

Targeted genomic sequencing of follicular dendritic cell sarcoma reveals recurrent alterations in NF- κ B regulatory genes

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Follicular dendritic cell sarcoma is a rare mesenchymal neoplasm with a variable and unpredictable clinical course. The genetic alterations that drive tumorigenesis in follicular dendritic cell sarcoma are largely unknown. One recent study performed *BRAF* sequencing and found V600E mutations in 5 of 27 (19%) cases. No other recurrent genetic alterations have been reported. The aim of the present study was to identify somatic alterations in follicular dendritic cell sarcoma by targeted sequencing of a panel of 309 known cancer-associated genes. DNA was isolated from formalin-fixed paraffin-embedded tissue from 13 cases of follicular dendritic cell sarcoma and submitted for hybrid capture-based enrichment and massively parallel sequencing with the Illumina HiSeq 2500 platform. Recurrent loss-of-function alterations were observed in tumor suppressor genes involved in the negative regulation of NF- κ B activation (5 of 13 cases, 38%) and cell cycle progression (4 of 13 cases, 31%). Loss-of-function alterations in the NF- κ B regulatory pathway included three cases with frameshift mutations in *NFKBIA* and two cases with bi-allelic loss of *CYLD*. Both cases with *CYLD* loss were metastases and carried concurrent alterations in at least one cell cycle regulatory gene. Alterations in cell cycle regulatory genes included two cases with bi-allelic loss of *CDKN2A*, one case with bi-allelic loss of *RB1*, and one case with a nonsense mutation in *RB1*. Last, focal copy-number gain of chromosome 9p24 including the genes *CD274* (PD-L1) and *PDCD1LG2* (PD-L2) was noted in three cases, which represents a well-described mechanism of immune evasion in cancer. These findings provide the first insight into the unique genomic landscape of follicular dendritic cell sarcoma and suggest shared mechanisms of tumorigenesis with a subset of other tumor types, notably B-cell lymphomas.

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Follicular dendritic cell sarcoma is a rare neoplasm of mesenchymal lineage that was first described by Rosai and colleagues.¹ Follicular dendritic cell sarcoma pursues a variable and largely unpredictable clinical course. Some patients (especially those with isolated nodal involvement) are cured by surgical excision, whereas others develop locally recurrent or metastatic disease. No reliable predictors of disease

course have been identified.^{2,3} In addition, little is known about the genetic alterations that drive tumorigenesis in follicular dendritic cell sarcoma. Although karyotypic abnormalities have been cited in case reports, no recurrent alterations have been identified.^{4,5} To our knowledge, the only study to investigate the genetic basis of follicular dendritic cell sarcoma was recently reported by Go *et al*,⁶ who looked for alterations in *BRAF* and identified V600E mutations in 5 of 27 cases (19%). The aim of the present study was to identify somatic genetic alterations in follicular dendritic cell sarcoma using a targeted next-generation sequencing assay that detects alterations in 309 known cancer-related genes. We evaluated 13 cases of follicular dendritic cell sarcoma reviewed at our institution and found recurrent alterations in genes involved in the regulation of NF- κ B activation (*NFKBIA* and *CYLD*),

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cell cycle progression (*CDKN2A* and *RB1*), and immune evasion (*CD274* and *PDCD1LG2*). These findings represent novel insights into the genomic landscape of follicular dendritic cell sarcoma and highlight commonalities in tumorigenesis with certain other tumor types, notably subsets of B-cell lymphoma.

Materials and Methods

Case Identification

An institutional review board-approved search of the pathology archives at Brigham and Women's Hospital from 2004 to 2015 yielded 13 cases of follicular dendritic cell sarcoma with sufficient formalin-fixed paraffin-embedded material for sequencing. The diagnosis of follicular dendritic cell sarcoma was confirmed by review of H&E slides and available immunohistochemistry. All cases were conventional follicular dendritic cell sarcomas, including the primary hepatic tumors, which did not show histological features of the inflammatory pseudotumor-like variant. By immunohistochemistry, all tumors were positive for CD21, and 12 of 12 evaluated cases were positive for CD35. In addition, 5 of 6 cases were positive for podoplanin (D2-40), and 3 of 10 were focally positive for epithelial membrane antigen. All studied tumors were negative for S-100 protein and keratins (5 and 7 cases, respectively). Additional information including patient demographics and clinical follow-up was obtained from the medical record when available.

Targeted Next-Generation Sequencing Assay and Data Processing

The tumor cellularity in the samples ranged from 50 to 80%. Targeted sequencing of 309 cancer-related genes (see Supplementary Table 1 for a complete list) was performed on DNA isolated from formalin-fixed paraffin-embedded tumor samples using hybrid capture with a custom probe set (Agilent SureSelect, Agilent Technologies, Santa Clara, CA) and massively parallel sequencing (HiSeq 2500, Illumina, San Diego, CA), as previously described.⁷ Data processing and analysis were performed using MuTect and GATK for single-nucleotide variants,^{8,9} VisCap Cancer for copy-number variations (Dana Farber Cancer Institute, Boston, MA), and BreakMer for large structural variations.¹⁰ Manual review and annotation were performed for all single-nucleotide variants, copy-number variations, and translocation calls made by the data pipeline. All single-nucleotide variants with a minor allele frequency >0.1% in the Exome Sequencing Project database were excluded as likely single-nucleotide polymorphisms. For single-nucleotide variants reported in the Exome Sequencing Project with a minor allele frequency of <0.1%, further

analysis with Mutation Assessor (Memorial Sloan Kettering Cancer Center, New York, NY) and PolyPhen2 (ref. 11) was performed to assess the predicted effect on protein function, with single-nucleotide variants retained only if they were classified as deleterious by both platforms. All retained alterations were then classified as likely pathogenic variants or variants of unknown significance according to the following criteria. Likely pathogenic variants were defined as frameshift, nonsense, or splice-site mutations; known pathogenic missense alterations; bi-allelic losses in tumor suppressors; or focal copy-number gains in oncogenes. Variants of unknown significance were defined as missense mutations with unknown functional effects or low-level copy-number gains and losses of uncertain function.

Immunohistochemistry and *In Situ* Hybridization

Immunohistochemistry was performed on selected cases following pressure cooker antigen retrieval (Target Retrieval Solution, pH 6.1; Dako, Carpinteria, CA) using a mouse anti-p16 monoclonal antibody (clone E6H4; 1:2 dilution; Ventana, Tucson, AZ) and a mouse anti-RB1 monoclonal antibody (clone G3-245; 1:100 dilution; BD Biosciences, San Diego, CA). *In situ* hybridization studies for Epstein-Barr virus-encoded mRNA (EBER) were performed on a Ventana Benchmark XT autostainer using an EBER 1 DNP probe (Ventana, cat # 760-1209A) and ISH iView Blue Plus anti-DNP detection system (Ventana).

Results

Clinical characteristics (including available follow-up information) for each case are summarized in Table 1. The mean patient age was 48 years (range 26–70 years). Eight patients were male and five were female. Nine tumors involved extranodal sites (liver–3, chest wall–1, omentum–1, bile duct–1, pre-sacral retroperitoneum–1, ovary–1, and supraclavicular fossa–1) and four tumors involved lymph nodes (cervical–2, axillary–1, and tonsil and neck–1). The predominantly extranodal distribution of disease in this cohort (as opposed to the lymph node predominant distribution typical of follicular dendritic cell sarcoma) is likely a reflection of the subspecialty expertise in soft tissue tumor pathology of the senior authors of this study. Eight cases were primary diagnoses without prior treatment, one represented residual disease after chemoradiation, one was a local recurrence after surgery, and three were metastases following surgery, chemotherapy, or radiation. Clinical follow-up data were available for six cases and included one patient dead from disease 37 months after primary diagnosis (case 4), three patients alive with disease 17, 56, and 12 months after primary diagnosis (cases 2, 5, and 13,

Table 1 Case characteristics

Case number	Age (years)	Sex	Site of primary tumor	Site of disease progression	Treatment history and clinical follow-up
1	60	Male	Chest wall	Chest wall ^{LR,S}	SR at time of primary diagnosis; SR and XRT for LR at 32 months; ANED at 61 months after first diagnosis
2	60	Female	Porta hepatis	Omentum ^{M,S}	CT at time of primary diagnosis; SDB for RPD and M at 17 months; no clinical follow-up available after SDB
3	48	Male	Supraclavicular fossa	Supraclavicular fossa ^{RPD,S}	CT and XRT at time of primary diagnosis; SR for RPD at 23 months; ANED at 27 months after first diagnosis
4	39	Male	Peri-duodenum	Bile duct ^{M,S}	SR then XRT at time of primary diagnosis; CT for multiple LR and M beginning at 11 months; DFD at 37 months after first diagnosis
5	56	Male	Liver ^S	NA	CT at time of primary diagnosis; SR at 9 months; CT for LR and M beginning at 21 months; AWD at 56 months after first diagnosis
6	26	Male	Tonsil/neck mass ^S	NA	None available
7	43	Female	Pre-sacral retroperitoneum ^S	NA	None available
8	52	Female	Liver ^S	NA	None available
9	28	Female	Cervical lymph node ^S	NA	None available
10	32	Male	Axillary lymph node ^S	NA	None available
11	70	Female	Ovary ^S	NA	None available
12	46	Male	Cervical lymph node ^S	NA	None available
13	65	Male	Upper abdomen	Liver ^{M,S}	CT at time of primary diagnosis; additional CT for RPD and M at 8 months; AWD at 12 months

Abbreviations: AWD, alive with disease; ANED, alive with no evidence of disease; CT, chemotherapy; DFD, dead from disease; LR, local recurrence; M, metastasis; NA, not applicable; RPD, residual or progressive disease; S, submitted for sequencing; SR, surgical resection; SDB, surgical debulking; XRT, radiation.

respectively), and two patients alive with no evidence of disease 61 and 27 months after primary diagnosis (cases 1 and 3, respectively). All 12 cases evaluated by *in situ* hybridization for EBER (including the two primary hepatic tumors) were negative. Sequencing quality for all samples exceeded the minimum benchmarks established in our laboratory, with a mean total coverage of 209 (range 52–310) and a mean percentage of sequences achieving greater than 30-fold coverage of 97% (range 83–99%). The copy-number variations data for one case (case 6) were not suitable for interpretation due to signal dispersion, which was likely caused by DNA degradation, and were excluded. Genes that included at least one likely pathogenic variant are summarized in Figure 1 along with any concurrent variants of unknown significance. All remaining alterations, including genes with variants of unknown significance but no likely pathogenic variants, are included in Supplementary Table 2.

Tumor suppressor genes critical for the negative regulation of NF- κ B activation showed recurrent likely pathogenic variants in 5 of 13 (38%) cases of follicular dendritic cell sarcoma (Figures 1 and 3). These alterations occurred in a mutually exclusive manner and included three cases with frameshift mutations or deletions in *NFKB1A* (cases 1, 11, and 12), which encodes a protein involved in the cytoplasmic sequestration of the NF- κ B complex. In addition, two cases demonstrated bi-allelic loss of *CYLD* (cases 2 and 4), which encodes a protein that regulates NF- κ B activation through the deubiquitination of upstream mediators. Of note, both

cases with loss of *CYLD* were metastases and carried concurrent likely pathogenic variants in at least one cell cycle regulatory gene (*CDKN2A* and/or *RB1*, as described below). In addition, three cases had variants of unknown significance in *NFKB1A*, including one case with a missense mutation (N108K, case 13) and two cases with one-copy losses (cases 7 and 10). One case also had a previously reported missense mutation in *TNFAIP3* (N102S, case 4), which encodes a protein that (similar to *CYLD*) regulates NF- κ B activation via deubiquitination of upstream mediators.¹² Interestingly, this variant occurs in the deubiquitinating domain of *TNFAIP3* and has been reported both in dbSNP (rs146534657) with a minor allele frequency of 1.3% and in several cases of B-cell lymphoma.¹² The observed allele fraction for this variant (60%) in case 4 could be consistent either with a single-nucleotide polymorphism or a somatic change demonstrating copy neutral loss of heterozygosity. Finally, low-level copy-number gain on chromosome 20q11 was noted in six cases in a region containing the anti-apoptotic gene *BCL2L1*, which is a primary transcriptional target of NF- κ B.¹³

Cell cycle regulatory genes also showed recurrent alterations in follicular dendritic cell sarcoma, with likely pathogenic variants in the canonical tumor suppressors *CDKN2A* or *RB1* noted in 4 of 13 (31%) cases (Figure 1). These included two cases with bi-allelic loss of *CDKN2A* (cases 2 and 4), one case with bi-allelic loss of *RB1* (case 4), and one case with a nonsense mutation in *RB1* (case 6). Each of these cases showed loss of p16 and Rb protein expression,

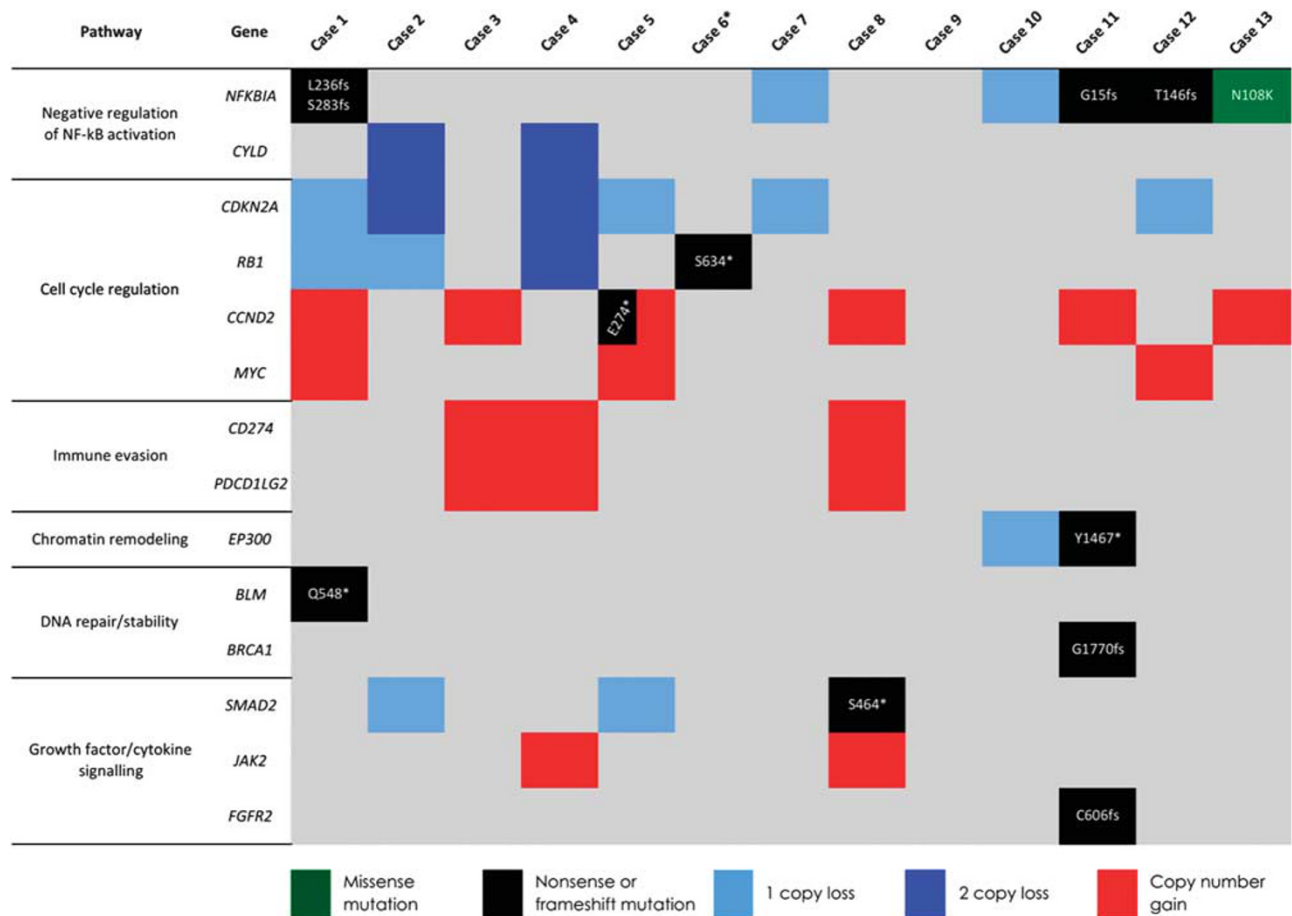


Figure 1 Summary of likely pathogenic alterations identified in 13 cases of follicular dendritic cell sarcoma. Included in the figure are all genes for which at least one likely pathogenic variant was observed. For each of these genes, all concurrent variants of unknown significance are also included. Genes with recurrent variants of unknown significance but no likely pathogenic variants are included in Supplementary Table 2. The use of two different colors for a particular gene indicates that two separate alterations were observed. *No copy-number variation data were obtained for case 6 due to signal dispersion likely caused by DNA degradation.

respectively, by immunohistochemistry (Figure 2). Additional notable alterations in cell cycle regulatory genes included a focal *MYC* copy-number gain in one case (case 12); copy-number gains involving *CCND2* (six cases), *CDK4* and *MDM2* (four cases), and *KRAS* (four cases); and one-copy losses in *CDKN2A* (four cases), *RB1* (two cases), and *TP53* (two cases). Although the functional consequences of the one-copy losses observed in tumor suppressors is uncertain due to the apparent absence of a second 'hit', alternative mechanisms of gene silencing (e.g., methylation) are not detected by the sequencing assay and could potentially contribute to bi-allelic inactivation in these cases. Additional notable alterations included three cases (cases 3, 4, and 8) with focal copy-number gains on chromosome 9p24 in a region containing the immune-regulatory genes *CD274* (PD-L1), *PDCD1LG2* (PD-L2), and *JAK2*. Pertinent negative findings include the absence of any *BRAF* V600E mutations in the cohort, which contrasts with the findings of Go *et al*⁶ cited above. Finally, translocation analysis did not identify any

recurrent or previously described translocations. A novel t(5;9)(p15;q22) involving *SYK* and *EXOC3* was noted in one case (case 2). However, as this translocation has not been reported previously and was only present in one case, its significance is uncertain.

Discussion

In the present study, likely pathogenic loss-of-function alterations in NF-κB regulatory genes occurred in 5 of 13 (38%) cases. These alterations occurred in a mutually exclusive manner, which is consistent with their functioning in the same pathway. In addition, loss-of-function alterations in NF-κB and cell cycle regulatory genes co-occurred in two of the three metastatic tumors included in the study (cases 2 and 4), suggesting that the accumulation of multiple pathogenic mutations in these pathways may possibly correlate with a more aggressive disease course. Notably, among the six

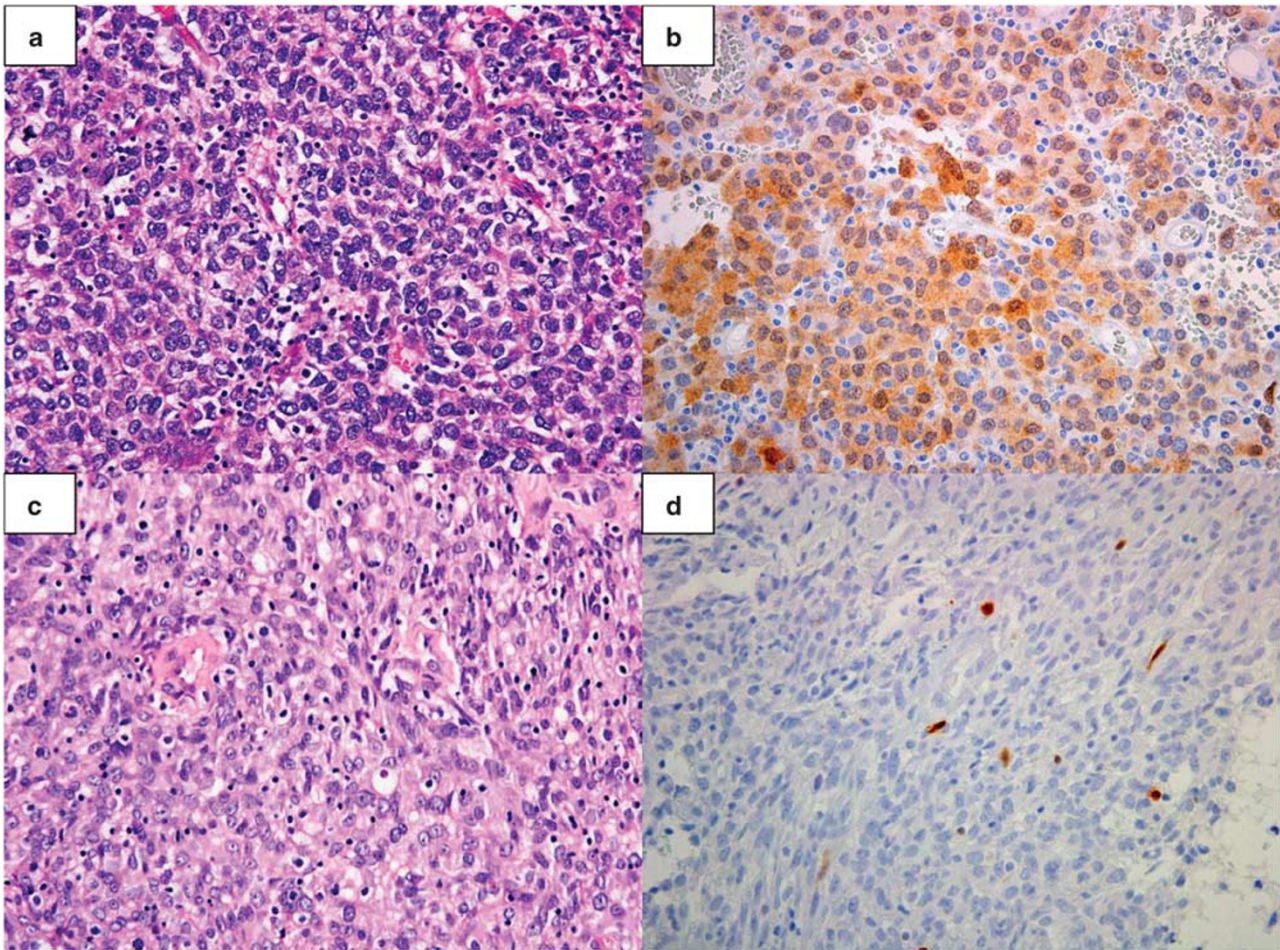


Figure 2 P16 and RB expression in follicular dendritic cell sarcoma. (a, c, e and g) Representative H&E images from case 1 (a), case 4 (c), case 2 (e), and case 6 (g). (b and d) Representative images of immunohistochemistry for P16 for case 1 (b) and case 4 (d). (f and h) Representative images of immunohistochemistry for RB for case 2 (f) and case 6 (h). All images original magnification x400. H&E, hematoxylin and eosin.

patients for whom clinical follow-up information was available, one patient (case 4) had both the highest apparent mutational burden (involving four concurrent likely pathogenic variants, which included loss-of-function alterations in *CYLD*, *CDKN2A*, and *RB1*, as well as copy-number gain of 9p24) and the most aggressive disease course (dying from metastatic disease 37 months after initial diagnosis). Although a larger study would be required to confirm any association between molecular features and clinical behavior, these findings suggest that molecular analysis could potentially provide a useful adjunct for the prediction of disease course in patients with follicular dendritic cell sarcoma.

Although mutations in cell cycle regulatory genes are frequently found across tumor types, loss-of-function alterations in NF- κ B regulatory genes, including *NFKBIA*, *CYLD*, and *TNFAIP3*, are less common but have been observed most frequently in lymphoid neoplasms, including Hodgkin lymphoma,¹⁴ diffuse large B-cell lymphoma (Figure 3),^{15,16} and plasma cell myeloma,¹⁷ as

well as select solid tumors, including adenoid cystic carcinoma¹⁸ and metastatic prostatic adenocarcinoma (Figure 3).¹⁹ *NFKBIA*, *CYLD*, and *TNFAIP3* are thought to exert tumor suppressor function primarily through the negative regulation of the canonical pathway of NF- κ B activation. Under normal conditions, NF- κ B activation through this pathway involves several key steps, including: (1) activation of the I κ B kinase in response to an appropriate extracellular stimulus; (2) phosphorylation, ubiquitination, and proteosomal degradation of I κ B-alpha (encoded by *NFKBIA*), which otherwise binds to NF- κ B in the cytoplasm and inhibits its nuclear translocation; (3) release and nuclear translocation of NF- κ B (itself composed of heterodimers of the proteins p50 or p52 and REL, RELA, or RELB); and (4) expression of NF- κ B target genes.¹³ Although I κ B-alpha regulates NF- κ B via direct interactions, the proteins encoded by both *CYLD* (CYLD) and *TNFAIP3* (A20) exert indirect regulatory activity through the removal of ubiquitin chains from upstream signaling molecules, such as RIP1 and

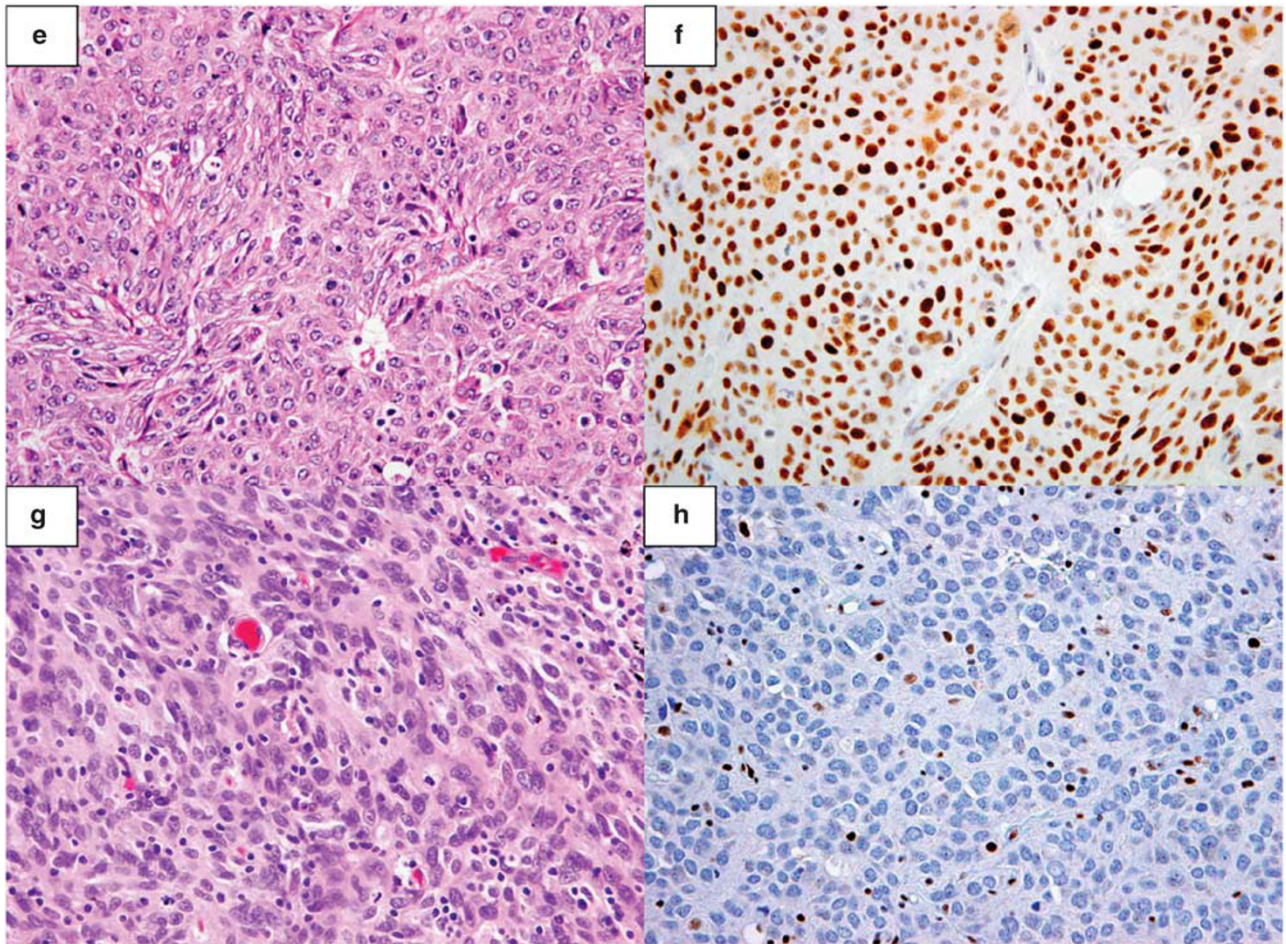


Figure 2 Continued.

TRAF2, which are important for activation of the I κ B kinase.^{20,21}

In the setting of cancer, loss-of-function alterations in *NFKBIA*, *CYLD*, or *TNFAIP3* lead to increased NF- κ B nuclear translocation and increased expression of NF- κ B target genes important for cell proliferation and cell survival.^{13,22} Foremost among these targets are the inhibitor of apoptosis and a subset of BCL2-family genes, including *BCL2L1*, which exert anti-apoptotic activity through the inhibition of caspases and pro-apoptotic BCL2-family genes, respectively.²³ Interestingly, the most frequent copy-number variation observed in the study was low copy-number gain on 20q11 in a region that includes *BCL2L1*, as well as several other candidate genes (observed in six cases). Although the impact of this alteration in follicular dendritic cell sarcoma is uncertain, a functional role of *BCL2L1* activation and copy-number gain has been demonstrated in other tumor types²⁴ and may suggest future avenues of investigation into potential mediators of anti-apoptotic activity in follicular dendritic cell sarcoma, as well as potential therapeutic targets, given recent interest in the

development of inhibitors of BCL-XL, the protein encoded by *BCL2L1*.²⁵

Finally, copy-number gain on chromosome 9p24 was observed in three cases in a region containing the genes *CD274* (PD-L1) and *PDCD1LG2* (PD-L2). Copy-number gain on 9p24 represents a well-described mechanism of immune evasion in several subtypes of lymphoma, including nodular sclerosing Hodgkin lymphoma and primary mediastinal diffuse large B-cell lymphoma, and is mediated in part by increased tumor expression of PD-L1 and PD-L2 and consequent activation of the PD-1 regulatory pathway on tumor-infiltrating lymphocytes.^{26–28} Although further study would be required to clarify the specific role of immune evasion in the pathogenesis of follicular dendritic cell sarcoma, the finding of 9p24 copy-number gain in a subset of cases raises the possibility that immunotherapy with PD-1/PD-L1/2 pathway blockade may represent a future avenue of therapeutic investigation.²⁷ In summary, the current findings represent novel insights into the genomic landscape of follicular dendritic cell sarcoma and provide a foundation for further experimental and therapeutic investigation in this tumor type.

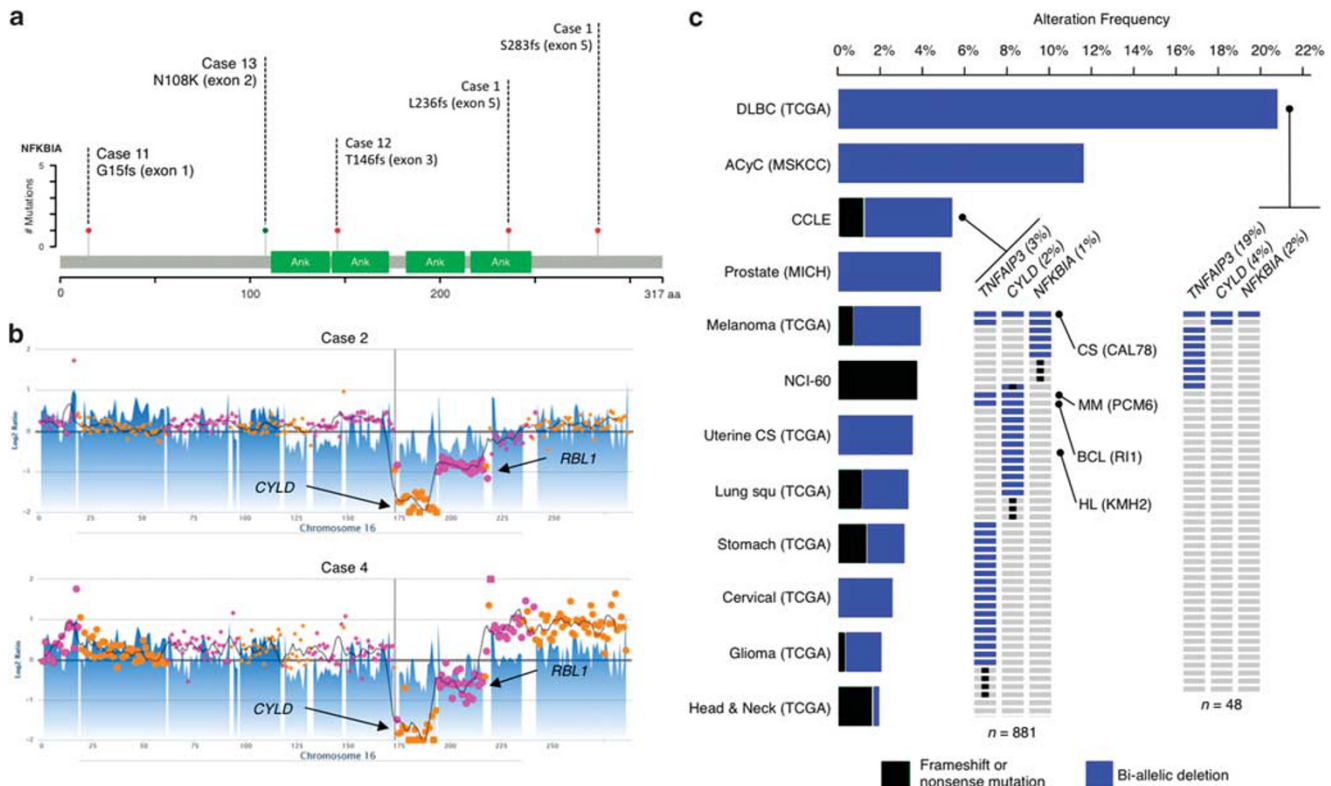


Figure 3 Alterations in the NF- κ B regulatory pathway in follicular dendritic cell sarcoma and other tumor types. **(a)** Gene level view of *NFKBIA* showing the location of each observed mutation. **(b)** Chromosome level view of chromosome 16 for cases 2 and 4 demonstrating bi-allelic loss of *CYLD*, as well as flanking one-copy loss of *RBL1*. **(c)** Frequency of loss-of-function alterations (bi-allelic loss, frameshift mutation, and nonsense mutation) in the NF- κ B regulatory genes *NFKBIA*, *CYLD*, and *TNFAIP3* across cancer genome studies available in cBioportal, which was used to generate the figure.¹⁵ Insets show frequency of alterations in each gene from selected studies. Letters and numbers listed in parentheses indicate the name of the corresponding cell line. ACyC, adenoid cystic carcinoma; BCL, B-cell lymphoma; CCLE, Cancer Cell Line Encyclopedia; CS, carcinosarcoma; DLBC, diffuse large B-cell lymphoma; HL, Hodgkin lymphoma; lung squ, lung squamous cell carcinoma; MM, multiple myeloma; MSKCC, Memorial Sloan Kettering Cancer Center; MICH, University of Michigan; TCGA, The Cancer Genome Atlas.

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Disclosure/conflict of interest

The authors declare no conflicts of interest.

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