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Case Report

Metaplastic thymoma with myasthenia gravis presumably caused by an accumulation of intratumoral immature T cells: a case report

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Abstract: Among human neoplasms, thymomas are well known for their association with paraneoplastic autoimmune diseases such as myasthenia gravis. However, regarding rare metaplastic thymoma, only one case of an association with myasthenia gravis has been reported. Here, we present the second case of a 44-year-old woman with metaplastic thymoma associated with myasthenia gravis. In metaplastic thymoma, intratumoral terminal deoxynucleotidyl transferase-positive T-cells (immature T-cells) are generally scarce, while they were abundant in the present case. We believe that these immature T-cells could be related to the occurrence of myasthenia gravis.

Keywords: Histology, immature T-cells, immunohistochemistry, metaplastic thymoma, myasthenia gravis

Introduction

Among human neoplasms, thymomas are concerned with the highest frequency of comorbidity with paraneoplastic autoimmune diseases, most common one of which is myasthenia gravis (MG) [1]. MG-associated thymomas sustain many functional features of the normal thymus, namely the ability of generating and exporting mature T-cells from immature precursors [2]. Because thymomas are enriched with autoantigen-specific T-cells, mature T-cell export from thymomas could result in disturbing the balance of circulating T-cell subset composition, and thereby contribute to the initiation of paraneoplastic autoimmune disease [3].

Frequent associations between World Health Organization type B1 and B2 thymomas and MG have been observed; however, other thymoma types such as thymic carcinoma have been less frequently associated with MG [4, 5]. Regarding rare metaplastic thymoma, only one case documenting an association with MG has been reported previously [4, 6].

Here, we present a second case of metaplastic thymoma associated with MG. We uncovered interesting findings that might explain this rare association, which we expand upon here.

Clinical summary

A 44-year-old female never-smoker, who was taking medication for a history of epileptic fits since the age of 18 years, began to notice severe fatigue and pain in the posterior cervical region and back. She complained of weakness in the cervical flexor muscle and left diplopia that had persisted four months. Her symptoms worsened gradually, and because of fatigue in the limbs, she experienced difficulties climbing stairs and washing her hair. Two months before admission, she had difficulty swallowing and noticed bilateral ptosis. She was referred to our hospital for a detailed examination.

A physical examination revealed paralysis of the left abducens nerve, bilateral ptosis, cervical flexor muscle weakness, and dysphagia, all of which worsened with recurrence motion. Her anti-acetylcholine receptor (AchR) antibody level was 78.6 nmol/L, whereas anti-muscle-specific kinase antibodies were not detected. Electromyography showed waning in the nasalis, trapezius, deltoid, and abductor pollicis bre-

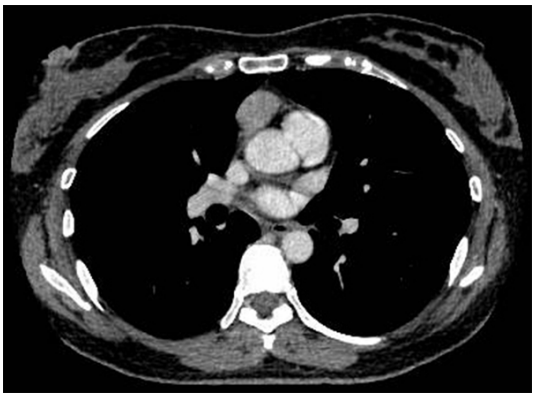

Figure 1. Contrast-enhanced computed tomography findings. There was a mildly enhanced mass in the anterior mediastinum. The mass had a smooth margin and did not invade any adjacent organs.

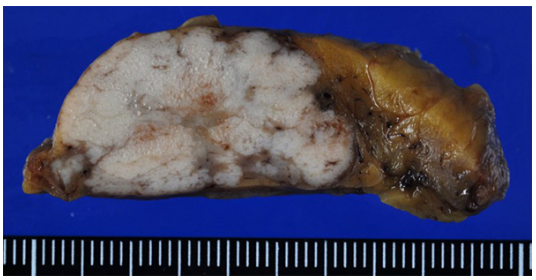

Figure 2. Macroscopic findings. A well-delineated, whitish, lobulated tumor was observed. There was no invasion to any adjacent organs.

vis muscles. Contrast-enhanced chest computed tomography detected a mass that measured 3.1 × 2.2 × 1.9 cm in the anterior mediastinum. The mass had a smooth margin and did not invade any adjacent organs (**Figure 1**). She was diagnosed with thymoma with MG and was administered pyridostigmine bromide and prednisolone (5 mg/day) to manage the worsening symptoms of MG. Eventually, she underwent an extended thymectomy. Her post-operative course was uneventful.

Pathological findings

Gross examination of the surgically resected specimen revealed a well-delineated, whitish, lobulated tumor that measured 3.6 × 2.6 × 2.2 cm in size (**Figure 2**). The tumor did not invade any adjacent organs.

Histologically, the tumor was multinodular and demarcated from the fat tissue by a thin fibrous capsule (**Figure 3A**). Focally, the tumor exhibited microinvasion in the surrounding fat tissue (**Figure 3B**). The tumor exhibited two cell types; adherent epithelioid cells and spindled cells. The epithelioid cells formed variably sized and shaped tumor nests (**Figure 3C**), had moderately enlarged nuclei with distinct nucleoli, and showed scattered characteristically bizarre nuclei with nuclear pseudoinclusions (**Figure 3D**). Spindled cell nuclei were mildly enlarged without distinct nucleoli (**Figure 3E**). Focally, a gradual transition from epithelioid cells to spindled cells was observed (**Figure 3F**). Interestingly, patchy, dense, intratumoral lymphocytic accumulation could be seen around the epithelioid cell-containing tumor nests (**Figure 3G**). There was no lymphovascular invasion, and the surgical margin was tumor-free.

Immunohistochemically, epithelioid cells were positive for AE1/AE3 (**Figure 4A**), p63 (**Figure 4B**), and E-cadherin (**Figure 4C**), whereas spindled cells were negative for these markers. Epithelioid cells were negative for epithelial membrane antigen (EMA) and vimentin, whereas spindled cells were positive for these markers (**Figure 4D**, **4E**). Densely accumulated lymphocytes were primarily positive for CD3 (**Figure 4F**), and terminal deoxynucleotidyl transferase (TdT) (**Figure 4G**). The Ki-67 (MIB-1) labeling index was >90% for lymphocytes but was <5% for tumor cells (**Figure 4H**). Judging by the number of CD4+ and CD8+ positive lymphocytes, we anticipated that many TdT-positive lymphocytes might have been double-positive; although, inside tumor nests, CD4+ cells were more prevalent than CD8+ cells and TdT-positive lymphocytes were scarce (**Figure 4I**, **4J**). CD20-positive B-cells were scarce and CD138-positive plasma cells were barely detectable.

Discussion

Because of the presence of TdT-positive cells, other thymoma types were included in the differential diagnosis. Type AB thymoma, which histologically resembles metaplastic thymoma, consists of distinct components of spindled and epithelioid cells, showing a biphasic growth pattern, with lymphocyte accumulation in the epithelioid cell regions [4]. Recently, Miki et al. discovered a characteristic subtype in type AB thymoma, which was called metaplastic sub-

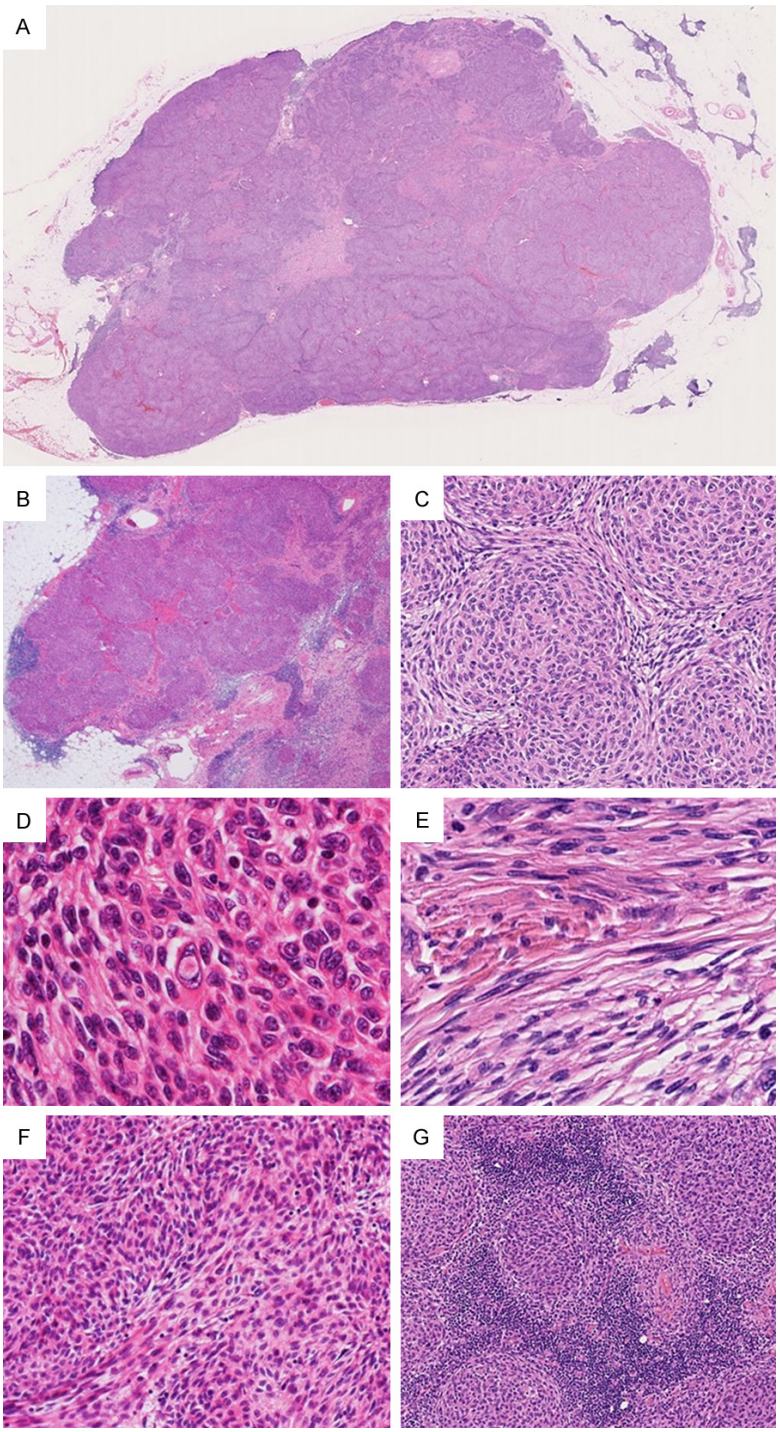

Figure 3. Histological findings. A. The tumor was multinodular and demarcated from the fat tissue by thin fibrous capsule. B. The tumor exhibited microinvasion into the surrounding fat tissue. C. There were two intratumoral cell types: adherent epithelioid cells and spindled cells. The epithelioid cells formed variably sized and shaped tumor nests. D. Epithelioid cell nuclei were moderately enlarged with distinct nucleoli, and showed scattered characteristically bizarre nuclei with nuclear pseudoinclusions. E. Spindled cell nuclei were mildly enlarged without distinct nucleoli. F. A gradual transition from epithelioid cells to spindled cells was observed. G. Dense, patchy, intratumoral lymphocytic accumulation was observed around the epithelioid cell-containing tumor nests.

type [7]. The conventional subtype of metaplastic thymoma was characterized by spindled cell components resembling type A thymoma, whereas the metaplastic subtype was characterized by spindled cell components consisted of fibroblast-like long spindled epithelial cells [7]. Immunohistochemically, spindled cell components of the metaplastic subtype exhibit decreased expression of AE1/AE3 and E-cadherin and increased expression of EMA and vimentin, compared with the conventional subtype [7]. This attenuated expression of AE1/AE3 and E-cadherin and augmented expression of EMA and vimentin were also observed in the spindled cell component of metaplastic thymoma [4, 8]. Although metaplastic thymomas are rare, the metaplastic subtype accounted for 74% (14/19) of type AB thymoma cases reported by Miki et al. [7]. Despite these observations, we interpreted our case as being different from the metaplastic subtype of type AB thymoma, because the epithelioid cells showed nuclear pleomorphism and pseudoinclusions, and lymphocyte accumulation was present not inside the epithelioid cell components, but at the epithelioid cell component circumference. By contrast, in type AB thymoma, lymphocyte accumulation is seen within the epithelioid cell component.

Immature T-cell infiltration is not generally expected in metaplastic thymomas [4]. Although our case was otherwise typical of metaplastic thymoma, presence of considerable amount of imma-

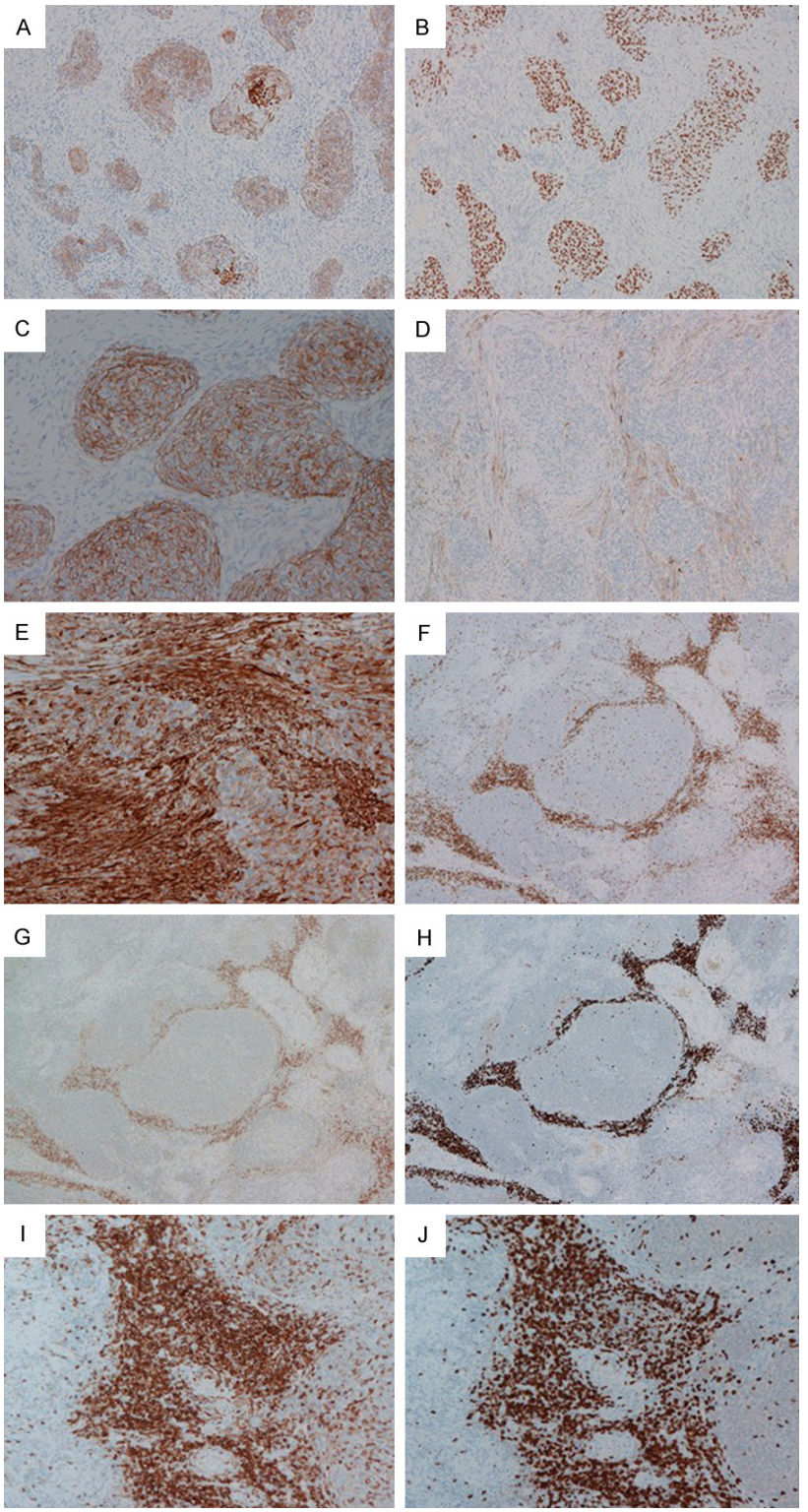

Figure 4. Immunohistochemical findings. A. Epithelioid cells were positive for AE1/AE3. B. Epithelioid cells were positive for p63. C. Epithelioid cells were positive for E-cadherin. D. Spindled cells were positive for EMA. E. Spindled cells were positive for vimentin. F. Densely accumulated lymphocytes were positive for CD3. G. Densely accumulated lymphocytes were positive for TdT. H. Ki-67 (MIB-1) strongly labeled densely accumulated lymphocytes, but labeling in tumor cells was <5%. I. Most densely accumulated lymphocytes were CD4+ lymphocytes. J. Most densely accumulated lymphocytes were CD8+ lymphocytes.

ture T-cells was noted. Immature T-cells are usually observed in many types of thymoma [4], which are unique in that they can generate mature T-cells from the immature ones. Intratumoral T-cell maturation is not the same as that of normal thymus in that the mature CD45RA+ T-cell subset is decreased in thymomas compared with that in normal thymus [9]. Furthermore, intratumoral mature T-cells usually have a higher autoantigen-specific potential toward the α-subunit of the AchR, a target of MG, than T-cells from normal thymus [9-11]. Based on these observations, it has been proposed that thymomas may generate and export autoantigen-specific T-cells by a process of aberrant positive or negative T-cell selection [10, 12, 13]. Because it is known that there is virtually no interaction between autoantigen-specific T-cells and autoantibody-producing B-cells inside thymomas [14], interaction between them at the periphery is supposed to be important for the development of MG. In our case, B-cells were rarely found within the thymoma, and antibody-producing plasma cells were almost completely absent. These findings support the postulation that interaction between autoantigen-specific T-cells and B-cells at the periphery leads to the generation of antibody-producing plasma cells.

A high percentage of intratumoral lymphocytes in MG-associated thymomas is CD4+CD8+ double-positive, a phenotype indicating cortical thymocytes [15]. Another observation that CD4+CD8+ double-positive lymphocytes co-exist in thymoma metastatic sites strongly suggests that T-cell development takes place, even outside the pre-existing thymic region [16]. In fact, thymoma-derived neoplastic epithelial cells is able to generate CD4+CD8+ cells from CD3-CD4-CD8-CD34+ T-cell precursors in vitro [17]. In our case, although double staining was not performed, we suspect that the majority of CD4+ cells and CD8+ cells might overlap, meaning double positivity. In addition, it is reasonable to consider that a large proportion of TdT-positive cells corresponded to CD4+CD8+ double-positive cells. However, in metaplastic thymoma, TdT-positive cells are usually difficult to find [4]. In the present case, we believe that the unexpected abundance of intratumoral TdT-positive cells could be related to the occurrence of MG. In the previously reported case of metaplastic thymoma associated with MG [6], constituent lymphocytes were not thoroughly examined; this case may also have shown an abundance of TdT-positive cells if examined.

The immunohistochemical expression patterns of tumor cells noted in the present study, suggested that epithelial mesenchymal transition (EMT) was involved in the development of the spindled cell component from epithelioid cell component as reported previously [8, 18]. During EMT, cells having epithelial characteristics lose them and acquire spindled morphology due to cytoskeletal reorganization, during which down-regulation of cytokeratin and E-cadherin and up-regulation of mesenchymal markers, such as vimentin, take place [19]. In the present case, there was a focal, gradual transition between the epithelioid cell component and spindled cell component, supporting the EMT theory in the occurrence of spindled cell component.

In conclusion, this is the second reported case of metaplastic thymoma associated with MG. The relative abundance of intratumoral TdT-positive cells for this thymoma subtype was possibly related to the occurrence of MG.

Disclosure of conflict of interest

None.

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