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BRIEF REPORT

Primary pulmonary myxoid sarcoma with an unusual gene fusion between exon 7 of *EWSR1* and exon 5 of *CREB1*

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Abstract

Primary pulmonary mesenchymal tumors are rare, yet they compromise a variety of entities. A novel low-grade malignant neoplasm coined primary pulmonary myxoid sarcoma (PPMS) has been introduced in the WHO classification of lung tumors. Molecular analysis in PPMS revealed recurrent gene fusions between *EWSR1* and *CREB1*, a member of the cAMP response element binding protein (*CREB*) family. However, only 23 PPMS have been reported in the literature reflecting their exceedingly low incidence. Here, we describe the case of a 41-year-old female patient with a lung tumor obstructing the right main bronchus. Histologically, the tumor was composed of spindle-shaped and epithelioid cells exhibiting a reticular growth pattern within a prominent myxoid matrix. Solid areas were also observed. Molecular analysis by next-generation sequencing identified a fusion transcript with an unusual gene fusion involving exon 7 of *EWSR1* and exon 5 of *CREB1*. Together, the diagnosis PPMS was established.

Keywords EWSR1 · CREB1 · Primary pulmonary myxoid sarcoma · NGS

Introduction

Sarcomas of the lung are overall rare and mostly consist of metastases from extrapulmonary sarcomas. Primary sarcomas of the lung are far less common. They account for approximately 0.5% of lung tumors [13].

In 2011, a novel sarcoma entity coined primary pulmonary myxoid sarcoma (PPMS) has been introduced [11]. PPMS is an exceedingly rare disease with 23 cases reported so far in the literature [1, 3, 5, 7, 8, 10, 11, 14, 15]. PPMS typically arise in middle-aged patients and distribute equally between genders [1]. They are often located adjacent to the airways. Histologically, PPMS exhibit a characteristic lobulated growth pattern composed of spindle- to stellate-shaped and polygonal to epithelioid cells, which are typically arranged in delicate lacelike strands and cords within a prominent myxoid stroma. Areas with a patternless, solid architecture have also been described in some cases [11]. Interestingly, molecular studies in PPMS highlighted recurrent gene fusions between *EWSR1* and *CREB1* [11]. Due to these relatively distinct clinical, histologic and molecular features, the World Health Organization (WHO) classification of tumors of the lung (4th edition 2015) recognizes PPMS as a standalone entity [13].

However, PPMS share histologic features with other sarcoma subtypes, e.g. extraskeletal myxoid chondrosarcoma. Furthermore, *EWSR1-CREB1* gene fusions are not exclusively found in PPMS. Moreover, PPMS tested negative for *EWSR1* and/or *CREB1* rearrangements by FISH- or PCR-

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based methods have been reported [1, 10, 11]. Thus, diagnosing PPMS may be challenging for pathologists, in particular when the diagnosis is solely based on histologic criteria. The rarity of PPMS and the overlapping histologic and molecular features with other sarcoma entities warrant further characterization of this intriguing sarcoma entity.

Herein, we describe the case of a female patient with a lung tumor, where comprehensive histologic and molecular profiling established the diagnosis PPMS. To our best knowledge, this is the first description of a gene fusion between *EWSR1* exon 7 and *CREB1* exon 5 in PPMS or other tumors associated with *EWSR1-CREB1* gene fusions.

Case report

A 41-year-old female with a past medical history of a mumps-related meningitis at age of 7 and endometriosis was referred to our hospital for evaluation and treatment of a right central lung mass identified after progressive persisting retrosternal pain and dyspnea. Chest computed tomography (CT) revealed a large endobronchial mass involving the right main stem bronchus (Fig. 1a). Bronchoscopy showed a round shaped tumor within the distal part of the right main bronchus, which was almost completely obstructed by the tumor (Fig. 1b). A bronchoscopy-guided biopsy was performed. Frozen section showed a solid growing neoplasm with no overt features of malignancy.

On gross examination of the lobectomy specimen, there was a grayish, tan-yellow, partly glassy, well-circumscribed, nodular tumor measuring 5.1 × 4.3 × 3.2 cm with a gelatinous cut surface (Fig. 2a). The tumor was predominantly located in the bronchial wall (Fig. 2b, c). Microscopic examination showed a relatively well-circumscribed tumor composed of a population of spindle-shaped, focally ovoid tumor cells growing in cords and fascicles embedded in a myxoid stroma, which stained positive with Alcian blue (Fig. 2a–h). Rare atypical ovoid cells with increased nuclear size and some hyperchromasia were identified (Fig. 2i). The mitotic activity was brisk with up to 11 mitoses per 10 high-power fields, and the Ki-67 labeling index reached 15% (Fig. 2j). The neoplastic cells were strongly positive for vimentin and focally weakly positive for epithelial membrane antigen (Fig. 2k, l). An expanded panel of immunohistochemical markers revealed weak positivity for CD99 but negativity for CK18, BerEp4, smooth muscle actin, desmin, S100 protein, CD31, CD34, ERG, podoplanin, HMB-45, inhibin, calretinin, STAT6, PAX8 and bcl-2. Fluorescence in situ hybridization studies for the *EWSR1* gene locus showed a positive break-apart signal in the neoplastic cell population. Next-generation sequencing (NGS) using a targeted RNA fusion assay (Archer® FusionPlex® Sarcoma kit, sequenced on the IonTorrent platform) was applied to decipher the underlying gene fusion [6].

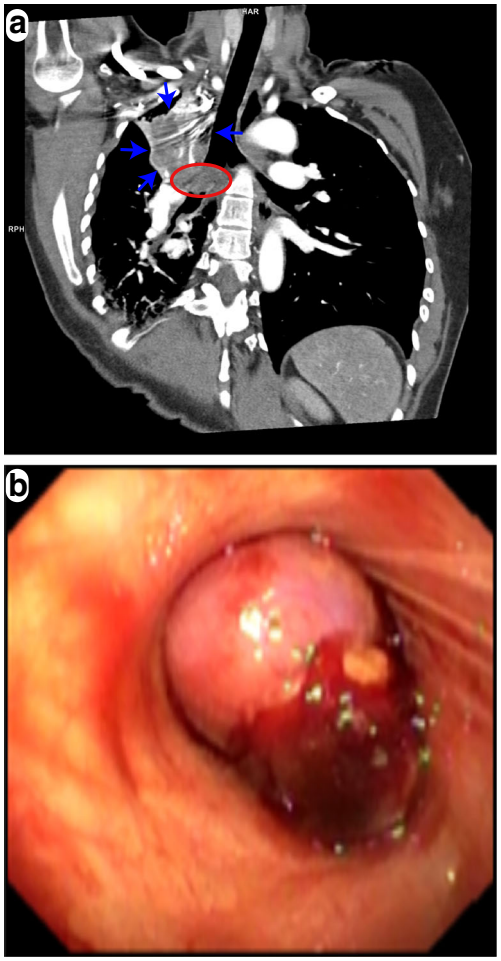

Fig. 1 **a** Chest computed tomography with coronal reconstruction reveals a lesion (red circle) surrounding the right main bronchus and atelectasis of the right upper lobe of the lung (blue arrows). **b** Bronchoscopy shows a nodular tumor almost completely occluding the right main bronchus

NGS revealed an in-frame fusion transcript involving *EWSR1* exon 7 (NM_005243) and *CREB1* exon 5 (NM_13442) in 43 reads (Fig. 3). In summary, the tumor was classified as PPMS. The patient has been disease free at the last follow-up visit 11 months after initial diagnosis.

Discussion

PPMS, first described in 1999 by Nicholson et al. and comprehensively defined by Thway et al. in 2011, is an exceedingly rare sarcoma entity [8, 11]. The diagnosis of PPMS is based on certain clinical (association with the bronchial system), histologic (lobulated growth and myxoid matrix) and molecular features (*EWSR1-CREB1* fusion). However, recent reports have highlighted variability in PPMS regarding these respective features [5, 9, 10]. We recommend the literature review of previously reported PPMS cases by Agaimy and colleagues [1].

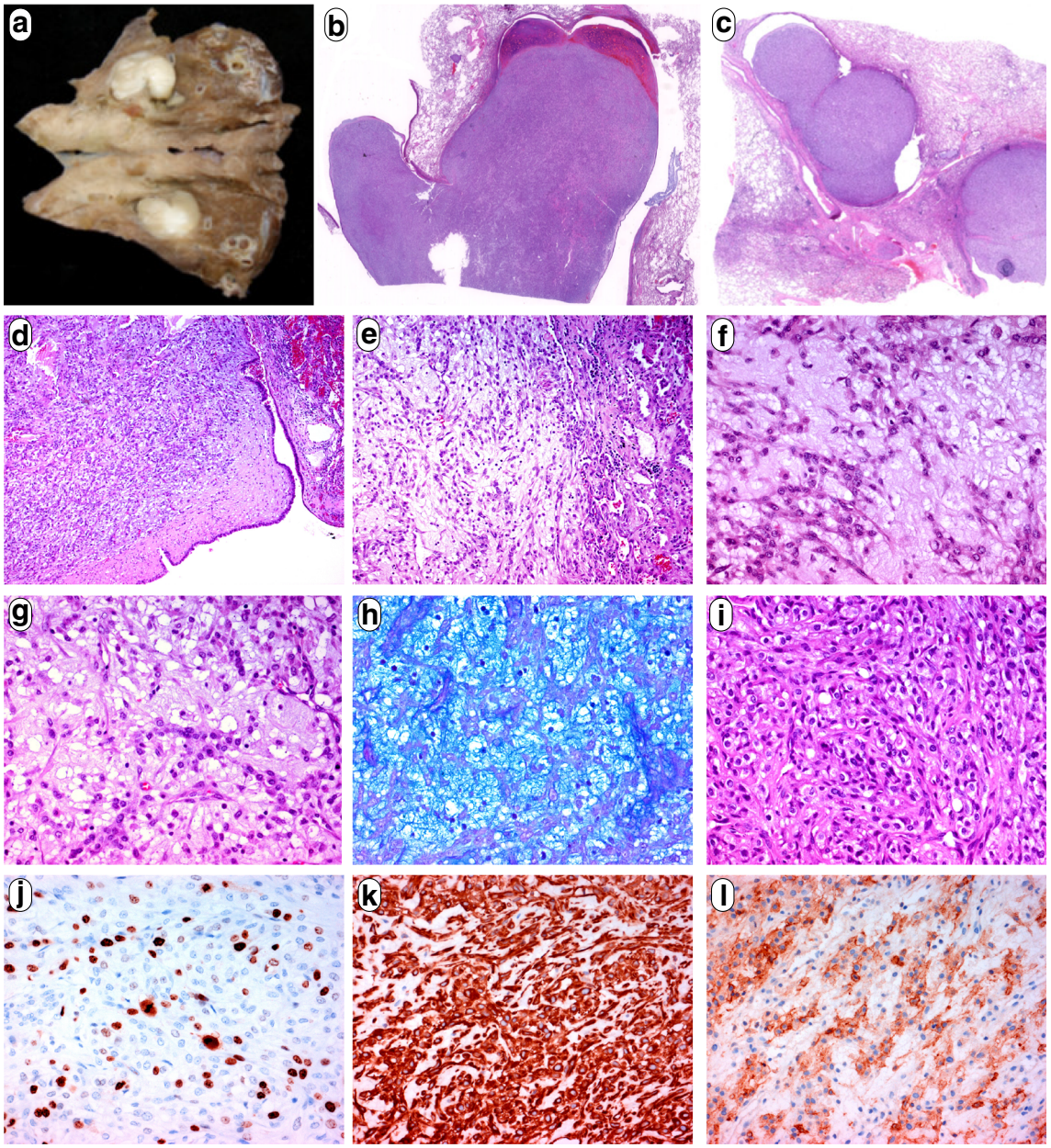

Fig. 2 **a** Postoperative gross specimen showing a yellow-tan-colored mass. Microscopic examination revealed a tumor located in close proximity to the bronchial system (**b** and **c**) that is composed of spindled to ovoid cells (**d**–**g**) embedded in a myxoid, Alcian-Blue positive (**h**) tumor matrix. Cellular areas with a solid growth were present (**i**). The proliferation index Ki-67 focally reached 15% (**j**). Tumor cells strongly expressed Vimentin (**k**) and diffusely expressed epithelial membrane antigen (**l**)

The molecular hallmark of PPMS is an interchromosomal gene fusion between the exon 7 of *EWSR1*, located on chromosome 22, with either exon 7 or exon 8 of *CREB1*, located on chromosome 2. These *EWSR1-CREB1* fusion transcripts have also been described in angiomatoid fibrous histiocytoma (AFH), in clear cell sarcoma (CCS)-like tumor of the gastrointestinal tract and in a subset of CCS of soft tissue. To our best knowledge we here report for the first time a gene fusion between exon 7 of *EWSR1* with exon 5 of *CREB1* leading to a novel *EWSR1-CREB1* fusion transcript. The chimeric fusion protein contains the transactivation domain (TAD) of EWSR1, the phosphorylated kinase-inducible-domain (pKID) of CREB1 and the basic leucin zipper domain (bZIP) mediating DNA binding and dimerization. The pKID domain is precluded from the hitherto known EWSR1-CREB1 fusions detected in the aforementioned tumor subtypes. The biological relevance of the pKID domain in the here described novel fusion protein remains to be determined.

It is worth noting that NGS-based fusion detection methods, depending on the applied assay, have the advantage to unveil previously unknown chimeric events. Since uncommon fusion transcripts, like in our case, probably

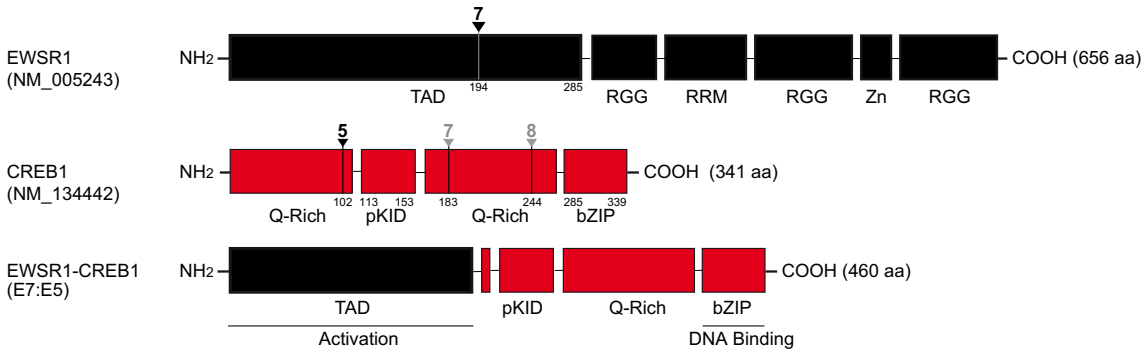

Fig. 3 Schematic figure of the novel EWSR1-CREB1 fusion protein with annotated functional domains. The breakpoints of the present case are marked with black triangles, the common breakpoints in CREB1 (exon 7 + 8) are marked with gray triangles. TAD transactivation domain, RGG glycine-arginine-rich region, RRM RNA-recognition motif, Zn zinc finger motif, Q-Rich glutamine-rich domain, pKID phosphorylated kinase-inducible-domain, bZIP basic leucin zipper domain, aa amino acids

would have been missed by conventional PCR-based detection methods, it seems reasonable to perform NGS (i.e. the Archer fusion assay) in cases possibly belonging to PPMS. Notably, only 11 of 23 cases classified under the category PPMS were positive for the *EWSR1-CREB1* fusion [1, 3, 5, 7, 8, 10, 11, 14, 15]. Further two cases had a break in *EWSR1* and one case had a break in *CREB1*. Five cases were negative in FISH studies for *EWSR1* and *CREB1* and therefore are believed to be devoid of the *EWSR1-CREB1* fusion. The remaining four cases were unsuitable for molecular analysis. It therefore remains open whether the novel fusion transcript described here might be present in so far molecularly unresolved PPMS. Anyway, the molecular mechanisms of these cases are still to be deciphered.

The detection of the *EWSR1-CREB1* gene fusion supports the diagnosis of PPMS, especially when the differential diagnosis extraskeletal myxoid chondrosarcoma, which exhibits a hyaluronidase-resistant Alcian blue-positive matrix in contrast to PPMS and carries an entity defining rearrangement in *NR4A3*, is under consideration [14]. Differential diagnoses of PPMS mostly represent pulmonary sarcoma metastasis, which typically follow an aggressive clinical course. The clinical behavior of PPMS seems more favorable with longtime follow-up reports of disease-free patients after surgery. However, aggressive biological behavior with metastasis has been reported in four PPMS cases, although three of those cases remained molecularly unresolved [1, 3, 11]. Histologic features of malignancy, e.g. necrosis or pleomorphism, did not correlate with more aggressive biologic behavior in these rare PPMS cases. Based on this background, the current WHO classification categorizes PPMS as low-grade malignancy [13].

Notably, *EWSR1-CREB1* fusions have also been described in AFH, which is another relevant differential diagnosis of PPMS [10]. Pulmonary AFH at unusual locations, e.g. the endobronchial system, have recently been described [2, 12]. Correctly distinguishing PPMS from pulmonary AFH may be critical due to a slightly increased risk for malignant behavior in PPMS [11]. However, both PPMS and AFH lack a specific immunophenotype with variable immunoreactivity for epithelial membrane antigen and CD99 in both entities. PPMS consistently lack muscle marker expression like smooth muscle actin or desmin, which have been found positive in at least 50% of AFHs. Muscle markers were consistently negative in our case. Overall, the relation between PPMS, AFH and intracranial myxoid mesenchymal tumors, a recently described subtype that shares histologic and molecular features with PPMS, remains unclear [4]. Another potential mimetic of PPMS is pulmonary myoepithelial tumors, which often have rearrangements in *EWSR1* or, more rarely, in *FUS*, both of which are fused to different fusion partners but do not fuse with *CREB1* in myoepithelial tumors [13]. Furthermore, myoepithelial tumors typically present with cytokeratin and S100 protein expression, both of which are commonly negative in PPMS [8, 11].

In conclusion, we describe the case of a female patient with PPMS, which carried a novel fusion transcript involving exon 7 of *EWSR1* and exon 5 of *CREB1*. Our case highlights the need for further investigations for a comprehensive understanding of this intriguing sarcoma group.

Author's contributions G.M. conceived the project. C.K. wrote the manuscript. L.T. coordinated data generation. A.S. and O.N. analyzed molecular data. C.P.H., R.E. and H.W. provided tumor samples and metadata. All authors read and approved the final manuscript.

Compliance with ethical standards

Informed consent Written informed consent for publication is obtained from the patient.

Conflict of interest The authors declare that they have no conflict of interest.

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