is very likely that such patients would have widespread metastatic disease and an obvious primary lung lesion.

An additional differential consideration is medullary thyroid carcinoma (MTC). Extensive involvement of the anterior neck or metastasis to the larynx could be misidentified as LCNEC. MTC can show a variety of histologic patterns with neuroendocrine chromatin, necrosis in large lesions, and a high mitotic rate [26]. A combination of histology, immunohistochemistry, and clinical presentation should allow for separation of MTC and LCNEC. Unlike LCNEC, MTC usually expresses calcitonin, TTF-1, and polyclonal carcinoembryonic antigen (CEA). Calcitonin expression should not be used in isolation as cases of calcitonin positive neuroendocrine carcinomas of the larynx have been reported [4, 5, 27], and, as noted previously,

it is well known that a significant minority of non-pulmonary neuroendocrine carcinomas can express TTF-1 [25, 28]. A laryngeal tumor as the presenting finding of MTC was reported by Sweeney in 1981 [29]. Sweeney's case showed an arytenoid mass, multiple positive lymph nodes, and no intrathyroidal carcinoma. In retrospect, however, it is likely that tumor was a primary laryngeal neuroendocrine carcinoma and not MTC. Clinical history and imaging are critical for proper diagnosis. The combination of a thyroid mass, multiple metastases, and midline lymph nodes would all favor MTC over LCNEC. Finally, serum calcitonin is elevated in almost all MTC but will not be elevated in laryngeal LCNEC.

Our collection of cases is the only series of such cases that we are aware of and demonstrates the importance of separating laryngeal LCNEC from AC. These patients have much more aggressive tumors, need appropriate counseling, and may need more aggressive treatment. A treatment approach similar to that of small cell neuroendocrine carcinoma may be indicated. Utilization of these well-defined classification criteria will help to identify these patients for larger series to further validate this impression.

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