Title
Primary mucinous adenocarcinoma of the thymic gland with intestinal differentiation and deletion in the HLA-locus: An immunohistochemical and molecular study with review of the Literature

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Abstract

Background: Primary adenocarcinoma of thymus is extremely rare. Case presentation: This is a case of primary adenocarcinoma with intestinal differentiation and focal mucin production in the thymus. Thymic cyst was associated with this tumor. Intestinal differentiation was confirmed by immunohistochemical stain with positivity for CDX-2, CK20, villin, MOC31 and focal positivity of CK7. Array comparative genomic hybridization (CGH) analysis showed a complex pattern of chromosomal imbalances including homozygous at the HLA locus in chromosomal region 6p21.32. Literature on this primary thymic tumor is reviewed.

Conclusion: Immunohistochemical study, clinical investigation as well as genetic studies may help diagnose this rare tumor.

Key words: Thymus gland, primary adenocarcinoma, intestinal differentiation, thymic carcinoma, thymoma, molecular study, CGH array, immunohistochemical study, Literature review
Introduction

Primary thymic carcinomas are very rare tumors. The most common histologic subtypes are squamous cell, adenosquamous/mucoepidermoid, basal cell, large cell undifferentiated, adenocarcinoma, carcinoma with adenoid cystic carcinoma-like features, lymphoepithelioma-like, clear cell, and sarcomatoid carcinomas\(^{(1)}\). Primary thymic adenocarcinoma was first reported in 1989\(^{(2)}\) but not accepted as a valid histologic subtype until 1997\(^{(3)}\). These are uncommon neoplasms. Papillary adenocarcinoma and mucinous adenocarcinoma are the most common variants\(^{(1,4-6)}\). Therefore, before a diagnosis of a de novo thymic adenocarcinoma, other possible diagnoses such as: metastatic carcinoma, thymoma and adenocarcinoma arising in a mediastinal teratoma should be excluded\(^{(1,7)}\). There is little genetic data for thymic carcinomas other than squamous cell carcinomas\(^{(8)}\), so we applied a genetic study by array comparative genomic hybridization (CGH) analysis on this case.
REPORT OF A CASE

A 28 year old woman presented with neck and right upper extremity pain accompanied by dyspnea of two years duration. Chest X-ray revealed mediastinal widening (figure A). Chest computed tomography (CT) scan (figure B) showed an anterior mediastinal mass invading in pericardium without extramediastinal extension. An initial clinical impression of a mediastinal germ cell tumor was considered but serum tumor markers such as alpha feto protein (α-FP), Beta-human chorionic gonadotropin (β-hCG), carcinoembryonic antigen (CEA) and CA-125 were normal. CA19-9 level was 2420 U/ml (normal: 0.37U/ml). A mediastinal biopsy was done and showed histologic features of adenocarcinoma. Endoscopy, colonoscopy, abdominopelvic sonography and imaging studies were negative for primary origin. The patient underwent mid-sternotomy. The mass was resected. The patient received chemotherapy (GEMOX) and radiotherapy with two recurrences in 2 years follow up. She is doing well and free of tumor after 6 months.
PATHOLOGIC FINDINGS

The mediastinal biopsy showed a moderately differentiated adenocarcinoma with a desmoplastic and necrotic stroma (figure C). Scattered small mucinous lakes were noted in the stroma. The stroma also showed foci of calcification and occasional psammoma bodies. Intracytoplasmic and stromal positive reactions were seen on mucicarmin stain. The excised tumor showed similar morphological and immunohistochemical characteristics. In one of the sections, a unilocunlar large cyst with columnar epithelium, in vicinity of the tumor was also identified (figure G,H). Small foci of remnant of thymic tissue were identified. Immunohistochemical (IHC) study showed positivity for CK, EMA, CK20 (figure D), CDX-2 (figure E), CK7, CD5 (figure F), villin and MOC31 but ER, PR, TTF-1, GCDFP-15, P63, CD30, PLAP, chromogranin and CD117 were all negative. All markers were from Novocastra (New Castle, UK); HMWK (clone 34 BE12), EMA (GP1.4), CDX2 (AMT28), CK20 (PW31), CK7 (RN7), CD5 (4c7), villin (CWWB1), MOC31 (MOC31), ER (6F11), PR (16), TTF-1 (SPT24), GCDFP-15 (23A3), PLAP (8A9), P63 ( 7JUL), CD30 (JCM182), chromogranin (5H7 ), CD117 (t595) and calretinin (CAL6).
Extensive histologic and immunohistochemical study of the whole specimen showed no evidence of teratomatous or germ cell elements.

**GENETIC ABBERATIONS IN FORMALIN-FIXED PARAFFIN-EMBEDDED TISSUE**

Array CGH on this case was performed using the Human Genome Microarray 180A platform (Agilent, Santa Clara, USA). The experimental procedures were performed according to the protocols provided by the manufacturer. Slides were scanned with the G2565CA Microarray Scanner (Agilent) at a scan resolution of 5 µm. Signal intensities from the generated images were measured and evaluated with Feature Extraction 10.10.11 and Agilent Genomic Standard Workbench Edition 6.5.0.58 (AGW6.5) software (Agilent) applying the Aberration Detection Method-2 (ADM-2) algorithm with a threshold of 6.0. It showed a complex karyotype with multiple gains and losses. Part of the HLA-locus in chromosomal region 6p21.32 seemed to be homozygously deleted (Figure I,J).
Discussion

Metastatic neoplasms to the mediastinum account for most of epithelial cell neoplasms. The second most common tumors are thymoma and thymic carcinoma. Primary adenocarcinoma is very rare in mediastinum, so before considering this diagnostic entity, most prevalent tumors such as metastatic adenocarcinomas, germ cell tumors and malignant teratomas must be ruled out\(^{(1,3,4,5,7,8)}\). Through clinical history, imaging studies, absence of extramediastinal tumor and histology, we report a case of primary adenocarcinoma with intestinal differentiation of the thymus with mucin production.

Immunostains are an important tool in the study of mediastinal tumors. For instance CD5 is a leukocyte marker expressed on differentiating thymocytes. It is said to be useful in differentiating thymic from non-thymic carcinomas\(^{(4,7,8)}\). Caution must be given because CD5 is positive in malignant pleural mesothelioma and adenocarcinomas of other (non thymic origin) organs\(^{(4,8)}\). It is also important to exclude tumors derived from the lung and pleura by evaluating TTF-1 and calretinin.
The exact origin of thymic adenocarcinoma is not clear. Glandular differentiation is rarely seen in ultrastructural studies of normal thymic epithelial cells\(^{(5)}\). Also, in the involuted thymus, glandular or tubular structures can be found\(^{(7)}\). Therefore, adenocarcinomas can result from extreme glandular differentiation during tumor progression.

Review of the literature on thymic adenocarcinoma is shown in table 1-2. Twenty-six cases have been reported. Patients have range in age from 15 to 82 years (mean 50 y ±17 y). Male/female ratio is 1.9/1. The most common morphologic subtypes are papillary carcinoma (38%), mucinous adenocarcinoma (34%), conventional adenocarcinoma (0.11%), NOS (0.07%) and papilotubular carcinoma (0.07%). Median age is 52.56 years. Associated findings are thymic cyst in 26% (mostly seen in mucinous subtype), thymoma in 11% (only in papillary subtype) and psammoma bodies in 15% (mostly seen in papillary subtype).

Serum tumor markers were increased. CEA in 23% (mostly seen in mucinous subtype), B-HCG in 0.38% (only in conventional subtype) and CA19-9 in 11% (one papillary, one NOS and two mucinous subtypes, including ours). Immunohistochemistry on different subtypes were performed in a limited number of papers and showed positivity in CK7 (7/11), CK20 (6/9), CEA (6/9), Leu M1 (4/5), B-HCG (1/2), CDX-2 (3/4), Muc2 (1/2), Muc5 (2/2), CD5 (8/12), P63 (1/2), CA-19-9 (3/3), CAM5.2 (2/2), CK5,6 (1/2), P53 (2/2), Her2 (1/2)\(^{(4-7,9,13-21)}\).
In our case the adenocarcinoma was associated with a large benign thymic cyst with columnar epithelium. It showed no dysplastic change. Most of the patients with thymic adenocarcinoma do not have any chief complaint (41%). In symptomatic group, the most common presenting sign is chest pain (0.17%). Other rare signs are cough (0.05%), dyspnea (0.05%), and shoulder pain (0.05%). Weight loss is a rare sign in this tumor (0.05%). Anterior mediastinum is the most common location (79%) followed by right, left, substernal and pretracheal mediastinum (each below %1). Prognosis cannot be accurately evaluated due to low incidence rate of thymic adenocarcinoma. Some patients underwent surgical resection (15 cases), chemotherapy (12 cases) or radiotherapy (19 cases). Clinical outcome showed local recurrences (2 cases), metastatic disease (3 cases) and death due to surgical complication or disease (9 cases). According to table 1, mucinous has much worse prognosis than papillary carcinoma (p value<0.05). There are diverse genetic heterogeneity in thymic tumors. Genetic characterization has concentrated on WHO types A, B3, and C that harbors few lymphocytes \(^{22}\). No chromosomal gain is seen in type A and AB thymomas\(^8\). Simultaneous gain of 1q and loss of chromosome 6, and 13q aberrations frequently detected in type B3 thymomas. Loss of heterozygosity (LOH) on chromosome 6 is the most frequent genetic abnormality in thymoma. Recent studies on genetic alterations of thymoma based on (LOH) analyses inferred two different genetic pathways of tumorigenesis of
thymoma, and heterogeneous genetic alterations in subtypes of thymoma, were identified by CGH and LOH analyses\(^{22,23}\). One of the important findings in the thymic epithelial tumor is frequent and multiple genetic aberrations of chromosome 6 that are found in 77.5 percent of them. There are five hotspots of frequent deletions indicating that several putative tumor suppressor genes on chromosome 6 are involved in the development of thymic epithelial tumor\(^{8,22,23}\). Deletion sites such as 6q21, 6q23, and 6q25-27 are well established in thymoma. As Zhou et al. reported; the most frequent LOH was found in the 6q23.3-25.3 chromosomal region and the second hot spot of deletions was located in the 6p21 region containing the major histocompatibility (MHC) classes I and II gene loci\(^{22,23}\).

In our case, array-CGH indicated that the \textit{HLA-DRB5}-locus in chromosomal region 6p21.32 was homozygously deleted, showing similar genetic aberrations with other thymic epithelial tumors.

**Conclusion**

This primary adenocarcinoma of the thymus with intestinal differentiation shows homozygous deletion of part of the \textit{HLA}-locus in chromosomal region 6p21.32 by array-CGH. It is associated with a benign thymic cyst. This is the first genetic study on a primary thymic adenocarcinoma (mucinous type), which shows a similar genetic aberration with other thymic epithelial tumors. We suggest adding
genetic study to clinicopathologic and imaging workup for confirmation of diagnosis of a primary thymic tumor.
References


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Figure legends

Figure A: Chest X ray showed mediastinal widening

Figure B: chest CT scan revealed anterior mediastinal mass that invade pericardium

Figure C: tumoral sheets and glandular structures in desmoplastic stroma (H&E stain, x 400)

Figure D: Malignant glands showed diffuse membranous positivity for CD5. (Immunoperoxidase )

Figure E: nuclear positivity for CDX-2 in malignant glands (Immunoperoxidase )

Figure F: Malignant glands showed diffuse positivity for CK20 (Immunoperoxidase )

Figure G: benign columnar lining thymic cyst adjacent to neoplastic glands (H&E stain, x 200).

Figure H: neoplastic glands arising in the vicinity of thymic cyst (H&E stain, x 200).

Figure I: Array-CGH-results displayed in a whole genome view showed deletion of chromosome 6.

Figure J: Array-CGH pattern suggesting homozygously deleted region
at the HLA-locus

in chromosomal region 6p21.32.
Additional files provided with this submission:

Additional file 1: table-1.docx, 34K
http://www.biomedcentral.com/imedia/1558339319870672/supp1.docx