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Epithelioid fibrous histiocytoma: molecular characterization of *ALK* fusion partners in 23 cases

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Epithelioid fibrous histiocytoma is a rare and distinctive cutaneous neoplasm. Most cases harbor *ALK* rearrangement and show ALK overexpression, which distinguish this neoplasm from conventional cutaneous fibrous histiocytoma and variants. *SQSTM1* and *VCL* have previously been shown to partner with *ALK* in one case each of epithelioid fibrous histiocytoma. The purpose of this study was to examine a large cohort of epithelioid fibrous histiocytomas by next-generation sequencing to characterize the nature and prevalence of *ALK* fusion partners. A retrospective archival review was performed to identify cases of epithelioid fibrous histiocytoma (2012–2016). Immunohistochemistry was performed to confirm ALK expression. Targeted next-generation sequencing was applied on RNA extracted from formalin-fixed paraffin-embedded tissue to identify the fusion partners. Twenty-three cases fulfilled inclusion criteria. The mean patient age was 39 years (range, 8–74), there was no sex predilection, and >75% of cases involved the lower extremities. The most common gene fusions were *SQSTM1-ALK* (*N*=12; 52%) and *VCL-ALK* (*N*=7; 30%); the other four cases harbored novel fusion partners (*DCTN1*, *ETV6*, *PPFIBP1*, and *SPECC1L*). The pattern of ALK immunoreactivity was usually granular cytoplasmic (*N*=12; 52%) or granular cytoplasmic and nuclear (*N*=10; 43%); the case containing an *ETV6* fusion partner showed nuclear staining alone. There was no apparent relationship between tumor morphology and the *ALK* fusion partner. In summary, *SQSTM1* and *VCL* are the most common *ALK* fusion partners in epithelioid fibrous histiocytoma; *DCTN1*, *ETV6*, *PPFIBP1*, and *SPECC1L* represent rare fusion partners. The proteins encoded by these genes play diverse roles in scaffolding, cell adhesion, signaling, and transcription (among others) without clear commonalities. These findings expand the oncogenic promiscuity of many of these *ALK* fusion genes, which drive neoplasia in tumors of diverse lineages with widely varied clinical behavior. This is the first documented account of *ETV6-ALK* and *SPECC1L-ALK* translocations in neoplasms.
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Epithelioid fibrous histiocytoma, also known as epithelioid cell histiocytoma, is a rare and enigmatic cutaneous neoplasm.[1,2] Clinically, lesions often resemble pyogenic granuloma.[1,2] Tumors tend to be small and solitary; they occur across a broad age-range, predominating between the third and seventh decades; and the extremities represent the most frequent sites of involvement.[1,2] Morphologically, epithelioid fibrous histiocytoma is frequently exophytic, with an epidermal collarette.[2] Centered in the dermis, tumors are comprised of plump epithelioid cells occasionally with a spindle cell component.[1,2] The nuclei are round-ovoid, with minimal atypia, vesicular chromatin and scattered bi- and tri-nucleation; mitotic activity is typically scant.

Originally considered a variant of fibrous histiocytoma,[1] subsequent studies emphasizing morphologic and immunohistochemical differences

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challenged this notion.[3] The discovery that epithelioid fibrous histiocytoma harbors canonical *ALK* translocations in a majority of tumors ultimately confirmed a biologic distinction.[4] Next-generation sequencing of two cases recently identified *VCL* and *SQSTM1* as distinct *ALK* fusion partners.[5] *VCL-ALK* rearrangements have previously been reported in a distinctive group of pediatric renal cell carcinomas,[6] while *SQSTM1-ALK* has been identified in ALK-positive large B-cell lymphoma.[7] A diverse array of neoplasms has been reported to contain *ALK* fusion products, including inflammatory myofibroblastic tumor,[8] lymphoma (eg, ALK-positive anaplastic large cell lymphoma, and ALK-positive large B-cell lymphoma),[9–11] lung adenocarcinoma,[12] renal cell carcinoma,[13] thyroid carcinoma,[14] and Spitz neoplasms.[15] *ALK* has the potential to fuse with numerous partner genes, and disparate tumor types (with widely variable clinical behavior) may possess the exact same fusion partner. In view of the tremendous potential for overlap among *ALK* fusion partners, the purpose of this study was to interrogate a large cohort of cases of epithelioid fibrous histiocytoma to characterize the nature and prevalence of *ALK* products in this neoplasm.

Materials and methods

Following institutional Research Ethics Board approval, we performed a retrospective review of our archives for cases of epithelioid fibrous histiocytoma diagnosed between 2012 and 2016. Each case was re-reviewed to confirm the diagnosis. Immunohistochemistry for ALK was performed as previously reported to confirm ALK expression.[4] The intent of this study was to characterize *ALK* partner genes; therefore, cases of epithelioid fibrous histiocytoma lacking ALK immunoexpression were not included. The pattern (i.e., subcellular localization) of ALK staining was recorded.

Each case was interrogated by a targeted next-generation sequencing fusion panel. Briefly, for each case 4–5 unstained slides cut from formalin-fixed paraffin-embedded tissue (4 microns) were scraped into Eppendorf tubes. RNA was extracted using the ExpressArt FFPE Clear RNA Ready kit (Amsbio, Cambridge, MA). RNA-seq libraries were prepared using 20–100 ng total RNA with the TruSight RNA Fusion Panel (Illumina, San Diego, CA), an enrichment-based assay that targets 507 known fusion-associated genes. Each sample was sequenced with 76 base-pair paired-end reads on an Illumina MiSeq at 8 samples per flow cell (~3 million reads per sample). The results were analyzed using the STAR aligner and Manta fusion caller as well as the JAFFA fusion caller utilizing BOWTIE2 aligner.[16,17]

Each fusion product was subsequently independently confirmed by reverse transcriptase polymerase chain reaction (RT-PCR). Briefly, RT-PCR was performed on the extracted RNA using random primers and Moloney Murine Leukemia Virus (M-MLV) reverse transcriptase according to manufacturer's instructions (Invitrogen, Burlington, Ontario, Canada). Polymerase chain reaction (PCR) was subsequently performed using primers specific for the fusion breakpoints (Supplementary Table 1). Forty cycles of PCR were performed at 94 °C for 30 s, 55 °C for one minute, and 72 °C for one minute using HotStarTaq DNA Polymerase (Qiagen, Toronto,

Table 1 Summary of key demographic, immunohistochemical, and molecular findings in epithelioid fibrous histiocytoma cohort

Patient	*Age (years)*	*Sex*	*Location*	*ALK Pattern*	*Fusion product*
1	74	M	Shoulder	granular C	*SQSTM1-ALK*
2	31	M	Thigh	granular C	*SQSTM1-ALK*
3	51	M	Thigh	granular C + N	*SQSTM1-ALK*
4	51	M	Lower back	granular C + N	*SQSTM1-ALK*
5	32	F	Thigh	granular C + N	*SQSTM1-ALK*
6	42	F	Lower leg	granular C	*SQSTM1-ALK*
7	52	M	Foot	granular C + N	*SQSTM1-ALK*
8	39	M	Thigh	granular C + N	*SQSTM1-ALK*
9	34	F	Buttock	granular C + N	*SQSTM1-ALK*
10	45	M	Lower leg	granular C + N	*SQSTM1-ALK*
11	41	F	Arm	granular C + N	*SQSTM1-ALK*
12	56	M	Thigh	granular C + N	*SQSTM1-ALK*
13	28	F	Shin	granular C	*VCL-ALK*
14	30	F	Foot	granular C	*VCL-ALK*
15	37	F	Thigh	granular C	*VCL-ALK*
16	23	F	Abdomen	granular C	*VCL-ALK*
17	38	M	Thigh	granular C	*VCL-ALK*
18	31	F	Lower leg	granular C	*VCL-ALK*
19	28	M	Leg, NOS	granular C + N	*VCL-ALK*
20	8	M	Shoulder	granular C	*DCTN1-ALK*
21	30	F	Leg, NOS	N	*ETV6-ALK*
22	21	F	Lower leg	granular C	*PPFIBP1-ALK*
23	66	F	Knee	granular C	*SPECC1L-ALK*

Abbreviations: C, cytoplasmic; F, female; M, male; N, nuclear; NOS, not otherwise specified.

Ontario, Canada). PCR products were sequenced on the Applied Biosystems 3730 DNA Analyzer using the Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Burlington, Ontario, Canada).

Results

A total of 24 cases fulfilled the inclusion criteria. Adequate RNA was obtained from all but one case, leaving 23 cases for molecular evaluation. Clinically, the mean patient age was 39 years (range: 8–74 years), and there was a similar number of females and males. The majority of tumors arose in the lower extremities (78%), with the thigh (30%) representing the most common site of involvement (Table 1).

Next-generation sequencing confirmed the presence of *ALK* rearrangement in all tumors (Table 1). In 12 cases (52%), *SQSTM1* was the *ALK* fusion partner; 7 cases (30%) had *VCL-ALK* as the fusion product. Four cases contained novel fusion partners that have not been reported previously in epithelioid fibrous histiocytoma: *DCTN1*, *ETV6*, *PPFIBP1*, and *SPECC1L* (Figure 1). All fusion breakpoints involved exon 20 of the *ALK* gene (NCBI Reference Sequence: NM_004304.4). The fusion partner exons were: *SQSTM1* exon 5 (NM_003900.4), *VCL* exon 16 (NM_014000.2), *DCTN1* exon 21 (NM_023019.3), *ETV6* exon 5 (NM_001987.4), *PPFIBP1* exon 9 (NM_003622.3), and *SPECC1L* exon 9 (NM_015330.4). Each of the fusion products was independently confirmed by RT-PCR followed by Sanger sequencing (Figure 2).

The patterns of ALK expression by immunohistochemistry were as follows: granular cytoplasmic staining ($N=12$; 52%), granular cytoplasmic staining combined with nuclear staining ($N=10$; 43%), and nuclear staining alone ($N=1$; 4%). The *SQSTM1-ALK* fusions tended to be more often associated with granular cytoplasmic and nuclear ALK staining (75%), whereas *VCL-ALK* fusions generally had granular cytoplasmic immunoreactivity without nuclear staining (86%). Of the cases with novel fusion partners, the tumor with *ETV6-ALK* fusion showed nuclear staining alone, while the remaining cases showing granular cytoplasmic staining patterns.

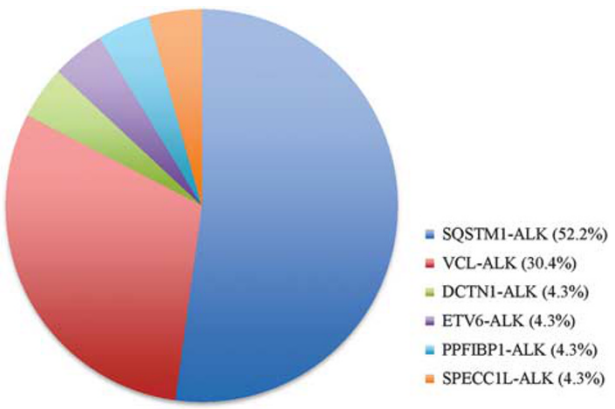

Figure 1 Pie chart demonstrating the relative percentage of each of the *ALK* fusion products within the study cohort.

There was no apparent association between morphology and the nature of the underlying *ALK* fusion partner. Tumors with *SQSTM1-ALK* and *VCL-ALK* fusions were morphologically and immunohistochemically similar to previous reports (Figure 3).[1,2,4] Each of the four cases with novel fusion partners were polypoid; cases 20, 22 and 23 contained an epidermal collarette. Case 20 (with *DCTN1-ALK*) was ulcerated with mixed epithelioid and spindle cell morphology (Figure 4). Immunohistochemistry for ALK showed diffuse granular cytoplasmic staining. Case 21 (with *ETV6-ALK*) was separated from the epidermis by a Grenz zone, and had a somewhat irregular peripheral tumor margin; this was the only case to show a strictly nuclear pattern of ALK staining (Figure 4f). Case 22 (with *PPFIBP1-ALK*) and Case 23 (with *SPECC1L-ALK*) both had granular cytoplasmic ALK expression (Figure 5). Morphologically, the latter tumor was somewhat less cellular than the other tumors, with collagenous stroma interspersed among the tumor cells (Figures 5d and e).

Discussion

Epithelioid fibrous histiocytoma is a benign cutaneous neoplasm with a characteristic morphology and immunophenotype.[1–3] The presence of *ALK* rearrangement, and concomitant ALK protein overexpression, previously confirmed this neoplasm to be biologically distinct from conventional fibrous histiocytoma and variants;[4,5] however, owing to its rarity, few cases have undergone rigorous molecular characterization. The goal of this study was to examine the nature and breadth of *ALK* fusion products in epithelioid fibrous histiocytoma. We show that *SQSTM1* and *VCL* are the most prevalent *ALK* fusion partners. In addition, we report four novel *ALK* partners in this rare neoplasm: *DCTN1*, *ETV6*, *PPFIBP1*, and *SPECC1L*. Moreover, this is the first documented report of neoplasms containing disease-defining *ETV6-ALK* and *SPECC1L-ALK* fusion genes.

ALK (anaplastic lymphoma kinase; CD246) encodes a transmembrane receptor tyrosine kinase within the insulin receptor superfamily. Alterations in this gene–including translocations, mutations, copy number changes and dysregulated expression–have been implicated in numerous tumor types (reviewed in detail elsewhere [18–20]). A heterogeneous group of neoplasms–spanning mesenchymal, epithelial, hematolymphoid and melanocytic lines of differentiation–have been reported to contain translocations involving *ALK*. Despite seemingly disparate ontogenies, many *ALK* fusion products are shared among different tumors. For example, *EML4-ALK* translocations occur in lung adenocarcinoma,[21]

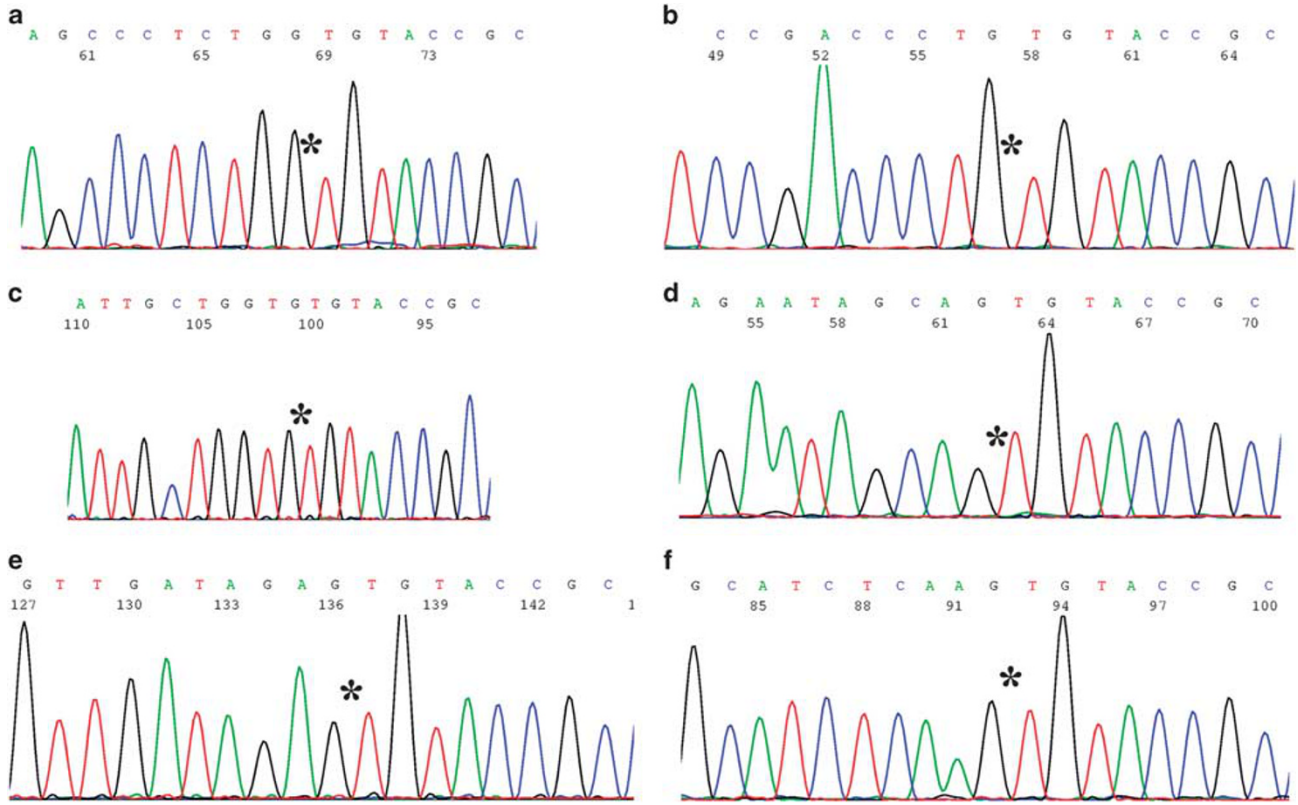

Figure 2 Representative images of Sanger sequencing for each of the *ALK* fusion partners identified in epithelioid fibrous histiocytoma: (**a**) *SQSTM1-ALK*, (**b**) *VCL-ALK*, (**c**) *DCTN1-ALK*, (**d**) *ETV6-ALK*, (**e**) *PPFIBP1-ALK*, and (**f**) *SPECC1L-ALK*. Asterisk denotes breakpoint.

inflammatory myofibroblastic tumor,[22] ALK-positive large B-cell lymphoma[23] and renal cell carcinoma.[24,25] There is tremendous variability among potential *ALK* fusion partners; further plasticity arises from the finding that other tyrosine kinase receptor genes may substitute for *ALK*, thereby yielding a potentially staggering number of fusion gene permutations. For example, in inflammatory myofibroblastic tumor *TFG* has been reported to partner with both *ALK*[26] and *ROS1*.[27]

This study identified *SQSTM1-ALK* as the most common fusion product in epithelioid fibrous histiocytoma. *SQSTM1* encodes sequestosome 1 (p62), a scaffolding protein with multiple roles including NFκB signaling and ubiquitin-mediated autophagy.[28] Mutations of this gene have been implicated in multiple diseases, including Paget disease of bone and frontotemporal lobar degeneration. In this study, the *SQSTM1-ALK* fusion was present in slightly over half of cases. In addition to epithelioid fibrous histiocytoma,[5] *SQSTM1-ALK* fusions have been reported previously in ALK-positive large B-cell lymphoma[7] and lung adenocarcinoma,[29] as well as inflammatory myofibroblastic tumor (Dickson, unpublished observation). Non-*ALK* fusion partners of *SQSTM1* have also been reported, including *NTRK3* in papillary thyroid carcinoma[30] and *FGFR1* in acute myelomonocytic leukemia.[31]

Slightly less than a third of cases in this study were characterized by *VCL-ALK* fusion products. *VCL* encodes vinculin, an adhesion protein linking the extracellular matrix to the actomyosin cytoskeleton. Vinculin is believed to play a role in cell migration, cell-matrix adhesion and cell-cell junctions.[32,33] Mutations in this gene have been implicated in dilated cardiomyopathy.[34] In addition to epithelioid fibrous histiocytoma,[5] *VCL-ALK* fusions have also been reported in some pediatric renal cell carcinomas associated with sickle cell trait.[6,13]

This is the first report of *DCTN1-ALK* in epithelioid fibrous histiocytoma. *DCTN1* encodes dynactin subunit 1, the largest subunit of the dynactin complex, which binds microtubules and cytoplasmic dynein. Dynactin is a microtubule-based biologic motor with diverse cellular functions, including cell division and intracytoplasmic vesicular and organelle movement.[35] *DCTN1-ALK* translocations have previously been identified in Spitz tumor,[36] pancreatic ductal carcinoma,[37] inflammatory myofibroblastic tumor,[38] and lung adenocarcinoma.[29]

ETV6 (ETS Variant 6; TEL) encodes an E-Twenty-Six (ETS) family transcription factor involved in DNA and protein binding, with important roles in development, differentiation and cell proliferation.[39] Mutations in this gene have been linked to forms of thrombocytopenia and hematologic malignancy.[40] This gene is promiscuous, forming fusion partners with numerous other genes in neoplasia. In addition

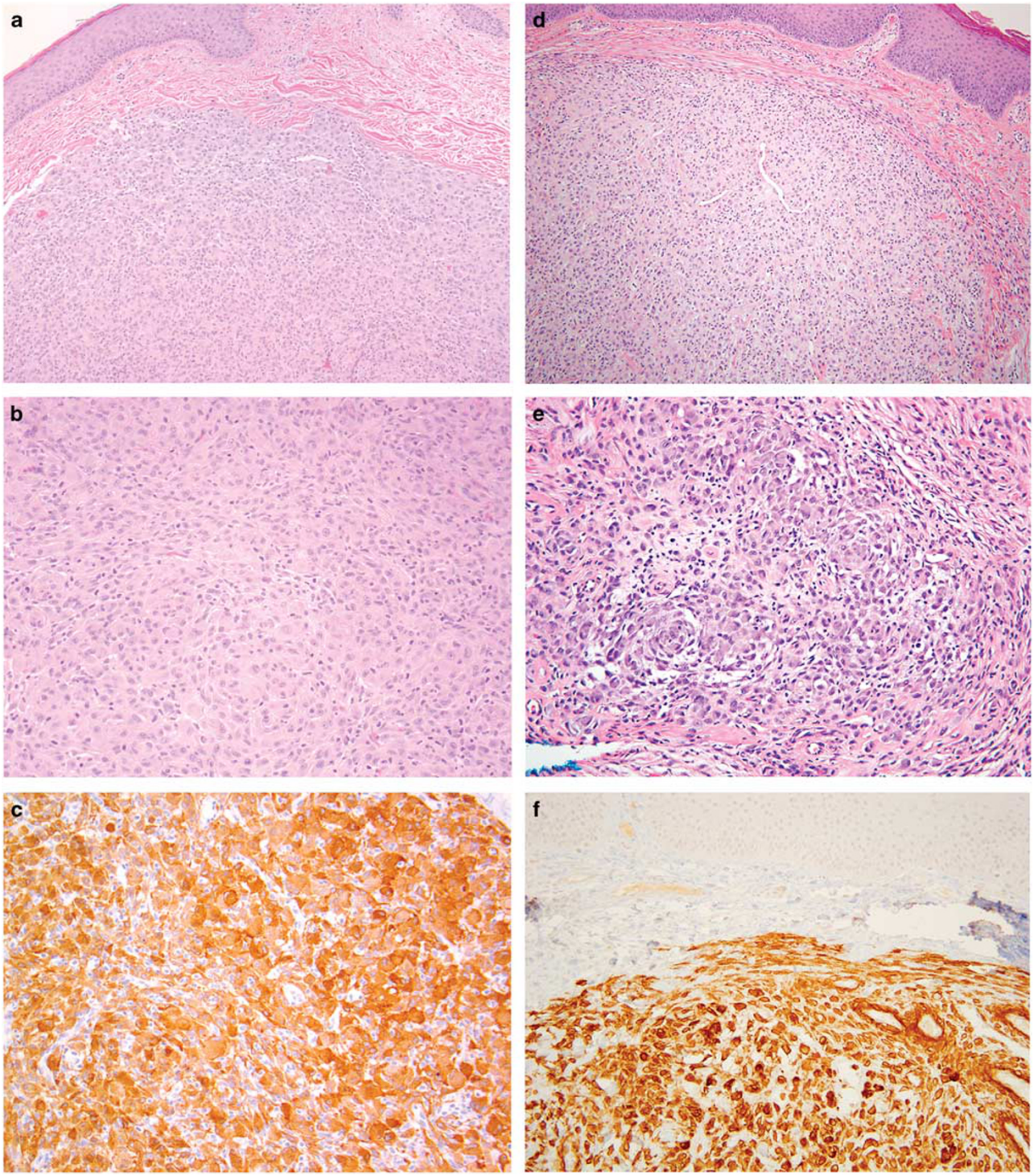

Figure 3 Representative photomicrographs of cases of epithelioid fibrous histiocytoma containing previously reported fusion products: (**a–c**) *SQSTM1-ALK* and (**d–f**) *VCL-ALK* fusion products. Note: granular cytoplasmic ALK staining in both tumors. (**a** and **d**) (hematoxylin and eosin, × 100); (**b** and **e**) (hematoxylin and eosin, × 200); (**c** and **f**) (immunohistochemistry for ALK, × 200).

to hematologic neoplasms, *ETV6-NTRK3* fusions have been reported in congenital/infantile fibrosarcoma,[41] congenital mesoblastic nephroma,[42] gastrointestinal stromal tumor,[43] infantile NTRK-associated mesenchymal tumors,[44] inflammatory myofibroblastic tumor,[45,46] mammary analog secretory carcinoma, secretory breast carcinoma,[47] sinonasal low-grade intestinal-type adenocarcinoma,[48] Spitz tumor,[49] and radiation-associated thyroid carcinoma.[50] *ETV6* fusions are not restricted to *NTRK3*; indeed, there are roughly 30 different *ETV6* fusion partners among hematologic

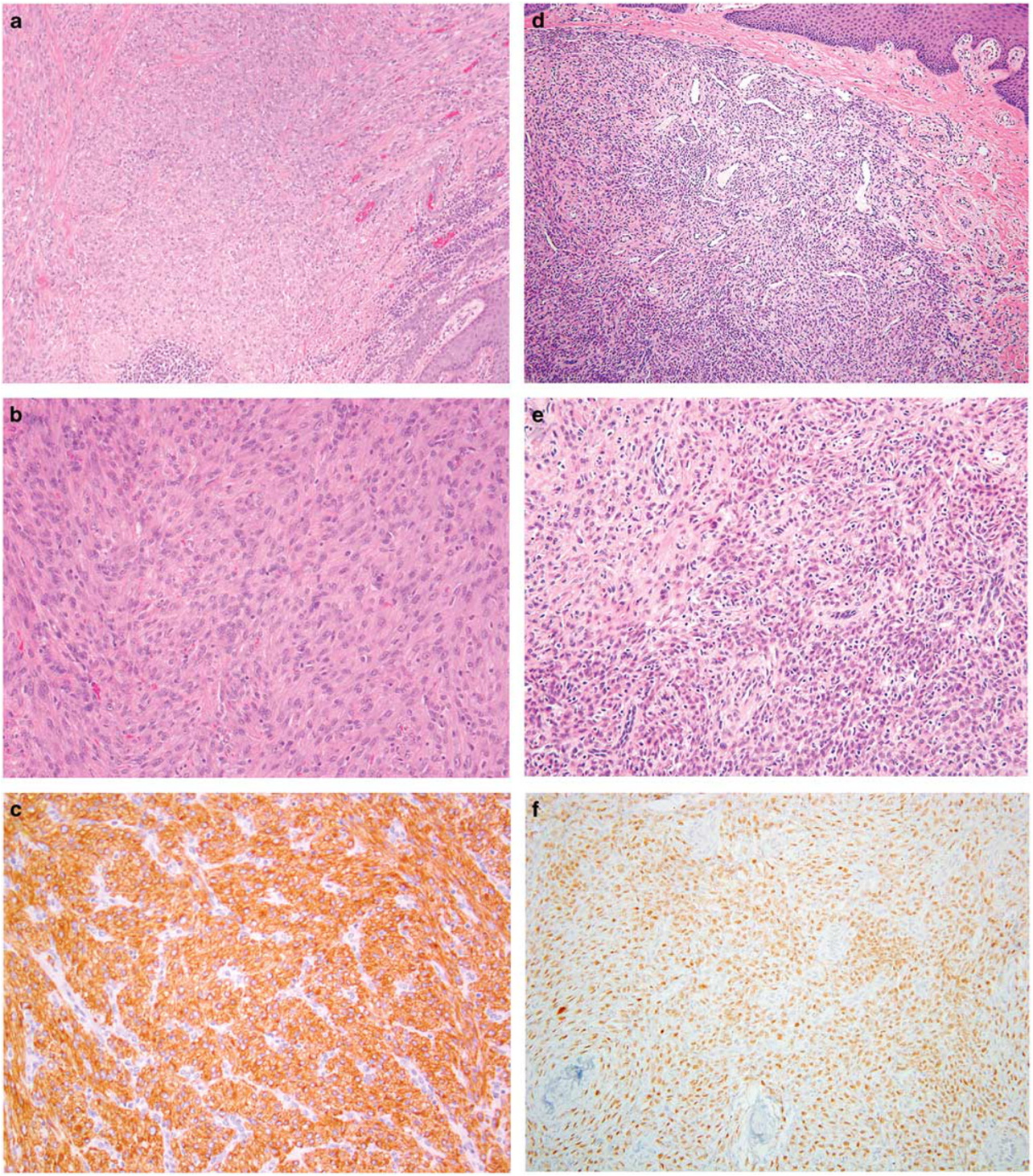

Figure 4 Representative photomicrographs of epithelioid fibrous histiocytoma with (**a–c**) *DCTN1-ALK* and (**d–f**) *ETV6-ALK* fusion products. Note: nuclear pattern of immunohistochemical staining with *ETV6-ALK* translocation. (**a** and **d**) (hematoxylin and eosin, × 100); (**b** and **e**) (hematoxylin and eosin, × 200); (**c** and **f**) (immunohistochemistry for ALK, × 200).

malignancies alone (reviewed in [39]). To our knowledge, this is the first documented account of a neoplasm bearing an *ETV6-ALK* fusion product. Interestingly, this fusion was associated with a nuclear pattern of ALK immunoreactivity, whereas the other tumors in this study showed either granular cytoplasmic or a combination of granular cytoplasmic and nuclear staining. Different patterns of ALK staining have been associated with specific fusion partners;[51] it remains to be determined whether this

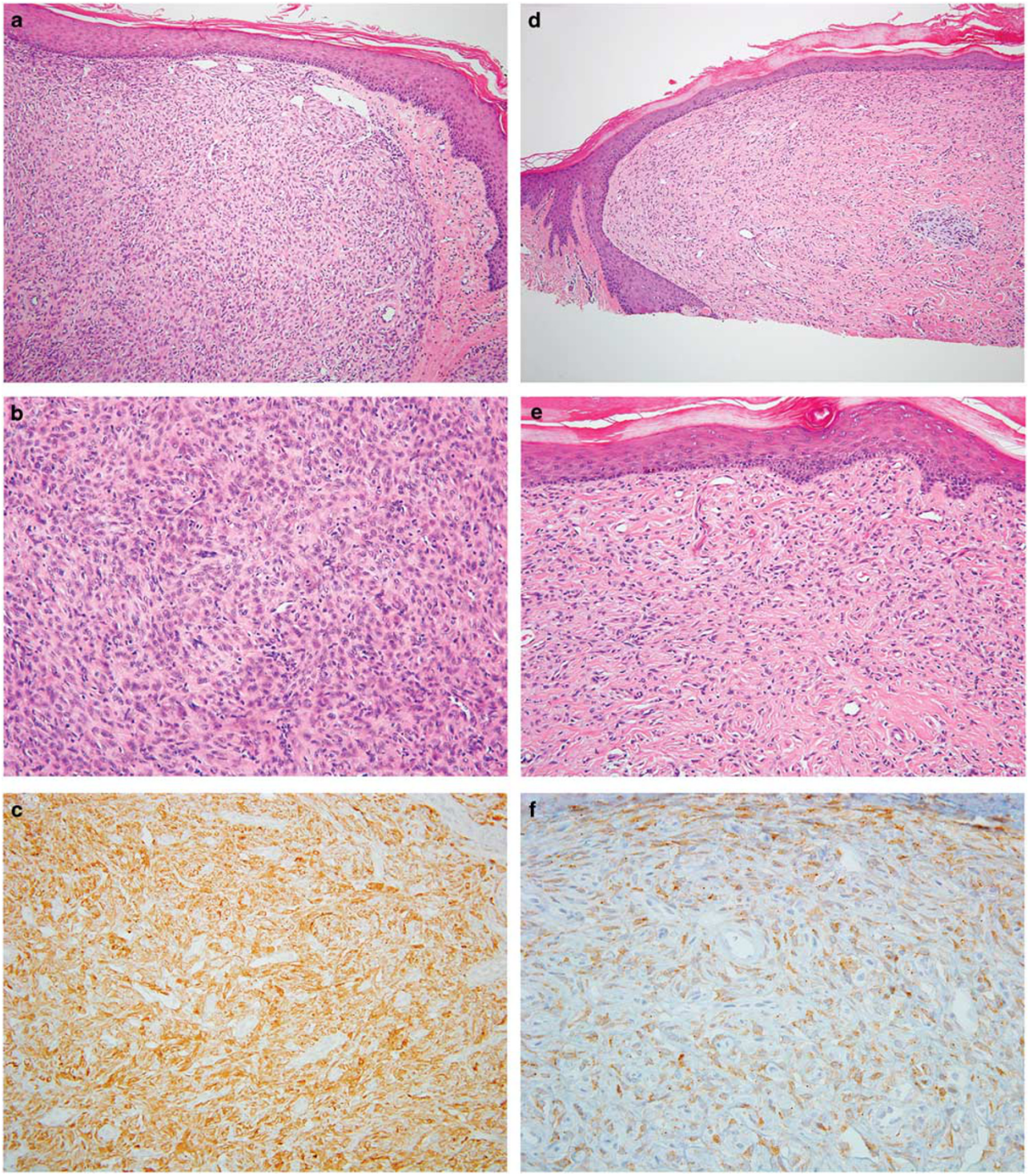

Figure 5 Representative photomicrographs of epithelioid fibrous histiocytoma with (**a–c**) *PPFIBP1-ALK*, and (**d–f**) *SPECC1L-ALK* fusion products. A delicate peripheral vasculature was a common finding among most tumors. Note: areas of interspersed collagenous stroma with *SPECC1L-ALK* translocation. (**a** and **d**) (hematoxylin and eosin, ×100); (**b** and **e**) (hematoxylin and eosin, ×200); (**c** and **f**) (immunohistochemistry for ALK, ×200).

pattern is unique–and/or universal–to cases with an *ETV6-ALK* fusion product in epithelioid fibrous histiocytoma.

PPFIBP1 encodes PPFIA Binding Protein 1 (liprin beta 1), a leukocyte common antigen-related (LAR) protein-tyrosine phosphatase-interacting protein. This protein is believed to be involved in cell adhesion, migration and development.[52] Translocations involving *PPFIBP1-ALK* have previously been reported in inflammatory myofibroblastic tumor;[53]

this is the first report of *PPFIBP1* rearrangement in epithelioid fibrous histiocytoma. Non-*ALK* fusion partners of *PPFIBP1* have also been identified, including *ROS1* in Spitz nevus.[54] *SPECC1L* (sperm antigen with calponin homology and coiled-coil domains 1) encodes a cytoskeletal cross-linking protein, with a purported role in cell adhesion, movement and division.[55,56] Mutations in this gene have been linked to abnormalities in craniofacial development.[55] This is the first report of *SPECC1L* forming a somatic gene fusion with *ALK* in disease. We have previously encountered a single case involving a *SPECC1L-NTRK2* fusion product; this was an undifferentiated spindle cell neoplasm with a prominent myxoid stroma (Dickson, unpublished observation).

Approximately 88% of epithelioid fibrous histiocytomas have evidence of *ALK* rearrangement.[4] Presumably, in some cases other genes, such as *ROS1*, may substitute for *ALK*. Mechanistically it has not been fully resolved why *ALK* is so readily capable of translocating with other partner genes in neoplasia. In some translocation-associated sarcomas, such as synovial sarcoma, multiple partners occur as a result of sequence homology within the partner gene; however, this does not appear to account for the breadth of *ALK* partners in epithelioid fibrous histiocytoma. Further molecular characterization of epithelioid fibrous histiocytoma, and other "ALKomas", is warranted.

In summary, these results suggest that *SQSTM1* is the most common *ALK* fusion partner, followed by *VCL,* in epithelioid fibrous histiocytoma. In addition, we report four novel *ALK* partners in these tumors: *DCTN1*, *ETV6, PPFIBP1*, and *SPECC1L*. To our knowledge, this is the first documented account of *ETV6-ALK* and *SPECC1L-ALK* translocations occurring in neoplasms. It is presumed that additional *ALK* fusion partners will be identified in the future; furthermore, since other genes, such as *ROS1*, may substitute for *ALK*, it is likely some of the aforementioned genes may be capable of generating non-*ALK* partners in epithelioid fibrous histiocytoma and other tumors. These data provide additional support for the separation of epithelioid fibrous histiocytoma from other types of cutaneous fibrous histiocytoma, although we have no good alternative nomenclature, at least for now.

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Disclosure/conflict of interest

The authors declare no conflict of interest.

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Supplementary Information accompanies the paper on Modern Pathology website (http://www.nature.com/modpathol)