Case report

EWSR1-fusion-negative, SMARCB1-deficient primary pulmonary myxoid sarcoma

Abbas Agaimy[1], Thomas Duell[2], Alicia T. Morresi-Hauf[3]

[1]Institut für Pathologie, Friedrich-Alexander Universität Erlangen-Nürnberg, Erlangen, Germany
[2]Klinik für Pneumologie, Asklepios Fachkliniken München-Gauting, Gauting, Germany
[3]Institut für Pathologie, Asklepios Fachkliniken München-Gauting, Gauting, Germany

Primary pulmonary myxoid sarcoma (PPMS) is a recently defined rare neoplasm with histological and molecular similarity to extraskeletal myxoid chondrosarcoma. To date, 20 cases have been reported. A 48-year-old man presented with a huge mass filling the right hemithorax and extending into the tracheobronchial system. Histological findings were consistent with PPMS. Immunohistochemistry was positive for vimentin, CD10, and EMA, but other lineage-specific markers were negative. SMARCB1 (INI1) expression was lost in the tumour cells. FISH analysis (*EWSR1, FUS, NR4A3,* and *SMARCB1*) revealed no abnormalities. This case suggests SMARCB1 loss as a possible alternative molecular event driving EWSR1-negative PPMS.

Key words: primary pulmonary myxoid sarcoma, EWSR1, myoepithelial carcinoma, SMARCB1, endobronchial tumour.

Introduction

Primary pulmonary myxoid sarcoma (PPMS) is a rare low-grade malignant neoplasm with distinctive clinicopathological, immunophenotypic, and molecular-genetic features. Since its first description by Nicholson *et al.* in 1999 as a low-grade malignant myxoid endobronchial tumour [1], no more than 20 well-documented cases appeared in the English-language literature (reviewed in Thway *et al*. [2]). The term PPMS was coined by Thway *et al*. in 2011, who reported a series of 10 cases including the two original cases and delineated the molecular-genetic profile of this exceptionally rare entity [2]. Primary pulmonary myxoid sarcoma is now included in the current World Health Organisation (WHO) classification of lung tumours as a separate entity [3]. In this report, we describe a new case of PPMS that showed prototypical histomorphological and immunophenotypic features of that entity. However, fluorescence *in situ* hybridisation (FISH) failed to show any evidence of EWSR1, FUS, or *NR4A3* gene fusions. Instead, immunohistochemistry showed complete loss of SMARCB1 (INI1) in the neoplastic cells indicating a role for SMARCB1-deficiency as a possible alternative molecular mechanism underlying some EWSR1-negative PPMS cases.

Case history

A 48-year-old Caucasian male with a significant smoking history and chronic obstructive pulmonary disease (COPD) presented with partial respiratory insufficiency. Clinical and imaging investigations (computer tomography of the chest) showed extensive retention pneumonia involving the right and partially the left lung. A huge mass measuring > 14 cm was seen on CT occupying the right hemithorax and extending into the right main bronchus, compressing the left main bronchus and reaching the trachea (Fig. 1).

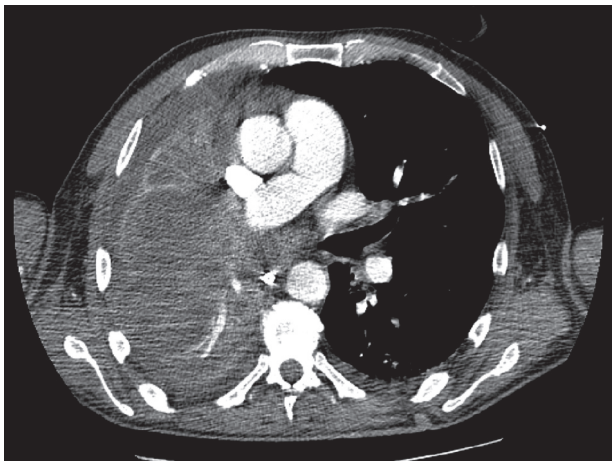

Fig. 1. CT thorax showing extensive pulmonary mass replacing the right hemithorax and extending into the main bronchus

Bronchoscopic biopsies were obtained from the endobronchial component. At the time of initial evaluation, there was no evidence of regional or distant metastases. The patient received several cycles of palliative chemotherapy (doxorubicin and trabectedin). Follow-up imaging showed initially stable disease but later investigations revealed signs of disease progression. The patient then received palliative radiotherapy again. CT examination showed a new focal lesion suggestive of cerebellar metastasis 13 months later. At last follow-up (23 months after initial diagnosis), he was alive with disease under palliative treatment.

Material and methods

The biopsy specimen was fixed in buffered formalin and embedded in paraffin for routine histological examination. Immunohistochemical studies were performed on 3-μm sections cut from paraffin blocks using a fully automated system ("Benchmark XT System", Ventana Medical Systems Inc., 1910 Innovation Park Drive, Tucson, Arizona, USA) and the following antibodies: pan cytokeratin (clone AE1/AE3, 1 : 40, Zytomed, Berlin, Germany), vimentin (V9, 1 : 100, Dako, Hamburg, Germany), CK7 (clone OV-TL, 1 : 1000, Biogenex), CK18 (clone CY-90, 1 : 500, Sigma), CK5 (clone XM26, 1 : 50, Zytomed), TTF-1 (clone 8G7G3/1, dilution, 1 : 500, Zytomed), ERG (clone EPR3864, prediluted/ready to use, Ventana Medical Systems), CD31 (clone JC70A, 1 : 20, Dako), CD10 (clone 56C6, 1 : 20, Dako), p63 (clone SFI-6, 1 : 100, DCS), desmin (clone D33, 1 : 250, Dako), α-smooth muscle actin (clone 1A4, 1 : 200, Dako), S100 protein (polyclonal, 1 : 2500, Dako), CD34 (clone BI-3C5, 1 : 200, Zytomed), CD30 (clone Ber-H2, 1 : 40, Zytomed), MUC4 (clone EP256, 1:500, Epitomics), TLE1 (polyclonal, 1 : 200, Santa Cruz), STAT6 (clone sc-621, 1 : 1000, Santa Cruz Biotechnology), and SMARCB1 (INI1) (MRQ-27, 1 : 50, Zytomed), according to the manufacturer instructions.

Results

The biopsy material was composed of multiple polypoid fragile gelatinous endobronchial exophytic tumour fragments measuring together 1.5 cm (Fig. 2A). Histological examination showed a low to moderate cellular neoplasm composed of bland looking medium-sized oval to rounded epithelioid cells arranged in a prominent reticular and microcystic lace-like chordoid pattern in a highly myxoid stroma (Fig. 2B, C). Mitoses were scant, with $<$ 2 mitoses/10 HPFs. There was prominent vascularisation of the stroma (Fig. 2B). Less than 10% of the biopsies were composed of cellular solid areas with similar cellular features as the myxoid areas (Fig. 2D). The solid cellular foci blended with the myxoid component and were occasionally surrounded by old haemorrhages. No pleomorphism, necrosis, or atypical mitoses were observed. Immunohistochemistry showed diffuse expression of vimentin (Fig. 3A) and variable expression of EMA (Fig. 3B) and CD10 (Fig. 3C), while pan cytokeratin (Fig. 3D) and all other lineage-specific markers listed above were negative. In addition, the neoplastic cells showed complete loss of nuclear SMARCB1 (INI1) expression by immunohistochemistry with retained positivity in the normal cells in the background (Fig. 3E, F). The bronchial mucosa showed no evidence of surface epithelial dysplasia and there was no epithelial tumour component.

FISH analysis using *EWSR1, FUS, NR4A3*, and *SMARCB1* double-colour probes revealed no recognisable translocation or copy number aberrations.

Discussion

Primary pulmonary myxoid sarcoma (PPMS) is exceedingly rare with no more than 20 well-documented cases in the English-language literature (Table I) [1, 2, 4, 5, 6, 7, 8]. The size of reported cases varied from 1.5 cm to 13 cm (median, 4 cm). All 16 tumours with detailed gross description presented as predominantly endobronchial masses leading to significant retention pneumonia. The disease affected females and males with equal frequency (11 females and 10 males) in the age range 26 to 68 years (median, 48 years). There was no difference in the disease distribution in the lower (n = 10) vs. upper (n = 8) lung lobes or in the left (n = 10) vs. right (n = 11) lung. Metastatic disease occurred in 4 patients a few to 13 months after diagnoses and was localised in the brain, kidney, and contralateral lung. Nine patients were alive without evidence of disease at last follow-up (12-180 months; median 69).

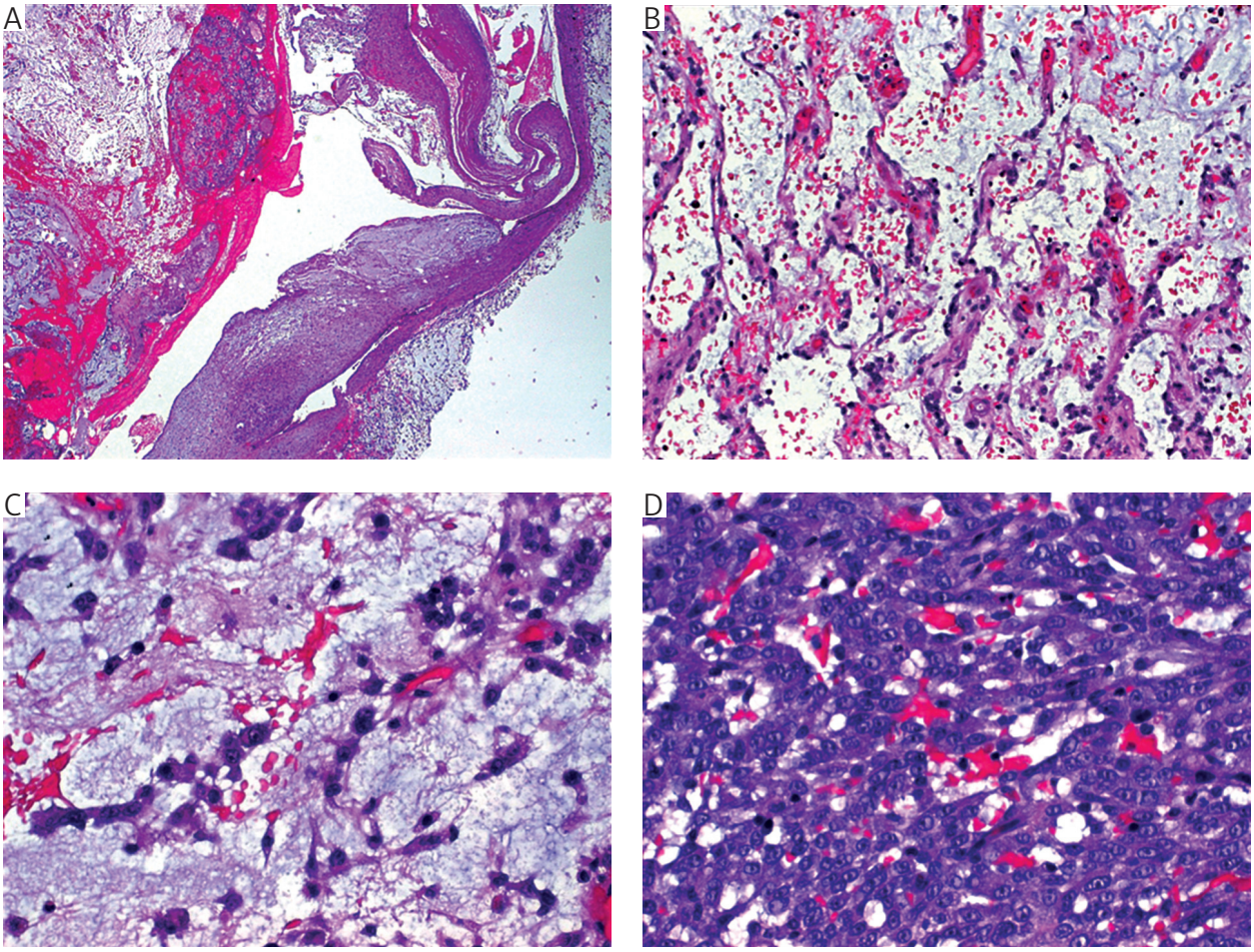

Fig. 2. A) Low-power view showing highly myxoid endobronchial tumour. B) Highly myxoid neoplasm with reticular and corded pattern in highly vascularised stroma. C) High-power view showing rounded monomorphic nuclei with distinctive central nucleoli. D) < 10% of the biopsy showed increase cellularity but the same cytological features

Variable immunohistochemical stains have been performed on individual cases. These revealed consistent expression of vimentin (18/18) and focal staining for EMA (9/17) and S100 protein (2/21). Desmin was expressed in a single case (1/19). None of the cases tested showed expression of pan cytokeratin (0/20), p63 (0/11), or smooth muscle actin (0/7).

Although available data is still limited, the molecular profile of PPMS seems to impact prognosis. Thus, all of the eight patients who remained disease-free at last follow-up had detectable *EWSR1* gene fusions, with seven of them having EWSR1-CREB1 translocations and one case showing positive EWSR1 status with unknown fusion partner. On the other hand, of the four patients who developed metastatic disease (one died of his disease a few months after diagnosis), three had wildtype EWSR1 status (including the current report).

Primary pulmonary myxoid sarcoma shows histological and molecular similarity to extraskeletal myxoid chondrosarcoma [9], myxoid angiomatoid fibrous histiocytoma [10, 11], and myxoid myoepithelial carcinoma of soft tissue [12]. Although, the unique growth pattern of this enigmatic entity with a predominantly endobronchial growth might suggest a salivary-analogue neoplasm [13], the consistent morphology lacking any salivary-like myoepithelial or mixed areas, absence of specific myoepithelial markers by immunohistochemistry, and the molecular features of this distinctive entity are more in line with true mesenchymal (soft tissue) origin than with a myoepithelial neoplastic entity. In terms of reproducible morphology and other distinctive clinicopathological features, PPMS represents a unique rare histologically low-grade sarcoma that needs to be distinguished from more aggressive primary and metastatic soft tissue tumours such as extraskeletal myxoid chondrosarcoma, chordoma, myxoid epithelioid sarcoma, and myoepithelial carcinoma as well as from lung sarcomatoid carcinoma with prominent myxoid changes [9, 13, 14].

Most difficult and even arbitrary is the distinction of PPMS from rare pulmonary cases of myxoid angiomatoid fibrous histiocytoma [10, 11] and

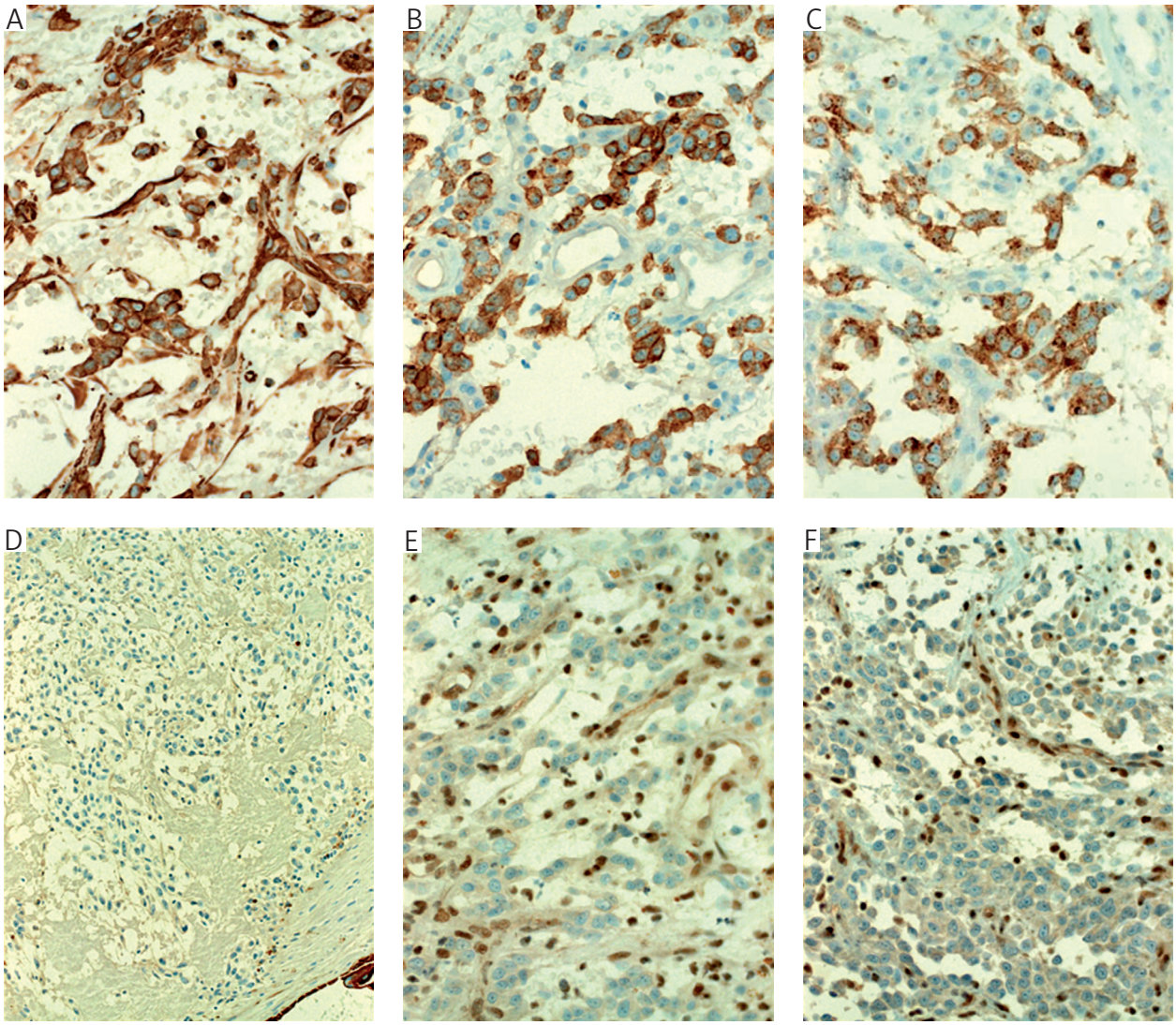

Fig. 3. In immunohistochemistry, the neoplastic cells expressed vimentin (A), EMA (B), and CD10 (C) but not pan cytokeratin (D; note positive staining in mucosa lower right). Both the highly myxoid (E) and cellular (F) area showed SMARCB1 loss (note strong staining in vessels and stromal cells in background)

extraskeletal myxoid chondrosarcoma [14]. Indeed, it is still unclear if some of the former represented unusual variants of PPMS. However, the most relevant differential diagnostic distinction when encountering a putative case of PPMS is to exclude pulmonary metastasis from occult or previous soft tissue primary because this may herald a more aggressive behaviour with significant prognostic implications.

The available literature (Table I) suggests genotypic heterogeneity in the group of PPMS. Absence of ESWR1 gene fusions in a subset of cases (mostly with more aggressive course) suggests different molecular subsets of lesions unified by the phenotype of PPMS. The current report is in line with the existence of different molecular subtypes in the spectrum of primary pulmonary myxoid sarcoma and indicates SMARCB1 loss as a possible alternative molecular driver in some PPMS. In this context, it should be emphasised that SMARCB1 loss seems to be exceptionally rare in pulmonary neoplasms because none of 316 non-small cell lung cancer specimens analysed in a recent study showed loss of SMARCB1 [15]. This is in sharp contrast to the reported SMARCB1 loss in variable subsets of mesenchymal, myoepithelial, and epithelial neoplasms in different organs [16]. Thus, it would be of relevance to investigate future PPMS cases for SMARCB1 status to see if this is mutually exclusive with the EWSR1 fusion (then as a primary driver event) or if it merely represents a secondary epigenetic event.

The authors declare no conflict of interest.

Table I. Clinicopathological features of reported primary pulmonary myxoid sarcomas (n = 21)

Author/ Ref	Age/ Gender	Site	Termino-logy used	Symptoms/ smoking history	Initial stage, size cm	Treat-ment	IHC	Outcome/ Follow-up (months)	EWSR1/ molecular testing
Nicholson *et al.* 1999* [1]	27 F	RLL	MMET	incidental, ex-smoker	4 cm, endobronchial	surgery	pos: vim neg: CK, EMA, S100, CD31, CD34, NSE, SMA, MSA, Leu7, myoglobin, desmin	NED (15 years)	NA
Nicholson *et al.* 1999*[1]	43 F	LUL/ lingula	MMET	chronic bronchitis, years, smoker	3.5 cm endobronchial	surgery	pos: vim neg: CK, EMA, S100, CD31, CD34, NSE, SMA, MSA, desmin,	NED (12 years)	NA
Inayama *et al.* 2001 [4]	60 M	RML, 2 years	LGPMS	on follow-up for bronchial asthma	9 cm, satellite nodules, endobronchial	surgery	pos: vim neg: CK, S100, CD34, SMA, desmin, CD68	NA	diploid on flow cytometry, no other studies done
Thway *et al.* 2011 [2]	45 F	RUL	PPMS	cough, bronchiectasis, mass	1.5 cm, endobronchial	surgery	pos: vim, S100 (focal) neg: CK, desmin, p63	NED (1 year)	EWSR1 break point
Thway *et al.* 2011 [2]	36 F	L	PPMS	neurologic symptoms	NA, NA	surgery	pos: vimentin. neg: CK, desmin, EMA, TTF1, S100	brain MTS, DOD (few mo)	EWSR1 negative CREB1 negative
Thway *et al.* 2011 [2]	32 F	RUL	PPMS	weight loss	NA, endobronchial	surgery	pos: vimentin. neg: CK, desmin, EMA, S100	NA	EWSR1-CREB1
Thway *et al.* 2011 [2]	28 M	LLL	PPMS	cough, haemoptysis, fever, weight loss	2.8 cm, endobronchial	surgery	pos: vimentin, EMA (w). neg: CK, desmin, S100, TTF1	left kidney MTS, NED (3 years)	EWSR1 negative CREB1 +
Thway *et al.* 2011 [2]	67 M	LLL	PPMS	NA	2.8 cm, endobronchial	surgery	pos: vimentin, EMA (w). neg: CK, desmin, S100, TTF1	NA	EWSR1-CREB1
Thway *et al.* 2011 [2]	68 F	RUL	PPMS	NA	2 cm, endobronchial	surgery	pos: vimentin. neg: CK, desmin, TTF1, S100	NA	EWSR1 negative CREB1 negative (poor preservation)
Thway *et al.* 2011 [2]	63 F	LUL	PPMS	haemoptysis	NA, endobronchial	surgery	pos: vimentin, EMA (wk). neg: CK, desmin, S100, TTF1	NED (4 years)	EWSR1-CREB1
Thway *et al.* 2011 [2]	51 M	RLL	PPMS	HIV+, incidental mass on X-ray	2 cm, NA	surgery	NK	NA	EWSR1-CREB1
Zhou *et al.* 2012 [5]	51 F	LUL, peripheral 10 years	EMC	severe iron deficiency anaemia, no lung symptoms	5 cm, 1/50	lobec-tomy	pos: vim, NSE, S100(+F) neg: CK, EMA, p63, SMA, MSA, desmin, KP1, calretinin	NED (32)	NA

Table I. Cont.

Author/ Ref	Age/ Gender	Site	Termino-logy used	Symptoms/ smoking history	Initial stage, size cm	Treat-ment	IHC	Outcome/ Follow-up (months)	EWSR1/ molecular testing
Matsukuma *et al.* 2012 [6]	31 M	LLL, partial endo-bronchial	PPMS	incidental on chest X-ray	2.7 cm	partial lobec-tomy	pos: vim, EMA(F), neg: CK, TTF1, Napsin A, S100, CD34, SMA, desmin, CD10, p63, calponin, CD117, h-caldesmon, HMB45, Synaptophysin, GFAP	NED (69)	EWSR1-CREB1
Smith *et al.* 2014 [7]	66 F	LUL endo-bronchial tumour	PPMS	obstructive symptoms, pneumonia	4 cm	surgery	pos. EMA(F) neg: desmin, CK, p63, S100	NA	EWSR1-CREB1
Smith *et al.* 2014 [7]	28 M	RLL	PPMS	cough, haemoptysis, 4 mo	8.5 cm, endobronchial	NS	pos: desmin, EMA(F) neg: S100, p63, CK	NA	EWSR1 (NS) (CREB1/ATF1 neg)
Smith *et al.* 2014 [7]	28 M	RUL	PPMS	chest pain, 2 weeks	6 cm, endobronchial	NS	pos: EMA(F) neg: S100, p63, CK	NA	FISH neg for EWSR1, CREB1 & tf1
Jeon *et al.* 2014 [8]	26 M	LLL	PPMS	cough, hemoptysis	9 cm endobronchial	surgery	pos: vim, EMA(F+), CD99(+F), neg: SMA, SMMHC, desmin, calpo-nin, h-caldesmon, S100, CK, CD34, synapto, CD56, p63	NED (19)	EWSR1-CREB1
Jeon *et al.* 2014 [8]	49 F	RLL	PPMS	known mass, increasing, 8 years	4 cm, endobronchial	surgery	pos: vim, SMA(F), CD99(F), neg: SMMHC, desmin, calponin, h-caldesmon, S100, EMA, CK, CD34, synaptophysin, CD56,p63	NED (117)	EWSR1-CREB1
Jeon *et al.* 2014 [8]	54 F	RLL	PPMS	incidental to mitral valve disease	4.5 cm, endobronchial	surgery	pos: vim, CD99(+F), neg: SMA, SMMHC, EMA, desmin, calponin, h-caldesmon, S100, CK, CD34, synapto, CD56, p63	NED (152)	EWSR1-CREB1
Jeon *et al.* 2014 [8]	65 M	LLL	PPMS	productive cough, chest pain	13 cm, endobronchial	surgery	pos: vim neg: SMA, SMMHC, EMA, CD99, desmin, calponin, h-caldesmon, S100, CK, CD34, synapto, CD56, p63	MTS right lung (7); NED (72)	EWSR1-CREB1
Current	48 M	right hemitho-rax	PPMS	cough	>14 cm, right hemi-thorax, endo-bronchial	biopsy	pos: vimentin, EMA, CD10 neg: AE1/AE3, CK5, desmin, SMA	cerebellar MTS (13 mo) AWD (23 mo)	EWSR1&FUS negative SMARCB1 FISH normal

AWD – alive with disease; CK – pan cytokeratin; DOD – died of disease; EMC – extraskeletal myxoid chondrosarcoma; F – focal; IHC – immunohistochemistry; LGPMS – low-grade pulmonary myxoid sarcoma; LLL – left lower lobe; LUL – left upper lobe; MMET – malignant myxoid endobronchial tumour; mo – months; MTS – metastasis; NA – not available; NED – no evidence of disease; neg, negative; NS – not specified; pos – positive; PPMS – primary pulmonary myxoid sarcoma; RLL – right lower lobe; RML – right middle lobe; RUL – right upper lobe; SMA – smooth muscle actin; SMMHC – smooth muscle myosin heavy chain; vim – vimentin; wk – weak

References

1. Nicholson AG, Baandrup U, Florio R, et al. Malignant myxoid endobronchial tumour: a report of two cases with a unique histological pattern. Histopathology 1999; 35: 313-318.
2. Thway K, Nicholson AG, Lawson K, et al. Primary pulmonary myxoid sarcoma with EWSR1-CREB1 fusion: a new tumor entity. Am J Surg Pathol 2011; 35: 1722-1732.
3. Travis WD, Brambilla E, Burke AP, Marx A, Nicholson AG eds. WHO Classification of Tumours of the Lung, Pleura, Thymus and Heart. 4th ed. IARC Press, Lyon 2015.
4. Inayama Y, Hayashi H, Ogawa N, et al. Low-grade pulmonary myxoid sarcoma of uncertain histogenesis. Pathol Int 2001; 51: 204-210.
5. Zhou Q, Lu G, Liu A, et al. Extraskeletal myxoid chondrosarcoma in the lung: asymptomatic lung mass with severe anemia. Diagn Pathol 2012; 7: 112.
6. Matsukuma S, Hisaoka M, Obara K, et al. Primary pulmonary myxoid sarcoma with EWSR1-CREB1 fusion, resembling extraskeletal myxoid chondrosarcoma: Case report with a review of literature. Pathol Int 2012; 62: 817-822.
7. Smith SC, Palanisamy N, Betz BL, et al. At the intersection of primary pulmonary myxoid sarcoma and pulmonary angiomatoid fibrous histiocytoma: observations from three new cases. Histopathology 2014; 65: 144-146.
8. Jeon YK, Moon KC, Park SH, et al. Primary pulmonary myxoid sarcomas with EWSR1-CREB1 translocation might originate from primitive peribronchial mesenchymal cells undergoing (myo)fibroblastic differentiation. Virchows Arch 2014; 465: 453-461.
9. Lucas DR, Stenman G. Extraskeletal Myxoid Chondrosarcoma. In: Fletcher CDM, Bridge JA, Hogendoorn CW and Mertens F (eds.). WHO Classification of tumors of soft tissue and bone. (Herndon, VA: Stylus Publishing) 2013; 223-224.
10. Thway K, Nicholson AG, Wallace WA, et al. Endobronchial pulmonary angiomatoid fibrous histiocytoma: two cases with EWSR1-CREB1 and EWSR1-ATF1 fusions. Am J Surg Pathol 2012; 36: 883-888.
11. Schaefer IM, Fletcher CD. Myxoid variant of so-called angiomatoid "malignant fibrous histiocytoma": clinicopathologic characterization in a series of 21 cases. Am J Surg Pathol 2014; 38: 816-823.
12. Jo VY, Fletcher CD. Myoepithelial neoplasms of soft tissue: an updated review of the clinicopathologic, immunophenotypic, and genetic features. Head Neck Pathol 2015; 9: 32-38.
13. Falk N, Weissferdt A, Kalhor N, et al. Primary Pulmonary Salivary Gland-type Tumors: A Review and Update. Adv Anat Pathol. 2016; 23: 13-23.
14. Kalhor N, Suster S, Moran CA. Primary pulmonary chondrosarcomas: a clinicopathologic study of 4 cases. Hum Pathol 2011; 42: 1629-1634.
15. Herpel E, Rieker RJ, Dienemann H, et al. SMARCA4 and SMARCA2 deficiency in non-small cell lung cancer: immunohistochemical survey of 316 consecutive specimens. Ann Diagn Pathol 26; 2017: 47-51.
16. Agaimy A. The expanding family of SMARCB1(INI1)-deficient neoplasia: implications of phenotypic, biological, and molecular heterogeneity. Adv Anat Pathol 2014; 21: 394-410.

Address for correspondence

Abbas Agaimy, MD
Pathologisches Institut
Universitätsklinikum Erlangen
Krankenhausstraße 8-10
91054 Erlangen
Germany
tel. +49-9131-85-22288
fax: +49-9131-85-22613
e-mail: abbas.agaimy@uk-erlangen.de