A case of epithelioid fibrous histiocytoma in the lung harboring EML4-ALK Fusion

Ting Sun
First Affiliated Hospital Zhejiang University
Bo Wang
First Affiliated Hospital Zhejiang University
Ke Sun (1200035@zju.edu.cn)
First Affiliated Hospital Zhejiang University

Case Report

Keywords: Epithelioid fibrous histiocytoma (EFH), Epithelioid cell histiocytoma (ECH), Anaplastic lymphoma kinase (ALK), EML4-ALK, RNAseq

Posted Date: April 20th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1521986/v1

Abstract

Background: Epithelioid fibrous histiocytoma (EFH) is a rare benign fibrohistiocytic tumor, has traditionally been considered a morphologic variant of cutaneous fibrous histiocytoma with prominent epit helioidcytomorphology. Indeed, besides characteristic epithelioid cytomorphology, several protein-expression and molecular genetic differences suggest that EFH may differ biologically from other variants. Most previously reported EFH caused by SQSTM1-ALK or VCL-ALK fusion gene. Herein, we report an EFH case of the lung harboring a rare EML4-ALK rearrangement other than in reported EFH.

Methods: Immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) test were operated on formalin-fixation and paraffin-embedding (FFPE) tissues to help for pathological diagnosis. RNAseq was conducted to find out the partner gene of ALK.

Results: Immunostaining showed that ALK staining of the lesional tumor cells were diffuse positive in a cytoplasmic and cell membrane staining and were also positive for histiocytic markers CD68 and CD163. While SMA, CK(pan), Desmin and CD4 were negative for staining. RNAseq further revealed that the tumor harbored an EML4-ALK fusion gene. FISH analysis demostrated that ALK gene breaked apart and fused with EML4 gene.

Conclusion: In conclusion, this is the first EFH localized in the lung. And the oncogenic driver of the EFH was rare EML4-ALK fusion gene other than in reported EFH. In. This enriches the spectrum of EFH and enlarges the horizon for the study of EFH. ALK expression is not seen in other variants of fibrous histiocytoma, thus, IHC and FISH tests of ALK provide a useful diagnostic tool to distinguish epithelioid fibrous histiocytoma from most histologic mimics in our case. We recommend that every fibrous histiocytoma, no matter the location of lesions, should be tested for ALK expression and gene rearrangement because of the helpness of pathological diagnosis and potential efficacy of ALK inhibitor therapy in case of recurrence or metastasis.

Full Text

Epithelioid fibrous histiocytoma (EFH), which is also known as Epithelioid cell histiocytoma (ECH), is a rare benign fibrohistiocytic tumor, the main diagnostic criteria is in which over 50% of the lesional cells presenting as epithelioid morphology previously[1,2]. Reported EFH were all skin-associated, EFH in the lung has not been reported.

Clinically, epithelioid fibrous histiocytoma most commonly arose on the shin of the extremities of young to middle-aged adults with a flesh-colored nodule appearance[1,2,3]. According to previous reports, epithelioid fibrous histiocytoma is usually exophytic and well circumscribed with an epidermal collarette and in the dermis relatively uniform epithelioid proliferate to polygonal cells with a amount of eosinophilic or amphophilic cytoplasm, vesicular nuclei with small nucleoli. The tumor cells are frequently binucleate or or trinucleate, multinucleate giant cells are less common compared with which in

regular fibrous histiocytoma. A remarkable feature in the stroma of epithelioid fibrous histiocytoma is their usually richly vascular, including small thin-walled vessels and larger thick-walled vessels[1,4].

A 47-year-old female with a nodule on upper lobe of the right lung, which had been present for 3 years, was referred to our hospital for surgical operation. The patient stated that the nodule has grown slowly over the past few years and without any significant clinical symptoms or medical history. The preoperative imaging revealed findings round-like solid nodule of 8-cm at the right-upper-lobe lung, with clear boundaries. The lesion was removed via wedge resection. The surgical specimen which was about 8×3×1.5cm^3 then was transported to the pathology department for pathological anatomy, inside which a 1.2-cm tumor with gray– yellow to gray–red in appearance, surrounding with partially fragmented tissues.

Histologically, the tumor was separated with a relatively smooth contour from ambient alveolar tissue with a definite boundary and was composed of compact fusiform cell at low magnification on fresh frozen slide (Fig. 1A). At high power, the tumor was composed of oval or short spindled cells with eosinophilic cytoplasm and ovoid to round nuclei with small distinct nucleoli, by a little number of lymphocytes and plasma cells infiltrating. The stroma of lesion was richly consisted of small thin-walled vessels. Tumor cells grew around the blood vessels and showed a whorled growth pattern (Fig. 1B).

Unlike the reported epithelioid fibrous histiocytoma located in skin, binucleate and trinuclear cells were not seen. Mitotic figures were found rarely and multinucleate giant cells are less common than in regular fibrous histiocytoma (Fig. 1C).

The next, a series of immunohistochemical staining were operated on tissue sections, tumor cells showed positive for anaplastic lymphoma kinase (ALK) (Fig. 1G) CD68 (Fig. 1E), CD163 (Fig. 1F) and focal tumor cells were immunoreactivity for EMA, the Ki-67 labeling index was approximately 10% in fibrous histiocytoma cells (Fig. 1L). Only a minority of lesional cells showed reactivity for S-100(Fig. 1H), immunohistochemical stains were negative for smooth muscle actin (SMA) (Fig. 1I), CK(pan) (Fig. 1J), Desmin (Fig. 1K), STAT6, Melan A, SOX10, CD1α, CD 4, CD21, CD23, CD34(Fig. 1D), SSTR2, and GLUT-1 in the lesional cells.

Tumor cells showed diffusely positive for ALK (clone 5A4, Leica Biosystems), which was moderate to strong in intensity (Fig. 1G). FISH analysis was performed to confirmed the presence of ALK rearrangement in the tumor using the ALK dual-color break apart rearrangement probe (Vysis/Abbott Molecular Diagnostics). About 40% of tumor cells demonstrated rearrangement singnal patterns, including 12% of tumor cells having one pair of split 3’ (red) and 5’ (green) signals besides a pair of fused-yellow signal and 28% having one or two pairs of 3’ and 5’ fused signals (yellow) plus one isolated 3’ signal (Fig. 2A).

To determine gene fusion partner of ALK and discover more molecular information of the tumor, next-generation sequencing (NGS), including 425 lung cancer related genes (Supplemental Table 1) was performed. As a result, EML4-ALK fusion created by EML4 exon 2 and ALK exon 20 (Fig. 2C) has been

recognized. EML4-ALK dual-color fusion rearrangement probe (Vysis/Abbott Molecular Diagnostics) were performed, showing positive result with fusion of the green (EML4) and red (ALK) signals (Fig. 2B).

Diagnosing a neoplasm as rare types of tumors requires the exclusion of associated commonly seen tumors. In our case, benign fibrous histiocytoma (dermatofibroma), histiocytic sarcoma, inflammatory myofibroblastic tumor (IMT) and ALK-positive histiocytosis were included in the differential diagnoses. ALK protein overexpression and ALK gene rearrangement previously confirmed this neoplasm to be biologically distinct from conventional fibrous histiocytoma and other variants[4,5,6]. ALK rearrangement has not been described in benign fibrous histiocytoma or other variants[4,7]. In addition to the negative staining for SMA and Desmin, immunophenotype of the neoplasm cells tended to be a histiocytic derivation other than mesenchymal tumor, including histiocytic sarcoma and IMT. Besides, the bland cytological features did not suggest a histiocytic sarcoma. As for ALK-positive histiocytosis, the most common gene fusion partners of ALK was KIF5B[8]. Histiocytosis with EML4-ALK fusion have been reported only in two cases recently reported separately by ROSS et al. and Bai et al. The fomer mentioned a case designated “Soft tissue histiocytosis (NOS)” in the supplemental online Tables S5[9]. Bai et al. reported ALK-positive histiocytosis with a novel EML4-ALK rearrangement in the lung of a Chinese woman[10]. Our case differs from ALK positive histiocytes in that Touton giant cells could be seen, and the lesional histiocytes were positive for CD4 in all of the reported cases. Notably, cytoplasmic ALK expression is observed with some cells of ALK-positive histiocytes appeaing as a distinct globular accentuation of staining[11].

As for EFH, the most common fusion products were SQSTM1-ALK and VCL-ALK, which account for greater than 70% of cases[7]. EFH with EML4 as ALK fusion partner has been reported only in a case reported by Dmitry et al[3]. Our case lacked the epidermal collarette surrounding and binucleate or trinucleate nuclear was not seen, which were indistinguishable from those seen in other cases of EFH. Although EFH of the lung have never been reported, here the present case was compatible with EFH based on the histological, immunohistochemical and cytogenetic findings. Owing to rarity and benign biologic behavior of EFH, few cases have employed rigorous molecular characterization and immunohistochemistry of ALK after resection. As additional diagnosis of EFH by means of ALK protein and molecular detection, morphologic and cytogenetic characteristics of EFH would be fully revealed in the future.

To date, 15 months later after resection, the patient has not received any treatment and did not show any clinical complications or recurrence. In addition, analysis of NGS revealed that RNA splicing factors splicing factor 3B subunit 1(SF3B1) mutation, which is subject to recurrent missense mutations in myelodysplastic syndrome (MDS) and among some pigmented tumors[12], suggesting an orally available modulator of the SF3b complex, H3B-8800[13,14], as a potential therapeutic regimen in case of recurrence or metastasis besides ALK- inhibitors.

Abbreviations

EFH: Epithelioid fibrous histiocytoma; ECH: Epithelioid cell histiocytoma; ALK: Anaplastic lymphoma kinase; EML4: Echinoderm microtubule-associated protein-like 4; FISH: Fluorescence in situ hybridization; IMT: Inflammatory myofibroblastic tumor; NGS: next-generation sequencing; SF3B1: splicing factor 3B subunit 1.

Declarations

Acknowledgements

We thank the patient for her kind cooperation, and we give our respect to her sincerely. Thank deputy director Ke Sun for noticing the case of rare disease in her pathological diagnosis work.

Authors' contributions

Ting Sun wrote the manuscript. Ting Sun and Bo Wang performed in situ hybridization. Ke Sun contributed to the pathologic diagnosis and provided useful comments. All authors have read and approve the final manuscript.

Funding

No Funding.

Availability of data and materials

The datasets analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

This study was approved by the Clinical Research Ethics Committee of the First Affiliated Hospital, Zhejiang University School of Medicine. The patient provided informed consent.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

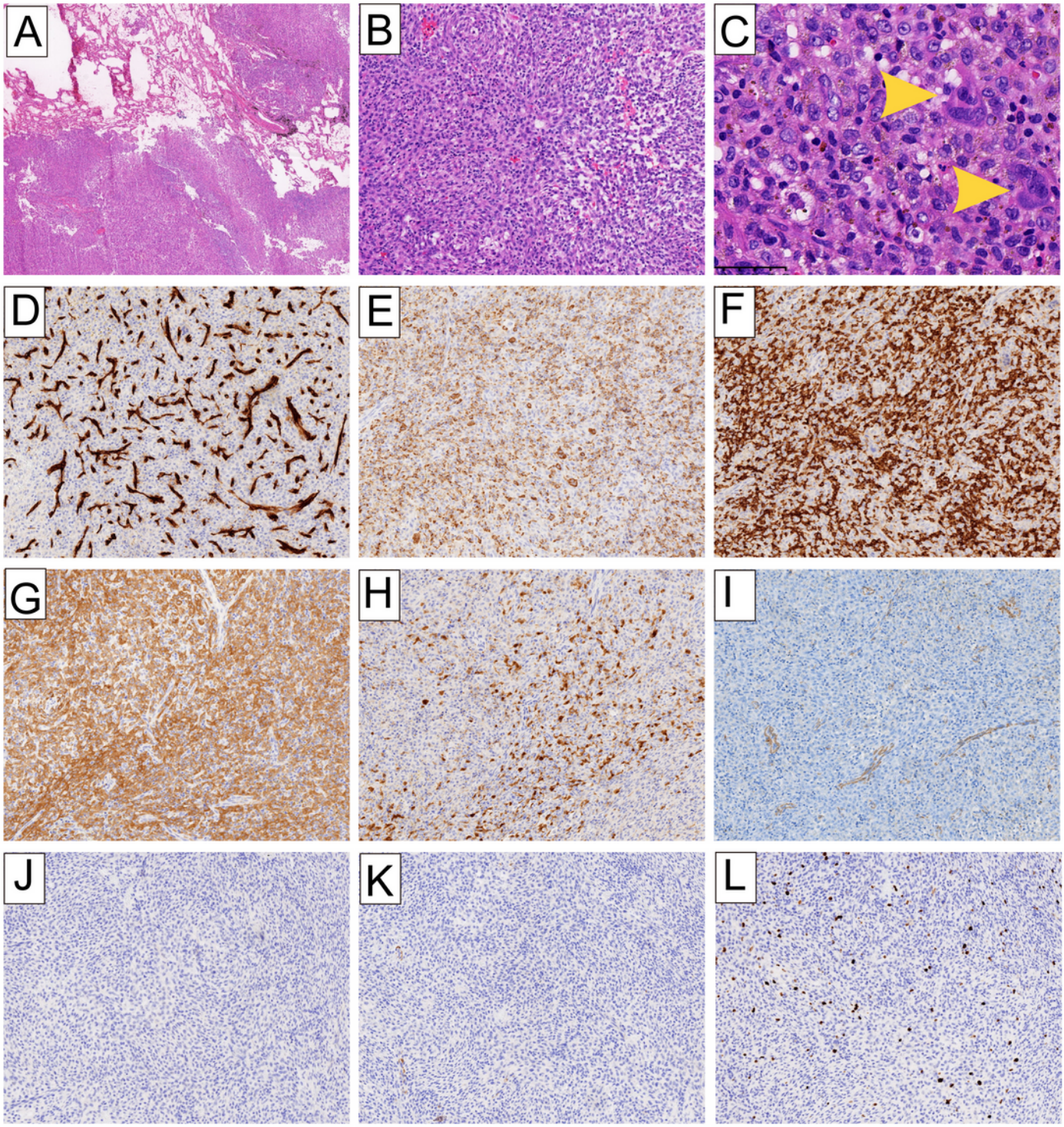

Figure 1

Morphologic, immunophenotypic features of epithelioid fibrous histiocytoma. (A) Low-power view shows EFH was circumscribed by ambient alveolar tissue with a relatively definite boundary (fresh frozen slide H&E, 20×). (B) The lesion consists of a compact growth of monotonous epithelioid and histiocytoid cells, with a whorled growth pattern around the vessels (100×). (C) Multinucleate giant cells could be seen occasionally in the lesion (arrowheads) (400×). (D) CD34 highlights the vascular endothelial cells, while

lesional cells are negative. The tumor shows positive for CD68 (E) and CD163 (F). ALK immunohistochemical staining shows moderate to strong reactivity diffusely (G). The lesional cells show partially positive for S-100 (H), and show negative for SMA (I), CK (J) and Desmin (K). Ki-67 labeling index was approximately 10% (L) (D-L, 100×).

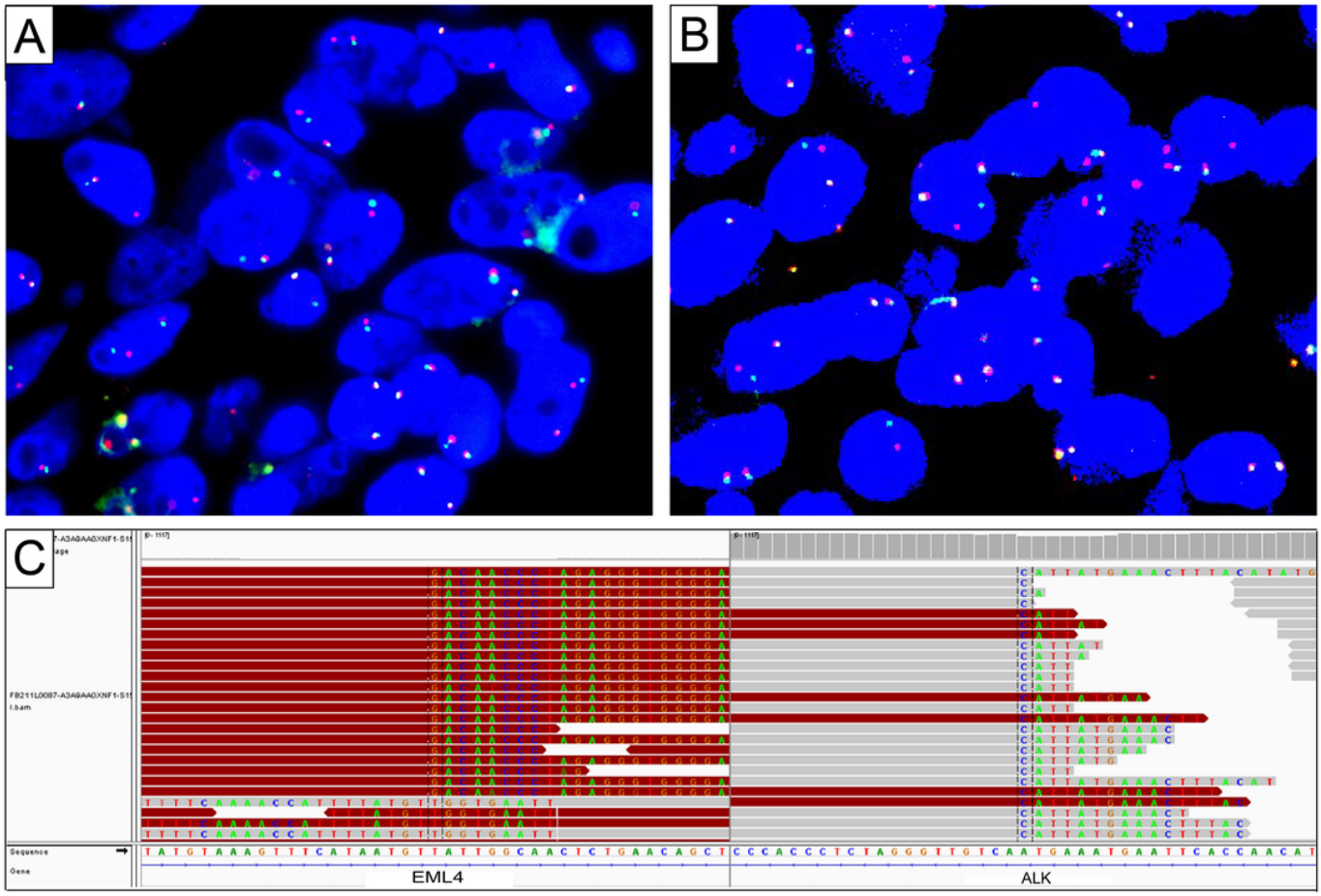

Figure 2

Molecular finding of EFH. ALK break-apart FISH assay reveals positive tumor cells containing one separate orange signal and one separate green signal or a separate red signal (A). EML4-ALK fusion FISH assay shows a positive pattern with fusion of the green (EML4) and red (ALK) signals in tumor cell nuclei (B). NGS reveals the EML4-ALK fusion joining EML4 exon 2 and ALK exon 20 (C).

Supplementary Files

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- SupplementalTable1.doc