

Case Report

Inflammatory Epithelioid CD34-Positive Low-Grade Myofibroblastic Sarcoma: A Novel Intrapulmonary Sarcoma

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Received: December 12, 2015; **Accepted:** December 29, 2015; **Published:** December 31, 2015

Abstract

Primary pulmonary mesenchymal tumors are rare. The differential diagnosis of CD34 and bcl2 positive epithelioid mesenchymal neoplasms is relatively narrow. The differential diagnosis for mesenchymal neoplasms associated with prominent inflammatory infiltrate is also limited. We present an incidentally identified intrapulmonary mesenchymal neoplasm with regional nodal metastases, characterized by monotonous epithelioid morphology, low-proliferative activity, prominent inflammatory reaction, strong CD34 expression, moderate bcl2 expression, negative epithelial and lineage specific markers, ultrastructural features of myofibroblastic differentiation, and negative specific molecular/genetic studies. This tumor defies classification according to existing guidelines. We consider that this lesion represents a previously un-described low-grade sarcoma with morphologic features intermediate between a solitary fibrous tumor and inflammatory myofibroblastic tumor, and propose the term intrapulmonary inflammatory epithelioid CD34-positive myofibroblastic sarcoma (CD34+ IEMS). The diagnostic work-up and differential diagnosis of this tumor are discussed in detail.

Keywords: Sarcoma; Myofibroblasts; CD34 antigen; Epithelioid cells; Lung neoplasms; Inflammation

Introduction

The CD34 antigen is a cell-adhesion transmembrane glycoprotein expressed by hematopoietic stem cells, endothelium, interstitial cells of Cajal, and a subset of fibroblastic dendritic cells present in the dermis, around blood vessels, within nerve sheaths, smooth muscle and hair follicles. Tumors derived from these structures are usually positive for CD34 [1,2]. Soft tissue tumors (STT) that characteristically express CD34 include dermatofibrosarcoma protuberans/ giant cell fibroblastoma (DFSP/GCF), solitary fibrous tumor (SFT), gastrointestinal stromal tumor (GIST), epithelioid sarcoma (ES), hemangioendothelioma/angiosarcoma (HE/AS), and as subset of malignant peripheral nerve sheath tumors (MPNST) [1,3,4]. Sarcomas typically associated with inflammation include inflammatory myofibroblastic tumor (IMT), epithelioid sarcoma (ES), and inflammatory variants of conventional sarcomas [1,4]. We report a case of an incidentally found intrapulmonary tumor with regional nodal metastases, characterized by epithelioid morphology, an associated prominent inflammatory infiltrate, strong expression of CD34 and vimentin, moderate expression of bcl2, negative epithelial markers, negative lineage specific markers, negative molecular-cytogenetic studies for known STT associated translocations and fusion genes, and electron microscopy (EM) features of myofibroblastic differentiation. We consider that this lesion represents a novel low-grade myofibroblastic sarcoma with morphologic features intermediate between a solitary fibrous tumor and inflammatory myofibroblastic tumor and propose the term intrapulmonary inflammatory epithelioid CD34-positive myofibroblastic sarcoma (CD34+ IEMS).

Case Presentation

A 67 year old man presented with a 3.2 cm intraparenchymal left upper lobe lung coin-lesion identified incidentally on a computerized tomography (CT) study (Figures 1A,B). A subsequent combined positron emission tomography (PET)-CT study showed low metabolic activity (standardized uptake value 2.1). The patient underwent a thorascopic left upper lobectomy. Gross examination showed a 3.2 x 2.9 x 1.8 cm intraparenchymal yellow-tan mass (Figure 1C) located 0.5 cm from the closest pleural surface and 2.0 cm from the bronchial resection margin. Touch imprints of the lesion showed syncytial groups of large epithelioid cells with abundant homogeneous cytoplasm, ovoid nuclei, vesicular chromatin and prominent nucleoli. The background showed mixed inflammatory cells (Figure 2A). Histologic sections showed a well circumscribed lesion consisting of syncytial monotonous large epithelioid cells admixed with numerous inflammatory cells that included lymphocytes, plasma cells, eosinophils and clustered foamy histiocytes (Figures 1D, 2B). Focally, myxoid degeneration with alveolar-like spaces was present (Figure 3), however immunohistochemical studies failed to show any residual lung parenchyma in these areas. Mitotic activity was low (<1/20HPF), necrosis was not apparent. Two lymph nodes (station 11 and hilar) had micrometastases. Immunohistochemical studies showed that the tumor was strongly and diffusely positive for CD34 and vimentin, and moderately for bcl-2 (Figure 2C). The tumor was negative for cytokeratin cocktail (CK) (AE1/AE3/Cam 5.2), CK5/6, CK7, CK20, TTF-1, P40, epithelial membrane antigen (EMA), WT1, calretinin, CD1a, CD3, CD20, CD23, CD31, CD45, CD68, CD117,

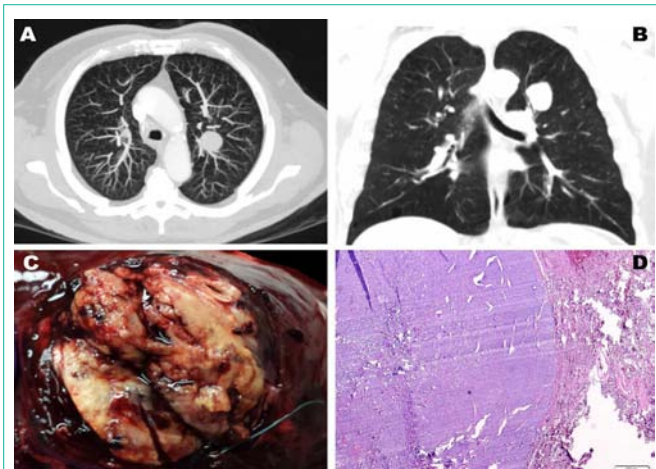


Figure 1: A, B. CT-scan with contrast, axial and coronal images show a well-delimited 3.2 cm intraparenchymal nodule in the left upper lobe. C. Gross images of the tumor show a well delimited yellow-tan fleshy mass. D. Low-power microscopic examination shows a solid mass with rounded pushing margins (hematoxylin & eosin, 2.5 X magnification).

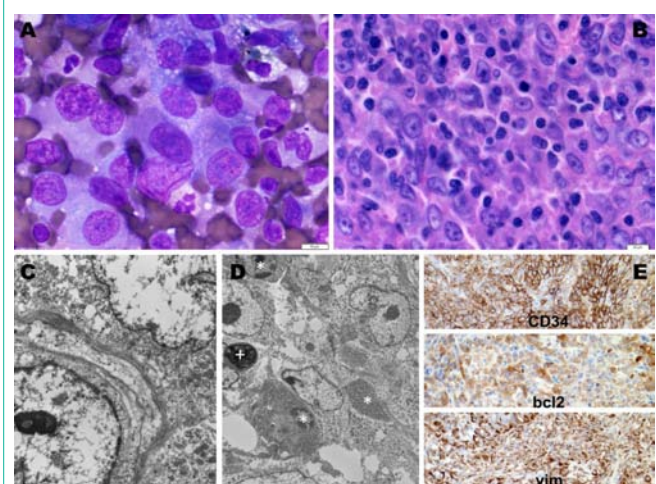


Figure 2: A. Touch imprint cytology shows syncytial groups of epithelioid cells with abundant homogeneous cytoplasm, ovoid nuclei, vesicular chromatin and prominent nucleoli (Diff-Quick®, 100 X magnification). B. Histologic sections show monotonous cohesive epithelioid cells, intimately admixed with mixed inflammatory cells. C. Electron microscopy shows plump tumor cells with rounded to ovoid nuclei, thin cytoplasmic filaments, scant rough endoplasmic reticulum and subplasmalemmal densities (direct magnification 23299 X). D. Three tumor cells and inflammatory cells consisting of plasma cells (*) and one lymphocyte (+) (direct magnification 7350 X). E. Immunostains show strong homogenous cytoplasmic expression of CD34 and vimentin, and variable bcl2.

ALK1, ERG1, S-100, muscle specific actin, desmin, SOX10, HMB45/tyrosinase/MART1 cocktail, STAT6, chromogranin, synaptophysin, myeloperoxidase, lysozyme and Epstein Barr virus encoded RNA chromogenic in situ hybridization (Table 1). P53 was positive in <10% scattered cells; Ki-67 proliferative activity was 8%. RT-PCR assay for NAB2-STAT6 gene fusion and fluorescent in situ hybridization (FISH) probes for SYT/SSX1 and SYT/SSX2 translocation, ALK-1 and EWS rearrangements performed at reference laboratories were negative. Electron microscopic study showed plump tumor cells with rounded nuclei with small indentations and prominent nucleoli. The cytoplasm had sparse organelles, consisting of thin filaments and rough

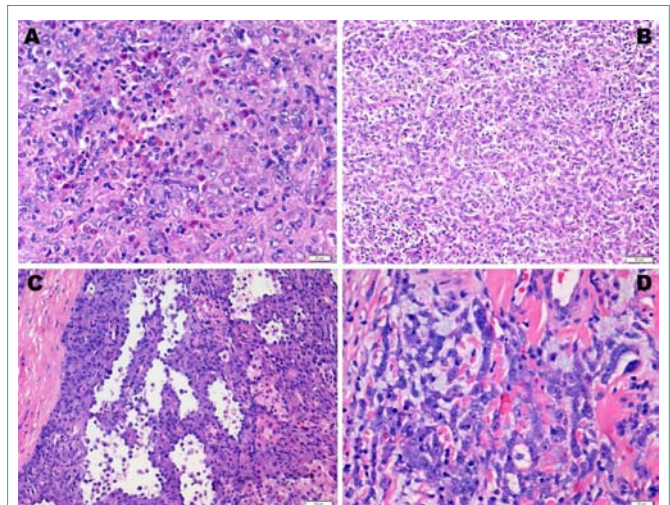


Figure 3: Histologic patterns: A. syncytial (predominant), B. vaguely storiform, C. alveolar D. retiform/myxoid (hematoxylin & eosin, 20-40X magnification).

endoplasmic reticulum; there were occasional subplasmalemmal densities. There were no well-formed intercellular junctions or basement membrane (Figure 2C). Tumor cells were separated by numerous inflammatory cells consisting of macrophages, plasma cells and lymphocytes (Figure 2D). The interstitium contained variable amounts of collagen. These features were interpreted as those of a low-grade sarcoma with fibroblastic/myofibroblastic differentiation.

Discussion

The morphologic, immunophenotypic, molecular-cytogenetic and ultrastructural findings of this case are diagnostic of a mesenchymal neoplasm. The co-expression of CD34, bcl2 and vimentin does not indicate a specific lineage; however the ultrastructural features support myofibroblastic differentiation [1,2,4]. The circumscription, low metabolic activity, monotonous histology, low mitotic/apoptotic activity, lack of necrosis and regional nodal metastases are those of a low-grade sarcoma. The differential diagnosis of CD34 positive low-grade sarcomas includes malignant SFT, GIST and DFSP/GCF. DFSP/GCF are the adult and pediatric versions of a low-grade “fibrohistiocytic” neoplasm. Clinically, these tumors have only been described in the dermis/hypodermis; histologically, they show poor circumscription and are not associated with prominent inflammation [1,4], immunohistochemically, they are usually negative for bcl2 [5,6] and biologically, these tumors can be locally aggressive, but unless they dedifferentiate, they usually do not metastasize. GIST may show a monotonous epithelioid morphology and may metastasize to the lung, but are not associated with prominent inflammation.

Immunohistochemically epithelioid GIST are often positive for bcl2, but expression of either CD117 or DOG1, which were negative in our case, is required for diagnosis. SFT most frequently arises from the pleura, but may rarely be intrapulmonary [7]; histologically it may show epithelioid morphology and is usually positive for CD34, bcl2 and vimentin. Most tumors show alternating hypo- and hypercellular areas of spindle cells in random or storiform arrangements; thick collagen bands or dense fibrosis, delicate staghorn vasculature or hemangiopericytoma-like morphology are present, at least focally [1,3,4,7], their morphology can be extremely

Table 1: Immunohistochemical stains.

Stain	Manufacturer	Catalog #	City, State, Country	Dilution	Retrieval
AE1/AE3	Novacastra	NCL-L- AE1/AE3	Newcastle upon Tyne, UK	1:1600	Enzyme
CAM 5.2	Cell Marque	452M-94	Rocklin, CA, USA	1:400	EDTA
CK 5/6	Cell Marque	356M-15	Rocklin, CA, USA	1:150	EDTA
CK7	Biocare Medical	CM061B	Concord, CA, USA	1:150	Enzyme
CK20	Novacastra	NCL-L-CK20-561	Newcastle upon Tyne, UK	1:200	EDTA
P40	Biocare Medical	ACI3066A	Concord, CA, USA	1:100	EDTA
EMA	Cell Marque	247M-94	Rocklin, CA, USA	1:1000	EDTA
TTF-1	Dako	M357529	Carpinteria, CA, USA	1:1400	EDTA
WT1	Cell Marque	348M-94	Rocklin, CA, USA	1:2000	EDTA
Calretinin	Novacastra	NCL-L- CALRET-566	Newcastle upon Tyne, UK	1:1200	EDTA
CD1a	Cell Marque	101M-15	Rocklin, CA, USA	1:10	EDTA
CD3	Thermo Fisher Scientific	RM9107-S1	Fremont, CA, USA	1:150	EDTA
CD20	Novacastra	NCL-CD20-7D1	Newcastle upon Tyne, UK	1:300	EDTA
CD23	Cell Marque	123M-14	Rocklin, CA, USA	1:80	EDTA
CD31	Cell Marque	131M-95	Rocklin, CA, USA	1:25	EDTA
CD34	Cell Marque	134M-14	Rocklin, CA, USA	1:400	EDTA
CD45	Cell Marque	145M-95	Rocklin, CA, USA	1:200	EDTA
CD68	Dako	M0876	Glostrup, Denmark	1:200	Enzyme
CD117	Cell Marque	117R-14	Rocklin, CA, USA	1:300	EDTA
ALK1	Cell Marque	204M-15	Rocklin, CA, USA	1:15	EDTA
ERG1	Cell Marque	434R-14	Rocklin, CA, USA	1:75	EDTA
S-100	Novacastra	NCL-L-S100P	Newcastle upon Tyne, UK	1:1600	EDTA
MSA	Cell Marque	201M-94	Rocklin, CA, USA	1:1400	EDTA
Desmin	Cell Marque	243M-15	Rocklin, CA, USA	1:60	EDTA
Bcl2	Cell Marque	226R-15	Rocklin, CA, USA	1:40	EDTA
Vimentin	Cell Marque	347M-14	Rocklin, CA, USA	1:250	EDTA
SOX10	Biocare Medical	ACI3099C	Concord, CA, USA	1:30	EDTA
HMB45	Cell Marque	282M-96	Rocklin, CA, USA	1:25	EDTA
Tyrosinase	Biocare Medical	CM155B	Concord, CA, USA	1:200	EDTA
MART1	Cell Marque	281M-85	Rocklin, CA, USA	1:300	EDTA
STAT6	Santa Cruz Biotechnology, INC	sc-621	Santa Cruz, CA, USA	1:1000	Citrate
Chromo	Biocare Medical	CM010A	Concord, CA, USA	1:600	EDTA
Synapto	Novacastra	NCL-SYNAP-299	Newcastle upon Tyne, UK	1:100	EDTA
MPO	Cell Marque	289A-74	Rocklin, CA, USA	1:1200	EDTA
Lzyme	Cell Marque	278A-14	Rocklin, CA, USA	1:1600	EDTA
P53	Thermo Scientific	MS-738-P0	Fremont, CA, USA	1:1250	EDTA
Ki-67	Cell Marque	275R-15	Rocklin, CA, USA	1:100	EDTA
Stainer	Manufacturer		City, Country		
Leica BOND-III	Leica Biosystems		Melbourne, Australia		

CK: Cytokeratin, EMA: Epithelial Membrane Antigen, TTF-1: Thyroid Transcription Factor 1, WT1: Wilms Tumor 1, CD: Cluster of Differentiation, ALK1: Anaplastic Lymphoma Kinase 1, ERG: ETS Related Gene; CD: Cluster of Differentiation; MSA: Muscle Specific Actin, bcl2: B-cell Lymphoma 2, SOX10: SRY-related HMG-box 10, HMB45: Human Melanoma Black 45, MART1: Melan A 1, STAT6: Signal Transducer and Activator of Transcription 6, Chromo: Chromogranin A, Synapto: Synaptophysin, MPO: Myeloperoxidase, Lzyme: Lysozyme; EDTA: Ethylenediaminetetraacetic Acid

variable [8]; however, dense inflammatory infiltrate is not a feature of this tumor. Recently, it has been demonstrated that virtually all

SFT have a NAB2-STAT6 fusion gene. The translocation may be demonstrated by molecular analysis or expression of STAT6 by

Table 2: Differential Diagnosis.

Tumor	Typical location	Epithelioid cells	Monotonous Histology	Inflammation	IHC			Cytogenetics Test	Metastatic potential
					CD34	Bcl2	Specific		
CD34+ IEMS	Lung	+	+	+	+	+		+	
SFT	Pleura	-/+	-	-	+	+	Stat6	NAB2-STAT6	-/+
IMT	Lung	-/+	-	+	-	-	ALK1	ALK1-rearrang.	-
DFSP/GCF	Skin	-	+	-	+	+		COL1A1-PDGFB	-
GIST	GI tract	-/+	+	-	+	+	C-Kit,DOG1		+
EAS	Viscera	+	-	-	+		ERG1,CD31		+++
EHE	Viscera	+	+	-	+		ERG1,CD31	WWTR1- CAMTA1	+
ES	Extremities	+	+	+	+		CK		+
SS	Extremities	-/+	+	-	-	+	CK	SYT-SSX1/SYT- SSX2	+
CCS	Extremities	+	+	-	-		Melan.Mark.	EWS-ATF1	+
SCa	Lung	+/-	-	+/-	-		CK, TTF1		+++

CD34+ IEMS: Inflammatory Epithelioid CD34 Positive Myofibroblastic Sarcoma; SFT: Solitary Fibrous Tumor; IMT: Inflammatory Myofibroblastic Tumor; DFSP: Dermatofibrosarcoma Protuberans; GCF: Giant Cell Fibroblastoma; GIST: Gastrointestinal Stromal Tumor; EAS: Epithelioid Angiosarcoma; EHE: Epithelioid Hemangioendothelioma; ES: Epithelioid Sarcoma; SS: Synovial Sarcoma; CCS: Clear Cell Sarcoma; SCa: Sarcomatoid Carcinoma; CK: Cytokeratin; Melan.Mark: Melanocytic Markers; Rearrang.: Rearrangement

immunohistochemistry [9,10]; these studies are more sensitive and specific than immunoreactivity for CD34 or bcl-2 for the diagnosis of SFT [9-12]. Our case did not show any classic areas of SFT; rather, it showed a prominent inflammatory infiltrate, and was negative for the NAB2- STAT6 fusion by immunohistochemistry and molecular studies, excluding this possibility. The differential diagnosis for CD34-positive epithelioid tumors includes ES, epithelioid HE (EHE) and epithelioid AS (EAS), and less likely, sarcomatoid carcinomas (SCa). Histologically, ES consists of monotonous epithelioid cells admixed with inflammatory cells, resembling our case; however ES usually affects the subcutis, tendon sheaths and fasciae of the extremities of young individuals as multinodular masses, on microscopy the tumor nodules almost invariably show prominent central necrosis surrounded by epithelioid tumor cells, resulting in a granuloma-like appearance. By immunohistochemistry, expression of cytokeratins and EMA is required for diagnosis, and on EM characteristic paranuclear aggregates of intermediate filaments are expected. Our case lacked necrosis, expression of epithelial markers and characteristic ultrastructural features. EHE and EAS may occur in the lung as primary tumors, or as metastases; EHE is a low-grade endothelial neoplasm that characteristically shows intracytoplasmic lumens with red blood cells, while EAS is a high-grade endothelial malignancy composed of epithelioid cells with high nuclear grade and marked cytologic atypia. Both neoplasms frequently express CK, and are required to express other vascular markers (CD31, ERG1) for diagnosis. The intrapulmonary location of our tumor obligated considering a SCa (Table 2).

Microscopically, SCa with epithelioid morphology are expected to have a high nuclear grade, pleomorphism, high mitotic/apoptotic activity, necrosis and/or specific sarcomatous differentiation. Expression of epithelial markers, at least focally, is expected; while expression of CD34 is common in sarcomas with epithelioid morphology, it is very rare in carcinomas [1,3]. Features favoring an epithelial origin on EM include numerous junctional complexes, basal lamina, microvilli and prominent vesicles. Our case lacked

these features. Finally, the possibility of aberrant expression of CD34 by sarcomas of fibroblastic/myofibroblastic or uncertain lineage like SS, IMT and CCS was considered. Epithelioid cells can be a component of SS and this tumor consistently expresses bcl2 and vimentin; however epithelioid cells in SS show true divergent epithelial/glandular differentiation and characteristic SYT/SSX1 or SYT/SSX2 translocation [1,3,4]. Our case did not have epithelial/glandular cells by morphology or immunohistochemistry, and FISH studies for these translocations were negative. IMT is a low-grade myofibroblastic neoplasm that may show local invasion and/or recurrences, but rarely metastasizes. It is usually associated with a prominent inflammatory infiltrate, and a heterogeneous zonal pattern with a mixture of fasciitis-like, fibrosarcoma-like, hyalinized areas, and variable number of ganglion-like cells that may have an epithelioid appearance.

Immunohistochemically expression of CD34 or bcl2 is uncommon, while variable actin is frequent. Expression of ALK1 correlates with ALK1 gene rearrangement, which is present in ~2/3 of the cases [3,4]. Monotonous epithelioid morphology, strong CD34 expression, negative FISH study for ALK1 rearrangements and presence of nodal metastases are strong arguments against this possibility [2,3]. CCS usually affects the tendons and aponeuroses of the extremities, but primary visceral tumors have been reported. Histologically, it consists of monotonous pale eosinophilic epithelioid cells in a vaguely nested pattern, without significant mitotic/apoptotic activity or necrosis similar to our case, however inflammation is not common. Immunohistochemically, expression of melanocytic markers is required; the differential diagnosis from melanoma is done through the demonstration of EWS rearrangements. Our tumor was negative for melanocytic markers and EWS break-apart probe.

Based on all the findings, this patient's neoplasm is considered a low-grade sarcoma with morphologic features intermediate between a SFT and IMT. Since the lesion was excised completely with adequate margins, no additional therapy is planned. The patient will be followed according to the National Comprehensive Cancer

Network Non-Small Cell Lung Cancer Guidelines.

Conclusion

We present a case of an intrapulmonary CD34-positive low-grade sarcoma with epithelioid cells and morphologic features intermediate between a SFT and IMT that defies classification according to existing guidelines. We propose the term intrapulmonary inflammatory epithelioid CD34-positive myofibroblastic sarcoma (CD34+ IEMS). Since this is first report of this entity the prognosis of this lesion is uncertain, but expected to follow the behavior of a low-grade myofibroblastic sarcoma. At this time, complete surgical resection without adjuvant therapy and long-term follow-up are considered reasonable for this lesion.

Acknowledgment

We thank Wendy Larson, HT at the Minneapolis VAHCS for performing all the immunohistochemical studies and Professor Andre Oliveira MD, PhD at Mayo Clinic Rochester for performing the NAB2-STAT6 fusion molecular studies.

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