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Case Report

The histogenic origin of melanoma arising in respiratory epithelium of a teratomatous germ cell tumor of the mediastinum: an enigma unraveled from an unlikely source

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Abstract: Mixed germ cell tumors are rare neoplasms that are known to occur in the anterior mediastinum. Characterized by two or more types of germ cell components, these tumors comprise upwards of 25% of mediastinal germ cell tumors. Even rarer are those harboring somatic-type malignancies such as carcinoma, sarcoma, and hematopoietic malignancies. To date, however, there are no known cases of melanoma arising in a malignant mixed germ cell tumor of the anterior mediastinum. We describe the first case of malignant melanoma with spindle and epithelioid components arising from respiratory epithelium in a mediastinal malignant mixed germ cell tumor of a 32-year-old male. In addition, we also provide evidence supporting the theory of neuroendocrine cells as the origin of melanoma arising in the respiratory epithelium. This case emphasizes the need to carefully evaluate all germ cell tumors, not only for a myriad of benign embryological components, but also for malignancies arising in these components, as they might change the prognosis and patient's course of treatment. This microscopic approach should bring to light the diversity of mixed germ cell tumors in addition to somatic malignancies with corresponding biologic potentials.

Keywords: Histogenic origin, melanoma, respiratory epithelium, teratomatous germ cell tumor, mediastinum

Introduction

The mediastinum is one of the most common sites of germ cell tumors (GCTs), second only to the gonads [1]. In fact, if a cytologically malignant epithelial lesion is present in a young to middle-aged adult male, it is considered such until biopsy-proven otherwise [2]. Most of the primary mediastinal GCTs arise within or immediately adjacent to the thymus and are subcategorized into three types: 1) pure seminomas, 2) non-seminomas, and 3) mixed GCTs [1].

Mixed GCTs are characterized by more than one GCT component, comprising 13-25% of all mediastinal GCTs. This subtype harbors somatic malignancies more often than both the seminomatous and non-seminomatous categories. Many different somatic-type malignancies arising in these GCTs have been described in the literature, including but not limited to rhabdomyosarcomas, leiomyosarcomas, and primitive neuroectodermal tumors, along with many carcinomas. In addition, sarcomas and carcinomas may coexist. Up to one-third of GCTs accompanied by a somatic malignancy arise in the mediastinum, 75% of which are found in mixed GCTs [3]. They may occur in the primary mediastinal lesion or solely in a metastatic focus, with most arising after chemotherapy completion or in distant recurrences. The majority exhibit elevated serum alpha-fetoprotein or beta-human chorionic gonadotropin levels; however, human placental lactogen, lactate dehydrogenase, and placental alkaline phosphatase (PLAP) may also be detected depend-

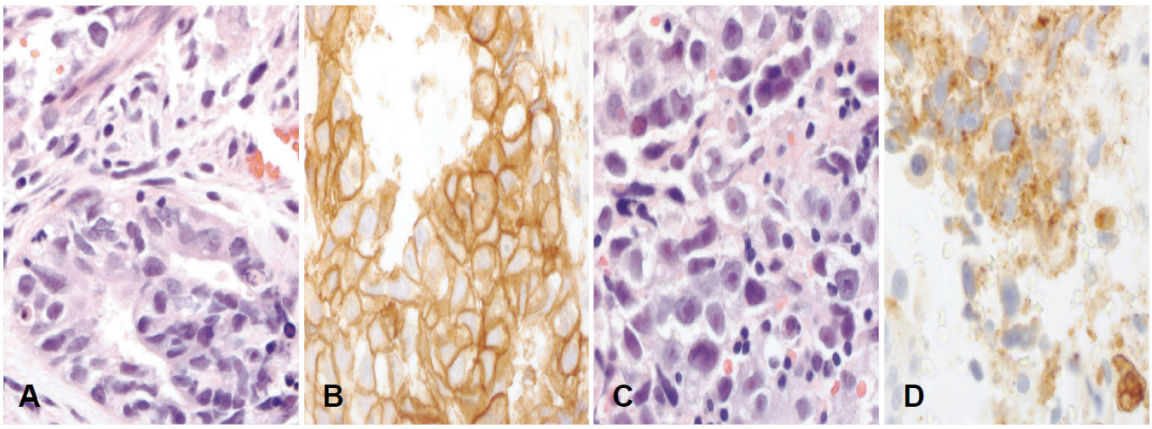

Figure 1. A thoracoscopic biopsy of mediastinal mixed germ cell tumor consisting of seminoma, embryonal carcinoma, and teratoma elements. (x400). A: Portion of the lesion showing a pseudoglandular arrangement of cells with indistinct borders and overlapping epithelioid, pleomorphic nuclei [hematoxylin and eosin (H&E)]. B: A CD30 immunostain highlights the neoplastic cells of embryonal carcinoma. C: Seminomatous portion of the lesion exhibiting pleomorphic epithelioid cells with central nuclei and prominent nucleoli, admixed with small lymphocytes. D: PLAP stain showing strong positivity in seminoma cells.

ing on the histologic subtypes present in the GCT [1-3]. They often locally invade into adjacent mediastinal structures and lungs.

GCTs with somatic-type malignancies that usually occur in young adult males, although they have been reported in patients as young as 4 and as old as 66 years of age. They are frequently symptomatic, often presenting with chest pain, dyspnea, cough, or superior vena cava syndrome. On imaging, a homogenous, solid component representing the somatic-type malignancy and a cystic component, indicative of the GCT, are seen. If hemorrhage and necrosis are present, the lesion may appear heterogeneous. When considering a diagnosis of mixed GCT with a somatic-type malignancy, growth patterns exhibited by the somatic-type malignancy are more important than the percent makeup. However, it is recommended to report on percent make-up of each.

The prognosis of mediastinal GCTs depends in large part on histology. In each of two studies with a total of 94 patients, the 5-year survival rate was significantly better for patients with seminomatous GCTs than for patients with nonseminomatous (87% vs. 33%, respectively) [4, 5]. Mixed GCTs with somatic-type malignancies are generally thought to be chemotherapy-resistant with short overall survival time. In one series, only 10 out of 30 patients with mixed GCTs with or without epithelial or sarcomatous components remained alive 5 years after diagnosis (33% 5-year survival), although longer survival has been observed in at least one smaller series of five patients [6, 7]. With such a bleak outlook in mixed GCTs with somatic-type malignancy, the presence of any molecular abnormality that can be therapeutically targeted may provide a ray of hope to patients. This underlies the importance of accurate histologic classification and subsequent molecular work-up of these tumors when appropriate.

To date, there are no known cases of melanoma arising in a malignant mixed GCT of the anterior mediastinum. Here, we report that malignant melanoma may arise in the context of not only a mediastinal mixed GCT, but also out of an unusual histopathologic background. Clinical and pathologic considerations essential to its diagnosis are also addressed.

Case description

The patient was a 32-year-old man with no history of melanoma or atypical melanocytic neoplasm, who presented to an emergency room with complaints of chest pain. An 11-cm right anterior mediastinal mass was identified on imaging. A thoracoscopic biopsy demonstrated a primary mixed GCT, consisting of seminoma, embryonal carcinoma, and teratoma elements. The embryonal component was characterized by medium-sized cells arranged in small sheets, cribriform, or pseudoglandular patterns with central necrosis (**Figure 1A**). The cells con-

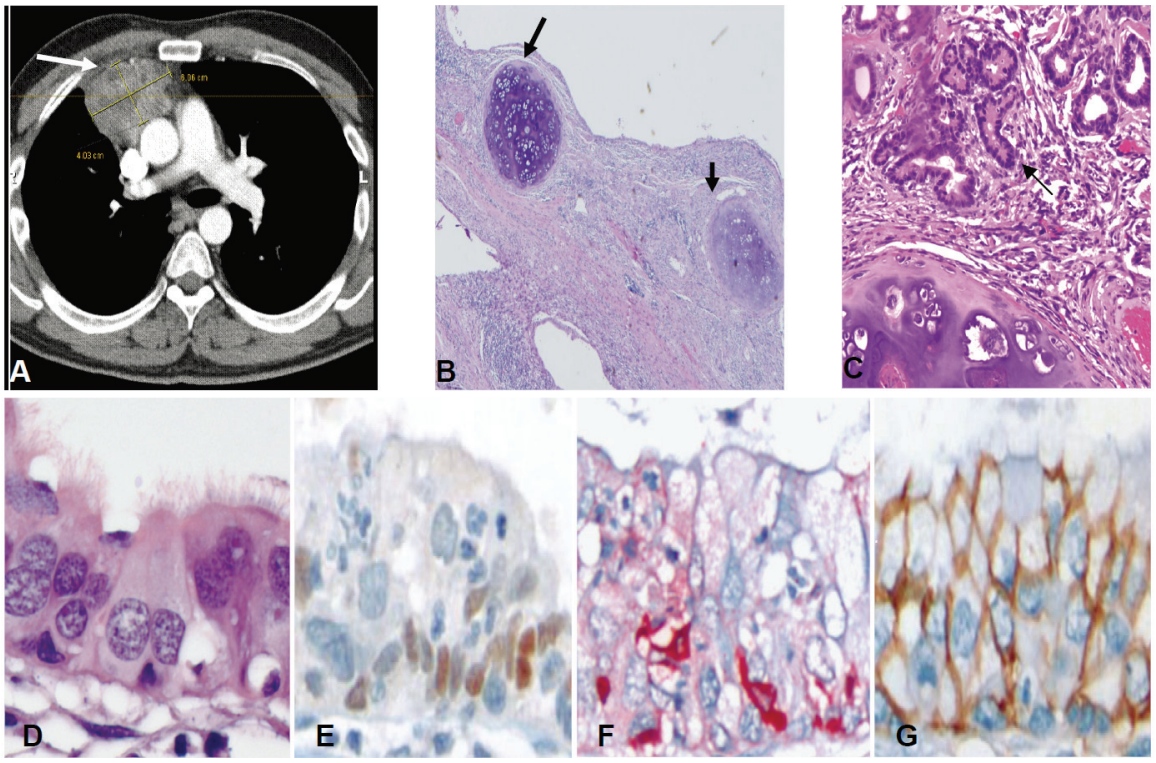

Figure 2. Mediastinal GCT status-post chemotherapy. A: CT scan shows a 6.0-cm heterogeneous mass in the anterior mediastinum (arrow). B: Teratomatous portion of tumor showing developing respiratory system with bronchial-like lumen (arrows highlight cartilage) (H&E, x100). C: Higher power view showing adjacent submucosal glands (arrow) (H&E, x400). D: Ciliated epithelium is seen lining the developing airways (H&E); The epithelium lining the bronchial-like lumen harbors TTF-1 (E), S100 (F), and CD56-positive cells (G), consistent with their respiratory and neural crest-derived neuroendocrine origins.

tained hyperchromatic, overlapping nuclei and indistinct cell borders. Immunohistochemical stains showed the cells to be positive for CD30 (**Figure 1B**), as well as PLAP, cytokeratin AE1/AE3, and CAM 5.2, but negative for LCA and TTF-1 (data not shown). The seminomatous portion consisted of benign lymphocytes intercalating among larger epithelioid cells with mildly hyperchromatic nuclei and prominent cherry-red nucleoli (**Figure 1C**). There was coexpression of PLAP and vimentin, but an absence of pan-cytokeratin, confirming the diagnosis. Immunostains for S100, HMB45, and MART1 were negative in both the teratoma and carcinoma components. The serum alpha-fetoprotein level was elevated at 6600 μg/L.

The patient received four cycles of BEP (bleomycin, etoposide, and platinum) and two cycles of TIP (paclitaxel, ifosfamide, and cisplatin) chemotherapy with an excellent biochemical and radiographic response. Alpha-fetoprotein levels were normalized, and a new CT scan showed a 6-cm, heterogeneous mass with extensive necrosis (**Figure 2A**). The mass was surgically resected.

Microscopically, 40% of the mass showed viable tumor cells with spindle and epithelioid features associated with small foci of mature teratoma comprising approximately 5% of the viable tumor. The teratoma component exhibited respiratory epithelial-lined spaces surrounded by islands of developing cartilage, submucosal glands, and lobules of primitive lung (**Figure 2B** and **2C**). Ciliated bronchial cells were easily appreciated on hematoxylin and eosin staining (**Figure 2D**), A TTF-1 immunostain was found to be positive in the epithelial mucosa (**Figure 2E**), which was also positive for pancytokeratin AE1/AE3-CAM 5.2 (data not shown). In addition, within the respiratory epithelium, also present were cells expressing both S100 and CD56 that were intimately intermingled with

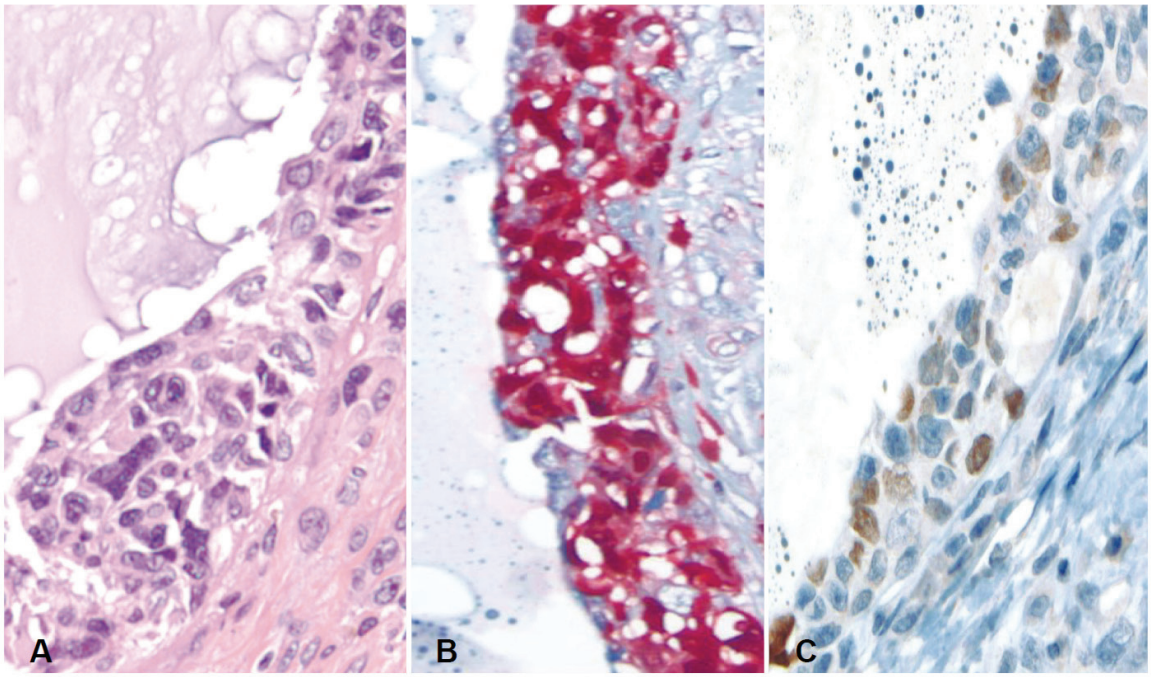

Figure 3. Focus of atypical cell proliferation within the epithelium lining the bronchial-like lumen. A: Atypical epithelioid cells with pleomorphic nuclei and prominent nucleoli intercalate among benign respiratory epithelial cells (H&E, x400). B: S100 highlights the atypical cells (x400). C: TTF-1 immunostain is positive in respiratory cells but negative in atypical cells (x400).

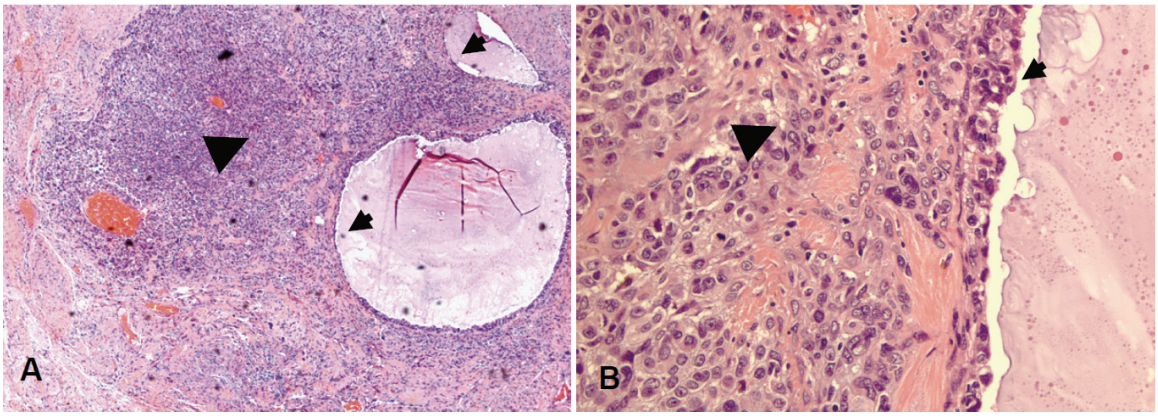

Figure 4. Invasive malignant melanoma in relation to the teratomatous lesion. A: A low-power (H.E., ×40) and B: a high-power (H.E., ×400) magnification view of teratomatous respiratory epithelium (arrows) and invasive melanoma (arrowhead).

TTF-1-positive respiratory cells, likely representing primitive neural crest-derived melanocytic cells (**Figure 2F** and **2G**).

Contiguous with the aforementioned teratomatous respiratory elements were foci of atypical cell proliferation within the epithelium lining the bronchial-like lumen with no invasive component. The atypical cells were positive for S100 but negative for TTF-1 (**Figure 3**) as well as pancytokeratin (not shown), likely representing an in situ melanocytic neoplasm rather than involvement by invasive melanoma. Interestingly, in other areas of the teratomatous component, the in situ melanocytic lesion was continuous with an invasive melanocytic lesion characterized by spindle and epithelioid cells, accounting for 30% of the viable tumor (**Figure**

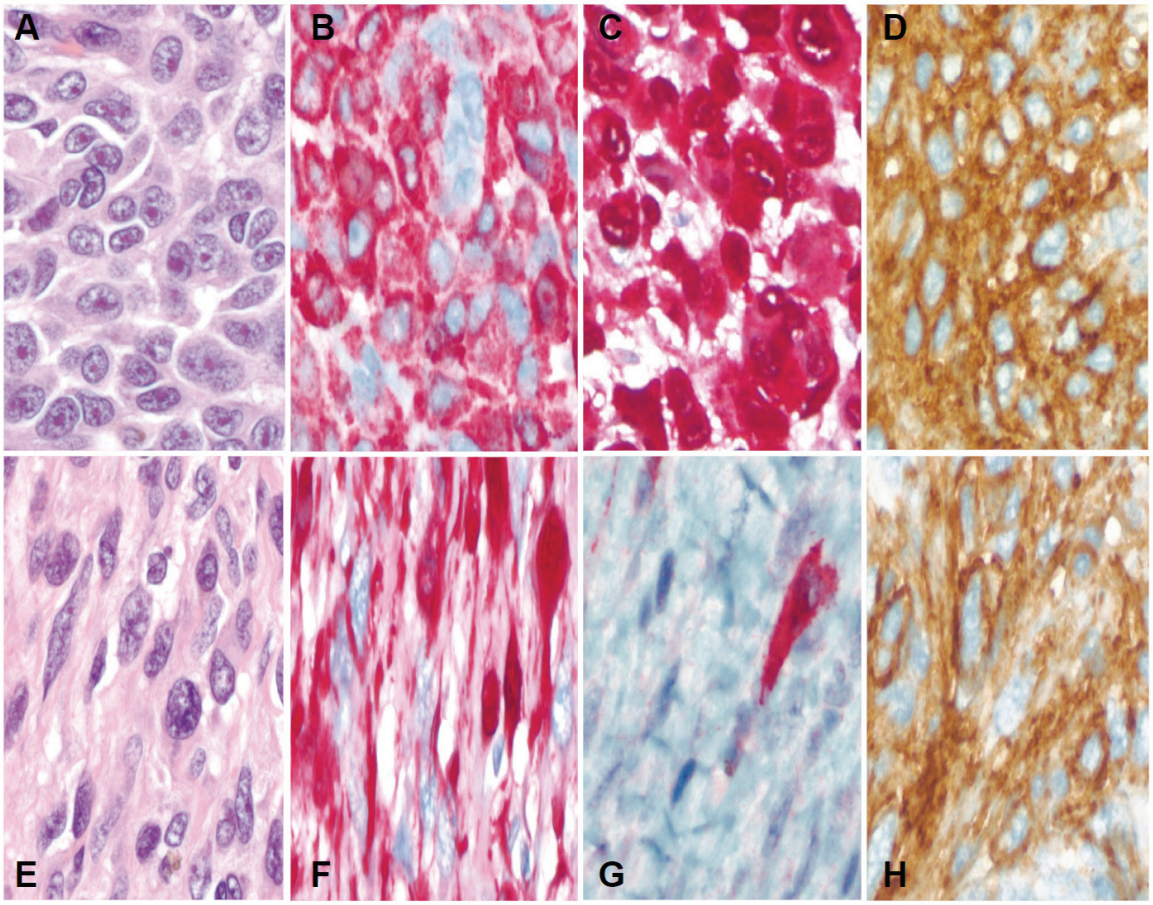

Figure 5. Invasive malignant melanoma with epithelioid and spindle cell features (x400). A: Epithelioid melanoma cells with prominent cherry-red nucleoli (H&E). Tumor cells are positive for HMB-45 (B), S100 (C), and CD56 (D). E: High-power view of the spindle cell melanoma component (H&E). F: S100 immunostain is positive in tumor cells. G: HMB45 is positive only in rare spindle cells. H: Spindle cell melanoma component is positive for CD56.

4). The nuclei were spindled to round, pleomorphic, hyperchromatic, or vesicular, with prominent cherry-red nucleoli. Brown pigment deposition was noted throughout. On immunohistologic examination, the epithelioid neoplastic cells expressed Melan-A, S100, and CD56 (**Figure 5A-D**), whereas the spindle cell areas were positive for S100 and CD56 while only rare cells were positive for Melan-A (**Figure 5E-H**). A Fontana Masson silver stain highlighted the melanin pigment in both the spindled and epithelioid cells, while iron stains were negative (not shown). The histologic and immunohistologic features of the tumor cells were diagnostic for malignant melanoma. Interestingly, the invasive component appeared to be intimately associated with foci of melanoma in situ in the primitive bronchial tissue, suggesting that it was arising from the neural crest-derived melanocytic cells located within the primitive respiratory epithelium.

Thirteen months after the initial diagnosis, a CT scan revealed multiple hepatic lesions. Ultrasound-guided liver biopsy showed several minute tissue fragments composed of enlarged plasmacytoid epithelioid cells with central, hyperchromatic, and pleomorphic nuclei (**Figure 6A**). Prominent cherry-red nucleoli were occasionally noted along with an abundance of cytoplasmic melanin. Immunohistochemistry revealed the neoplastic cells to be S100, HMB-45, Melan-A, and tyrosinase positive, but pan-keratin negative, findings diagnostic of metastatic malignant melanoma (**Figure 6B** and **6C**).

Activating mutations in the *BRAF* oncogene (V600E) have been reported in 33% to 47% of primary melanomas and 41% to 55% of metastatic melanomas [8]. Vemurafenib is a new serine threonine kinase inhibitor, which was recently approved for the treatment of patients with unresectable or metastatic melanoma

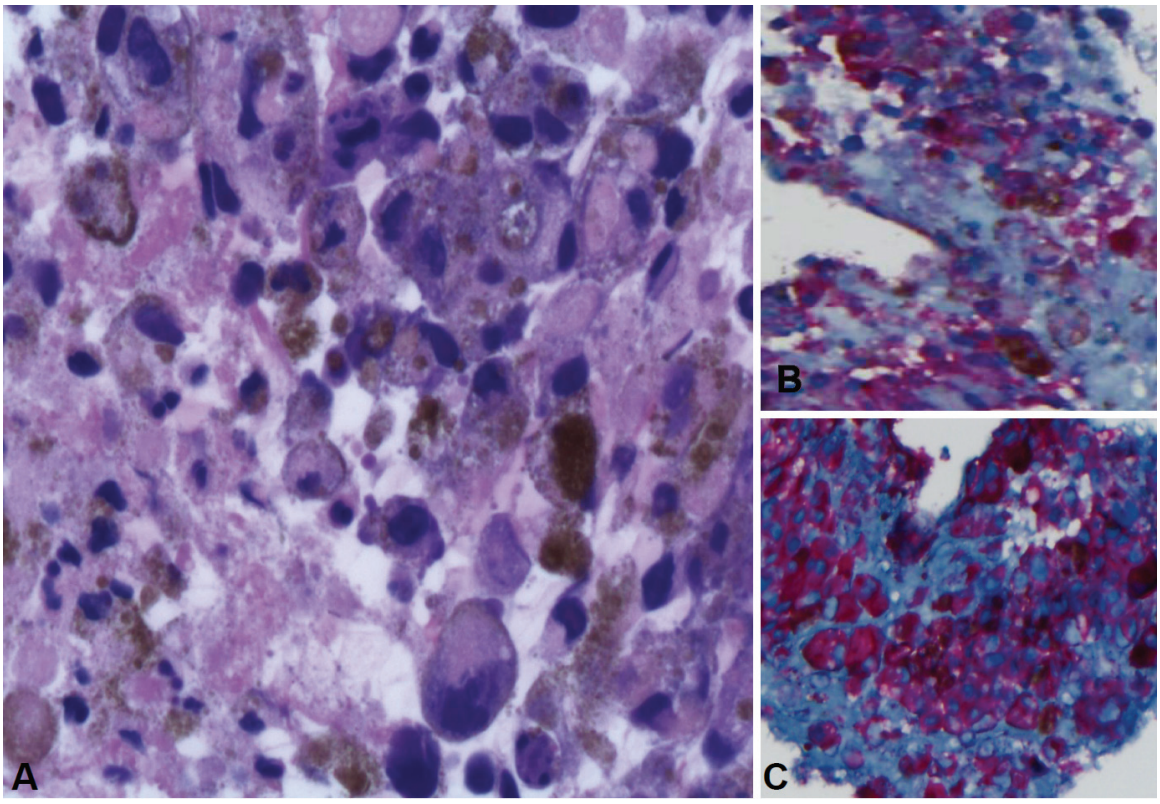

Figure 6. Metastatic melanoma to liver (x200). A: Liver parenchyma is completely replaced by atypical melanocytes, and melanin pigment is obvious. B: S100 stain highlights the metastatic melanoma cells. C: HMB-45 stain is strongly positive, confirming the diagnosis of metastatic melanoma.

with the *BRAF* V600E mutation. To determine whether the patient would benefit from vemurafenib therapy, *BRAF* mutation analysis was performed. However, the results showed that the tumor cells were wild-type for *BRAF* codon 600.

Given the presence of positive mediastinal margins, a decision was made to proceed with radiation therapy in this area. The patient was considering enrollment in several clinical trials but to date remains on radiation therapy alone.

Discussion

Melanoma is a malignant neoplasm of pigment-producing cells (melanocytes) located predominantly in the skin; however, mucosal localizations, such as the oral cavity, esophagus, ano-genital mucosa, trachea, and in ovarian cystic teratoma, have been also described [9-15]. Primary melanoma of the lung and trachea is a very rare neoplasm that frequently arises in the respiratory epithelium. To be considered as a primary mucosal melanoma, the tumor should adhere to strict guidelines [14, 15] discussed below.

Malignant GCTs of the mediastinum are uncommon, representing only 3% to 10% of mediastinal tumors and only 1% to 5% of all germ cell neoplasms. It is believed that these tumors arise as a consequence of abnormal migration of germ cells during embryogenesis. Previously, carcinoma, sarcoma, and hematologic malignancies have all been observed in GCTs.

Here, we describe, to our knowledge, the first case of malignant melanoma arising from the primitive respiratory epithelium of a malignant GCT. A literature search performed in Pubmed using the key terms “mixed GCT” and “melanoma” did not reveal any previous cases of malignant melanoma arising in a mediastinal GCT. In the case presented here, the malignant melanoma was detected in the resection of a mediastinal GCT that had been reduced in size by six months of chemotherapy. Interestingly, the melanoma appears to have arisen from melanocytes located in teratomatous respiratory

elements. Specifically, S100-positive cells were present within teratoma harboring primitive bronchial epithelium.

This case also met criteria set forth when diagnosing primary melanoma of the respiratory tract: 1) junctional changes with a "dropping off" or "nesting" of malignant cells containing melanin, just beneath the bronchial epithelium; 2) invasion of the bronchial epithelium by the melanoma cells in an area where the epithelium is not ulcerated; 3) a malignant melanoma associated with these epithelial changes; 4) a solitary tumor; 5) no past history of excision or fulguration of a cutaneous, mucous membrane, or ocular lesion, and; 6) no demonstrable tumor anywhere else at the time of diagnosis [14, 15].

There are several theories to explain the occurrence of primary respiratory melanoma. It is well-known that melanocytes derive from neural crest cells, which in turn arise out of the neuroectodermal layer of the developing embryo. These cells, along with other neuroendocrine cell types, fall under an umbrella category known as the "dispersed neuroendocrine system" (DNES) [10, 15]. Cells of neural crest origin/DNES are found in various body sites, such as skin, anorectal and vaginal mucosa, ocular cavity, and the larynx. Thoughts regarding the origin of melanocytes in the respiratory mucosa vary. One theory touts that they migrate here during embryological development along with the respiratory system, forming from an outpouch of the 6th branchial arch [10, 15]. Others believe that they arise from uncommitted DNES cells, which normally migrate to the respiratory mucosa and give rise to terminally differentiated neuroendocrine cells present within the bronchial epithelium. Lastly, melanocytes are thought to possibly derive from submucosal glandular cell metaplasia.

We believe the case discussed here may provide viable proof concerning the origin and development of melanoma in bronchial or tracheal mucosa. Findings seen here favor the hypothesis of a pluripotent stem cell of the DNES, evidenced by bland appearing cells coexpressing CD56 (a neuroendocrine marker) and S100 (a neural and melanocytic marker) present among TTF-1-positive bronchial cells in developing respiratory-like epithelium (see **Figure 2**). Whichever proves most plausible matters not because the mere fact that this occurs supports the possibility of melanoma arising from the developing respiratory system in a teratoma.

In addition to various GCT malignancies, the differential diagnosis of malignant melanoma arising in a GCT potentially includes melanotic neuroectodermal tumors of infancy and malignant mixed GCTs with a benign or atypical nevoid component. The melanotic neuroectodermal tumor of infancy has characteristic histologic features useful in its distinction from melanoma: a biphasic pattern of large polygonal cells and arranged at the periphery of alveolar-like structures with much smaller neuroblastoma-like cells comprising the center. The larger cells typically have melanin pigment and even express HMB-45 on immunohistochemical analysis. However, in contrast to melanoma, the tumor is usually S100 negative [16, 17].

The differential diagnosis of the mixed GCT with an atypical or benign nevoid component is relatively straight-forward. In such cases, the histologic criteria useful in the distinction of nevi and melanoma in the skin should be applied to melanocytic proliferations in the teratomatous skin portions of a GCT. Other neoplasms included in the differential diagnosis are melanocytic carcinoid tumors, melanotic paragangliomas, and melanotic schwannoma, which have been excluded by histopathological and immunohistochemical studies.

The prognosis of mediastinal teratoma is excellent after complete or microscopically incomplete resection [18], whereas the prognosis of mucosal melanoma in general is rather poor. Radiotherapy has been tried in mucosal melanoma of the head and neck [12] with disappointing results. Chemotherapy has been used mainly for palliation only, while in selected cases adjuvant interferon-α therapy has been utilized. In this case, melanoma cells did not respond to conventional chemotherapy and 13 months after the initial diagnosis, a CT scan revealed multiple liver metastases.

The presence of a *BRAF* mutation is important in determining an optimal therapeutic approach, as there are inhibitors available for the treatment of melanoma. About half of all primary and metastatic cutaneous melanomas harbor a mutation in the serine/threonine protein kinase BRAF involving amino acid substitu-

tion at position 600. The majority (about 80%) of *BRAF*-mutated melanomas have the V600E missense mutation, while V600K and V600G/R account for most of the remaining cases [19-22]. The *BRAF* V600E mutation is present most commonly in melanomas that occur on body sites with intermittent sun exposure as opposed to those that occur with chronic sun damage [8, 19, 23]. Although *BRAF* mutation in metastatic melanoma portends a poor prognosis, it predicts response to targeted BRAF inhibitors [8, 19, 20]. Primary melanomas from acral and mucosal sites less frequently harbor *BRAF* mutations, found in only 10-15% of such cases [8, 19, 23]. Consistent with these results, it is not surprising that the melanoma in our case, which arose from teratomatous respiratory mucosa, was negative for a *BRAF* mutation.

Conclusions

To our knowledge, this is the first case highlighting the development of melanoma out of teratomatous respiratory components of a mediastinal GCT. Additionally, this unusual occurrence may provide evidence of the melanocytic origin from neuroendocrine cells normally found in respiratory epithelium. Since the majority of GCTs with somatic-type malignancies develop post-treatment, it is not surprising to see a similar situation in the present case [3]. This case emphasizes the importance of identification of newly arising malignancies in GCTs, as discussed here, as it may carry important prognostic and therapeutic implications.

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