# A multilocular thymic cyst associated with mediastinal seminoma: evidence for its medullary epithelial origin highlighted by POU2F3-positive thymic tuft cells and concomitant myoid cell proliferation

AUTHOR(S):

Sugimoto, Akihiko; Yamada, Yosuke; Fujimoto, Masakazu; Minamiguchi, Sachiko; Sato, Takuma; Akamatsu, Shusuke; Marx, Alexander; Haga, Hironori

CITATION:

Sugimoto, Akihiko ...[et al]. A multilocular thymic cyst associated with mediastinal seminoma: evidence for its medullary epithelial origin highlighted by POU2F3-positive thymic tuft cells and concomitant myoid cell proliferation. Virchows Archiv 2021, 479(1): 215-220

ISSUE DATE:

2021-07

URL:

http://hdl.handle.net/2433/276591

# A multilocular thymic cyst associated with mediastinal seminoma: Evidence for its medullary epithelial origin highlighted by POU2F3-positive thymic tuft cells and concomitant myoid cell proliferation

Akihiko Sugimoto[1], Yosuke Yamada[1,*], Masakazu Fujimoto[1], Sachiko Minamiguchi[1], Takuma Sato[2], Shusuke Akamatsu[2], Alexander Marx[3], Hironori Haga[1]

[1]Department of Diagnostic Pathology, Kyoto University Hospital, Kyoto, Japan

[2]Department of Urology, Kyoto University Hospital, Kyoto, Japan

[3]Institute of Pathology, University Medical Centre Mannheim and Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany

***Corresponding Author**
Yosuke Yamada
Department of Diagnostic Pathology
Kyoto University Hospital
Kyoto, Japan
Email: yyamada@kuhp.kyot-u.ac.jp
Tel: +81-75-751-4946
ORCID: 0000-0001-7952-2706

**Word count (from Introduction to Discussion):** 1494 words

**ABSTRACT (150 words)**

Multilocular thymic cyst (MTC) and germ cell tumors are common diseases that impact the mediastinum. Correctly diagnosing these diseases can be difficult because several other conditions can mimic them. We report a male patient with MTC associated with mediastinal seminoma. A needle biopsy of the mediastinal tumor revealed numerous epithelioid cell granulomas that mimicked sarcoidosis or mycobacterial infection. However, large atypical cells positive for Oct3/4 and KIT were noted between the granulomas; thus, we diagnosed the patient with mediastinal seminoma. The resected tumor, after chemotherapy, consisted of multiple cystic lesions, and a residual germ cell tumor was first considered. However, thymic medulla-specific elements, namely, POU2F3-positive thymic tuft cells and rhabdomyomatous myoid cells accompanying the epithelium, led to the correct diagnosis of MTC. Our case underscores the importance of recognizing the histological features associated with mediastinal seminoma and provides novel findings for MTC pathogenesis, namely, the presence of thymic tuft cells.

**Keywords**

Multilocular thymic cyst; mediastinal seminoma; myoid cells; thymic tuft cells; POU2F3

## INTRODUCTION

Multilocular thymic cyst (MTC) and germ cell tumors are common diseases of the mediastinum. However, it can be difficult to correctly diagnose these diseases because several neoplastic and non-neoplastic conditions are included in the differential diagnoses. Although MTC is believed to be derived from the thymic medulla [11], the detailed characteristics of MTC, beyond its morphology, are not well understood. We report a case of MTC associated with mediastinal seminoma that presents novel findings for the pathogenesis of MTC.

## CASE REPORT

A male patient in his twenties, with a history of appendectomy, presented with right hypochondriac pain. The patient had a brother with mediastinal teratoma. Myasthenia gravis (MG)-related symptoms, such as ptosis or muscle weakness, were not observed. Imaging revealed a 13 cm tumor in the anterior mediastinum that consisted of multiple cystic lesions and a solid component with contrast enhancement (Figure S1a). Multiple liver tumors were also noted, for which metastasis of the mediastinal tumor was suspected. The serum alpha-fetoprotein (AFP) value was elevated in the patient (274 ng/ml [normal AFP value < 15 ng/ml]), but the values for lactate dehydrogenase (LDH) and human chorionic gonadotropin (hCG) were normal.

The patient underwent a needle biopsy of the mediastinal tumor. The biopsy revealed numerous discrete epithelioid cell granulomas that mimicked mediastinal sarcoidosis or mycobacterial infection (Figure 1a). However, large atypical cells were noted between the granulomas, and some atypical cells exhibited distinct central nucleoli and apoptosis (Figure 1b). The atypical cells were immunohistochemically positive for Oct3/4, KIT (Figure 1, panels c and d), SALL4, and podoplanin (not shown); the cells were negative for AFP, CD3, CD20, and pan-cytokeratin (CK) (not shown). Thus, a mediastinal seminoma accompanied by a granulomatous reaction was diagnosed. A large number of CD3-positive small lymphocytes (Figure 1e) and CK5-positive reticular squamous epithelium (Figure 1f), presumably thymic medullary epithelium, accompanied the tumor.

The patient received chemotherapy for 3 months (4 courses of bleomycin, etoposide, and platinum therapy), because the presence of non-seminomatous components (i.e., mixed germ cell tumor) was suspected due to the elevated serum AFP value [8]. After chemotherapy, the mediastinal tumor decreased in size; additionally, the solid component and the multiple liver tumors disappeared (Figure S1b). The mediastinal tumor was subsequently surgically resected.

The resected tumor was 18.0 x cm x 10.0 cm x 2.5 cm in size. It contained 6.0 cm x 6.0 cm x 2.0 cm multicystic lesions in a background of fibrous or hyalinized tissue (Figure S1b), with deposition of hemosiderin and cholesterol crystals where the tumor cells were

probably once located and disappeared with chemotherapy (Figure 2a and 2b). Each cystic lesion was lined with squamous epithelium without cytological atypia, which often showed reticular architecture (Figure 2c). Immunohistochemistry (IHC) revealed that these squamous cells were positive for CK5 (Figure 2d), CK19, claudin-4, and p40; the Ki-67 labeling index was approximately 2% (not shown). It is of note that a small number of POU2F3-positive cells were interspersed within the squamous epithelium (Figure 2e and 2f). Additionally, aggregation of desmin-positive myoid cells, which simulated rhabdomyoma, was also observed intermingling with the epithelium (Figure 2g and 2h). Components suggesting residual germ cell tumor were not observed morphologically or immunohistochemically (AFP, KIT, hCG, Oct3/4, and SALL4) (not shown). Thus, a diagnosis of MTC was made. The patient has been carefully followed, without any postoperative therapies, and has remained disease-free for approximately 6 months.

## DISCUSSION

Because of the relatively high proportion of germ cell tumors among mediastinal lesions, germ cell tumors should be considered an important part of the differential diagnosis in patients that present with a mediastinal tumor; this is particularly important if a patient is considered relatively young for the development of thymic epithelial tumors [12].

Correctly diagnosing germ cell tumors is sometimes difficult. Although needle

biopsies are commonly performed for mediastinal tumors, the obtained specimens are often small. Also, the histology of germ cell tumors is diverse due to the variable histological subtypes with different malignant potential [12]. Furthermore, the accompanying reactive inflammatory response might present an additional obstacle to obtaining a correct diagnosis [12, 13]. As initially reported by Moran et al. [7], the granulomatous reaction that accompanies a seminoma should be recognized because this finding can easily be observed in low power fields and may hamper careful evaluation of the area between the granulomas. The importance of recognizing this reaction should be emphasized in that granulomatous diseases, such as sarcoidosis and mycobacterial infections, often involve the mediastinal lymph nodes, and their treatment strategies differ from those for mediastinal tumors. In addition to large atypical cells with a distinct central nucleolus, apoptotic bodies, which were observed more easily than atypical cells in our case, might be a clue for pathologists to suspect seminomas. The finding that seminoma cells and the thymic medullary epithelium were tightly attached in our case may suggest that mediastinal germ cell tumors originate in the thymic medulla and that this area functionally supports the proliferation of tumors [9].

MTC is the most common non-neoplastic cystic lesion of the mediastinum [11] and can be associated with various mediastinal tumors, including germ cell tumors [4]. Similar to mediastinal germ cell tumors, correctly diagnosing MTC can be difficult because of its histological diversity and features that are similar to other cystic lesions of the mediastinum.

In the present case, a residual germ cell tumor was considered in the differential diagnosis because it was possible that if the tumor was a mixed germ cell tumor, the chemotherapy-sensitive seminoma components would disappear and the chemo-refractory mature components would remain or develop (i.e., growing teratoma syndrome) [12]. However, the epithelial components were entirely cystic without cytological atypia; further, the squamous layers that lined the cystic lesions contained a small number of POU2F3-positive cells, presumably thymic tuft cells.

Recent single-cell analyses uncovered unexpected heterogeneity in the constituents of the thymus, which included thymic tuft cells [2, 6]. The detailed functions of these cells are still not fully known, but they shape the microenvironment of the thymus, particularly as it relates to innate immunity [2, 5, 6]. Thymic tuft cells are exclusively located in the thymic medulla in low proportions (approximately 5%), particularly in or around the Hassall's corpuscle [2, 6, 14]; these findings were confirmed in the thymus around the MTC in our case (Figure S2a-d). These cells are reported to frequently have a bulbous morphology in mice [2], but it is difficult to morphologically discern these cells from other medullary epithelial cells in humans. Thus, IHC for POU2F3, the master regulator of tuft cells [15], is helpful in their detection. Indeed, it was challenging to morphologically discern tuft cells from neighboring epithelial cells in the MTC and the surrounding thymus in our case.

The MTC in our case is unique because it is associated with seminoma, and

chemotherapy was performed before the resection. To address whether the presence of POU2F3-positive cells is attributable to this special condition or is an ordinary finding in MTCs, we performed IHC on three non-tumor-related MTCs lined mainly by squamous epithelium. We observed that all of the MTCs contained a small number of POU2F3-positive cells within the cysts (Figure 3a and 3b), suggesting that this finding is a common feature of MTCs. Because serial sections were used for hematoxylin and eosin staining and POU2F3-IHC in these control cases, we were able to morphologically detect probable thymic tuft cells in the MTC and the surrounding thymus. In the MTC, these cells exhibited a flattened appearance and were difficult to discern from neighboring epithelial cells (Figure 3a and 3b). In the normal thymus, they partly exhibited a seemingly bulbous appearance (Figure 3c and 3d).

Myoid cells are also specific constituents of the thymic medulla and might be involved in MG as the first target of autoimmunity [10]. A recent publication suggested that myoid cells might be derived from medullary epithelial cells, which may explain the strong affinity these cells have for the epithelium [1]. A unique finding in our case was the tumor-like florid expansion of the cells, which Chetty previously reported as rhabdomyomatous MTC [3]. Except for these rhabdomyomatous components, no other components suggesting teratoma, such as skin, cutaneous appendages, or seromucous glands, were evident within the tumor. Further, the rhabdomyomatous cells were always intermingled

with the epithelial cells of the MTC. These findings are sufficient to exclude the possibility that these cells are components of a teratoma.

These findings, namely, thymic tuft cells and myoid cells within the MTC, strongly support the notion that MTC is the cystic expansion of the thymic medullary epithelium [11], and suggest that this cystic lesion may preserve some functions related to the thymic medulla. Further studies contributing to a better understanding of MTC biology, such as the relationship with thymus-associated autoimmune diseases (e.g., MG), are warranted.

In summary, our case emphasizes the importance of knowledgeable evaluation in obtaining a correct diagnosis for mediastinal germ cell tumors and cystic conditions. Furthermore, we provide novel information on the pathogenesis of MTC, namely, the presence of thymic tuft cells.

## FIGURE LEGENDS

**Figure 1. Microscopic findings of the mediastinal tumor**

Numerous discrete epithelioid cell granulomas are observed (panel a). Between the granulomas, large atypical cells with distinct central nucleoli are noted (arrow). Several apoptotic bodies (inset) are also evident (panel b). These atypical cells are positive for Oct3/4 and KIT (panels c and d). Between the granulomas, many CD3-positive T lymphocytes are evident (panel e), as is CK5-positive reticular epithelium (panel f). (panels a and b: hematoxylin and eosin section; panels c-f: immunohistochemistry [c: Oct3/4; d: KIT; e: CK5; f: CD3])

**Figure 2. Microscopic findings of the mediastinal tumor after chemotherapy**

The tumor consists of multiple cystic lesions in a background of fibrous tissue (panel a). Cholesterol crystals and calcification are evident in the fibrous area (panel b). The cystic lesions are lined with CK5-positive squamous epithelium (panels c and d) without cytological atypia (panel e), and a small number of POU2F3-positive cells are present in the epithelium (panel f). Aggregation of desmin-positive myoid cells is also easily observed (panels g and h).

(panels a-c, e, g: hematoxylin and eosin section; panels d, f, h: immunohistochemistry [d: CK5; f: POU2F3; h: desmin])

**Figure 3. Microscopic findings of a multilocular thymic cyst (MTC) that occurred independently of neoplastic lesions**

The cysts are lined with flattened epithelium that contains a small number of POU2F3-positive cells. Their morphology is generally indistinguishable from that of neighboring epithelial cells (panels a and b). Non-neoplastic thymus around the MTC contains POU2F3-positive thymic tuft cells. They are located in or around the Hassall's corpuscle, and some exhibit a seemingly bulbous morphology (panels c and d). The low power view of the MTC (panel e).

(panels a, c, e: hematoxylin and eosin section; panels b, d: immunohistochemistry [POU2F3])

## SUPPLEMENTARY INFORMATION

**Figure S1. Contrast-enhanced radiological findings of the mediastinal tumor before and after chemotherapy, and macroscopic findings of the resected tumor**

A 13 cm multicystic tumor with a solid component is evident in the anterior mediastinum (panel a). After chemotherapy, the size of the tumor is decreased (panel b). The cut surface of the resected tumor exhibits multicystic lesions in a background of whitish/fibrous tissue (panel c).

**Figure S2. Presence of POU2F3-positive thymic tuft cells in the normal thymus around the tumor**

POU2F3-positive thymic tuft cells are exclusively located in the terminal deoxynucleotidyl transferase (TdT)-negative thymic medulla (panels a and c: hematoxylin and eosin section; panels b and d: immunohistochemistry [b: TdT; d: POU2F3]).

## DECLARATIONS

**Funding**: None declared

**Conflicts of interest**: None declared

**Availability of data and material**: Not applicable

**Authors' contributions:**

Drafting the manuscript and figures: AS and YY. Acquisition and analysis of clinical data: TS and SA. Correction and approval of the manuscript: all authors.

**Code availability**: Not applicable

**Ethics approval**: Not applicable

**Consent to participate**: Not applicable

**Consent for publication**: Not applicable

## REFERENCES

1. Bautista JL, Cramer NT, Miller CN, Chavez J, Berrios DI, Byrnes LE, Germino J, Ntranos V, Sneddon JB, Burt TD, Gardner JM, Ye CJ, Anderson MS, Parent AV (2021) Single-cell transcriptional profiling of human thymic stroma uncovers novel cellular heterogeneity in the thymic medulla Nat Commun 12:1096. doi: 10.1038/s41467-021-21346-6
2. Bornstein C, Nevo S, Giladi A, Kadouri N, Pouzolles M, Gerbe F, David E, Machado A, Chuprin A, Tóth B, Goldberg O, Itzkovitz S, Taylor N, Jay P, Zimmermann VS, Abramson J, Amit I (2018) Single-cell mapping of the thymic stroma identifies IL-25-producing tuft epithelial cells Nature 559:622-626. doi: 10.1038/s41586-018-0346-1
3. Chetty R, Reddi A (2003) Rhabdomyomatous multilocular thymic cyst Am J Clin Pathol 119:816-821. doi: 10.1309/QDJC-A1HX-QLHL-CFTM
4. Inui M, Nitadori JI, Tajima S, Yoshioka T, Hiyama N, Watadani T, Shinozaki-Ushiku A, Nagayama K, Anraku M, Sato M, Fukayama M, Nakajima J (2017) Mediastinal seminoma associated with multilocular thymic cyst Surg Case Rep 3:7. doi: 10.1186/s40792-016-0278-7
5. Kadouri N, Nevo S, Goldfarb Y, Abramson J (2020) Thymic epithelial cell heterogeneity: TEC by TEC Nat Rev Immunol 20:239-253. doi: 10.1038/s41577-019-0238-0
6. Miller CN, Proekt I, von Moltke J, Wells KL, Rajpurkar AR, Wang H, Rattay K, Khan IS, Metzger TC, Pollack JL, Fries AC, Lwin WW, Wigton EJ, Parent AV, Kyewski B, Erle DJ, Hogquist KA, Steinmetz LM, Locksley RM, Anderson MS (2018) Thymic tuft cells promote an IL-4-enriched medulla and shape thymocyte development Nature 559:627-631. doi: 10.1038/s41586-018-0345-2
7. Moran CA, Suster S (1995) Mediastinal seminomas with prominent cystic changes. A clinicopathologic study of 10 cases Am J Surg Pathol 19:1047-1053. doi: 10.1097/00000478-199509000-00008
8. Nazeer T, Ro JY, Amato RJ, Park YW, Ordonez NG, Ayala AG (1998) Histologically pure seminoma with elevated alpha-fetoprotein: a clinicopathologic study of ten cases Oncol Rep 5:1425-1429. doi: 10.3892/or.5.6.1425
9. Oosterhuis JW, Stoop H, Honecker F, Looijenga LH (2007) Why human extragonadal germ cell tumours occur in the midline of the body: old concepts, new perspectives Int J Androl 30:256-263; discussion 263-254. doi: 10.1111/j.1365-2605.2007.00793.x
10. Roxanis I, Micklem K, McConville J, Newsom-Davis J, Willcox N (2002) Thymic myoid cells and germinal center formation in myasthenia gravis; possible roles in pathogenesis J Neuroimmunol 125:185-197. doi: 10.1016/s0165-5728(02)00038-3
11. Suster S, Rosai J (1991) Multilocular thymic cyst: an acquired reactive process. Study of 18 cases Am J Surg Pathol 15:388-398
12. Travis WD, Brambilla E, Burke AP, Marx A, Nicholson AG (2015) WHO classification of Tumours of Lung, Pleura, Thymus and Heart. IARC, Lyon

13. Weissferdt A, Suster S, Moran CA (2012) Primary mediastinal "thymic" seminomas Adv Anat Pathol 19:75-80. doi: 10.1097/PAP.0b013e318248b725

14. Yamada Y, Simon-Keller K, Belharazem-Vitacolonnna D, Bohnenberger H, Kriegsmann M, Kriegsmann K, Hamilton G, Graeter T, Preissler G, Ott G, Roessner ED, Dahmen I, Thomas R, Ströbe P, Marx A (2021) A tuft cell-like signature is highly prevalent in thymic squamous cell carcinoma and delineates new molecular subsets among the major lung cancer histotypes J Thorac Oncol. doi: 10.1016/j.jtho.2021.02.008

15. Yamashita J, Ohmoto M, Yamaguchi T, Matsumoto I, Hirota J (2017) Skn-1a/Pou2f3 functions as a master regulator to generate Trpm5-expressing chemosensory cells in mice PLoS One 12:e0189340. doi: 10.1371/journal.pone.0189340