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MYC and TP53 Alterations but Not MAPK Pathway Mutations Are Common Oncogenic Mechanisms in Follicular Dendritic Cell Sarcomas

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Context.—Despite their stromal origin, follicular dendritic cells (FDCs) share many functions with hematopoietic system cells. FDC neoplasms are currently classified by the World Health Organization along with those of a histiocytic nature. However, the molecular alterations driving oncogenesis in FDC sarcomas (FDCSs) are beginning to be unveiled, and do not seem to concur with those described in histiocytic neoplasms, namely MAPK pathway activation.

Objective.—To identify molecular alterations driving tumorigenesis in FDCS.

Design.—We investigated the role of MYC and TP53 in FDC-derived tumor oncogenesis and assessed comprehensively the status of the MAPK pathway in 16 FDCSs, 6 inflammatory pseudotumor (IPT)–like FDCSs, and 8 IPTs.

Results.—MYC structural alterations (both amplifications and rearrangements) were identified in 5 of 14 FDCSs (35.7%), all associated with MYC overexpression. TP53 mutations were identified in 4 of 14 FDCSs (28.6%), all of which displayed intense and diffuse p53 expression. None of these alterations were identified in any IPT-like FDCS or in IPT cases. No MAPK pathway gene alterations were identified in any of the cases studied.

Conclusions.—The presence of MYC and TP53 alterations and the lack of association with Epstein-Barr virus segregate classical FDCS from IPT-like FDCS, pointing at different oncogenic mechanisms in both entities. Our results suggest a possible oncogenic role of MYC and TP53 alterations in FDCS. The absence of MAPK pathway alterations confirms the lack of a significant role of this pathway in the oncogenesis of FDC-derived neoplasms.

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Follicular dendritic cell sarcoma (FDCS) is an uncommon sarcomatoid proliferation of follicular dendritic cells1 presenting both in lymph nodes and in extranodal sites. The 2017 revised 4th edition of the World Health Organization (WHO) Classification of Tumours of Hematopoietic and Lymphoid Tissues2 clusters FDCS with neoplasms derived from histiocytic and hematopoietic dendritic cells on the basis of the common functional properties of their normal counterpart, such as antigen processing and presentation to lymphoid cells, rather than on their cell of origin. Of note, FDCS has been described in cases of hyaline-vascular Castleman disease (HVCD), suggesting a possible pathogenic relationship.3,4 An extranodal form of FDCS associated with Epstein-Barr virus (EBV) infection, involving mainly liver and spleen and histologically resembling inflammatory pseudotumor (IPT) rather than sarcoma, is included in the FDCS diagnostic category as a variant.5

The knowledge of the pathogenesis of FDCS and the genetic alterations that drive its tumorigenesis is limited. Nevertheless, some studies have associated FDCS with widespread chromosomal instability and tumor suppressor–driven biological alterations, along with dysregulation of cell cycle, recurrent alterations in nuclear factor κB (NF-κB) regulatory genes, mitogen–activated protein kinase (MAPK) activation, and immune evasion.6–14
Studies carried out in large series of histiocytic neoplasms have shown a high frequency of alterations in the MAPK pathway in these entities.\textsuperscript{15–18} Mutually exclusive BRAF- and MAP2K1-activating mutations have been observed in up to 72% of Langerhans cell histiocytosis (LCH)\textsuperscript{19,20} and in up to 26% of histiocytic sarcomas.\textsuperscript{21} BRAF V600E mutation has been described in up to 18.5% of FDCS\textsuperscript{22} but it is relatively rare in hematologic malignancies.\textsuperscript{22} The MAPK pathway, together with the phosphoinositide 3-kinase/protein kinase B (PI3K/AKT) pathway, constitutes the main control mechanism of cell survival, differentiation, proliferation, metabolism, and motility in response to extracellular stimuli. The synergistic activation of both pathways has already been described in the pathogenesis of histiocytic sarcoma.\textsuperscript{23}

Recent studies have postulated that overexpression of cyclin D1 in LCH would be a potential surrogate marker of MAPK pathway activation.\textsuperscript{23,24} In addition, LMP1 and LMP2, the major oncoproteins in EBV, can activate multiple signal pathways, such as PI3K/AKT and MAPK, further supporting the need to thoroughly interrogate these pathways. A recent study that characterized the molecular alterations in FDCS using a comprehensive gene panel suggested that, unlike in histiocytic neoplasms, FDCS onconeogenesis might not be driven by MAPK pathway alterations.\textsuperscript{25} This is the only study that has analyzed the MAPK pathway in FDCS. Also, the significance of MYC, whose synergic role with the Ras-Raf-MAPK cascade has been described in B lymphomagenesis,\textsuperscript{25,26} has not been addressed in dendritic cell–derived neoplasms.

In the present work we investigated the presence of genetic and molecular alterations in major oncogenic pathways, with special emphasis on those whose oncogenic role in both histiocytic and lymphoid neoplasms is well established, such as the MAPK pathway, the PIK3/AKT pathway, the MYC oncogene, and p53. Together with FDCS we include IPT-like FDCS and compare both groups with a series of IPIs.

**MATERIALS AND METHODS**

**Case Selection and Histopathologic Review**

Cases diagnosed as FDCS, IPT-like FDCS, or IPT during the period 2000–2018 were retrieved from the files of 4 different institutions. Histopathologic review was carried out on available hematoxylin-eosin and immunostained slides according to the criteria of the 2017 revised 4th edition WHO Classification of Tumours of Hematopoietic and Lymphoid Tissues.\textsuperscript{2} The study was approved by the local research ethics committee and was performed in accordance with the Declaration of Helsinki.

**Immunohistochemistry and EBV Study**

Most cases were studied using whole tissue sections (for cases with limited material, tissue microarrays were constructed as described previously)\textsuperscript{27} and were characterized with a panel of immunohistochemical antibodies including lymphoid markers, follicular dendritic cells (FDCs), histiocytic markers, EGFR, Ki-67, cyclin D1, p-ERK1/2, MYC, and p53 (Supplemental Table 1; see the supplemental digital content containing 3 tables and 2 figures). Two-micrometer-thick sections were used in a peroxidase-labeled detection system, standard antigen retrieval protocols, and an automated immunostainer (Autostainer Link 48, Dako, Glostrup, Denmark, or Benchmark Ultra, Roche Diagnostics, Basel, Switzerland) as previously described.\textsuperscript{28} Lymphoid markers, FDCs, histiocytic markers, and EGFR immunohistochemical stains were scored as 0 (absence of positivity), 1 (positivity in <30% of neoplastic cells) and 2 (positivity in ≥30% of neoplastic cells). Scores 1 and 2 were considered positive. For p-ERK, Ki-67, cyclin D1, p53, and MYC stains, the percentage of neoplastic cells exhibiting nuclear staining was manually counted in 300 neoplastic cells for each case. Cases were considered positive for ERK and cyclin D1 if nuclear staining was present in 50% or more of the neoplastic cells. For p53, positivity cutoff was set to 10% or more of neoplastic cells according to previously published data\textsuperscript{29} and MYC positivity cutoff was set to 40% or more of neoplastic cells based on the WHO recommendations. EBV RNA was detected by in situ hybridization using EBV-encoded small RNA (EBER1 and 2) probes (Leica BOND-MAX system, Leica Biosystems, Newcastle upon Tyne, United Kingdom) as previously described.\textsuperscript{30}

**Fluorescence In Situ Hybridization**

A locus-specific identifier (LSI) Sure fluorescence in situ hybridization (FISH) probe for p71.2 and a SureFISH chromosome 7 CEp (Agilent Technologies, Glostrup, Denmark) were used to assess alterations in the EGFR gene. A SureFISH MYC break-apart probe (Agilent Technologies) and a Vysis LSI MYC break-apart or Vysis LSI IGH/MYC/CEP 8 Tri-Color Dual Fusion probe (Abbott Molecular Inc, Abbott Park, Illinois) were used to assess alterations in the MYC gene. For TP53, a Vysis TP53 SpectrumOrange probe and a centromeric chromosome 17 CEP probe (D17Z1) (Abbott Molecular Inc) were used. In all cases FISH was performed on 2-μm-thick sections of formalin-fixed, paraffin-embedded (FFPE) tissues according to manufacturer’s instructions.

Cells were analyzed under an Axio Imager.Z2 epi-fluorescence microscope (Carl Zeiss Microimaging GmbH, Göttingen, Germany) equipped with a triple band-pass filter to simultaneously visualize 4’,6-diamidino-2-phenylindole, fluorescein isothiocyanate green, and orange-red. Digital images were captured on a CoolCube1 CCD camera (MetaSystems, Altlüsheim, Germany). A minimum of 100 interphase nuclei per sample were evaluated. MYC copy number gains were defined as 3 or more signals/nucleus, whereas cases with 4 or more signals/nucleus were considered amplified. MYC-rearranged cases were defined by the presence of a single fusion and separate green and red signals or a fusion signal and unpaired green or red signal(s) in 15% or more of the cells. EGFR gene copy number was classified into normal disomy, low trisomy (3 copies in <40% of cells), high trisomy (3 copies in ≥40% of cells), low polysomy (≥4 copies in 10%–40% of cells), or high polysomy (≥4 copies in ≥40% of cells) and EGFR gene amplification (EGFR/CEP7 ratio ≥2 or clusters of EGFR signals in >10% of cells) according to the criteria defined by Varella-Garcia et al\textsuperscript{11} and were further divided into 2 major groups: low EGFR copy number (disomy, low trisomy, high trisomy, and low polysomy) and high EGFR copy number (high polysomy and gene amplification).\textsuperscript{30} TP53 deletions were determined by a TP53/CEP17 ratio of 0.5 or lower in 30% or more of the neoplastic cells.

**DNA Extraction and Next-Generation Sequencing**

Genomic DNA was obtained from 15 FDCS, 6 IPT-like FDCS, and 8 IPT FFPE samples with QIAamp DNA FFPE Tissue Kit (Qiagen, Valencia, California) and was quantified fluorometrically in a Qubit instrument (Invitrogen, Carlsbad, California). Targeted libraries were prepared with the Accel-Amplicon 56G Oncology Panel v2 kit (Swift Biosciences, Ann Arbor, Michigan) from 25 ng of DNA following the manufacturer’s instructions and were sequenced on a MiSeq platform using a 150-cycle paired-end run (2 × 150) at an average coverage above 4450 reads per sample (Supplemental Figure 1). The Accel-Amplicon 56G Oncology Panel v2 assay includes comprehensive coding exon coverage for TP53, as well as contiguous overlapping coverage for EGFR, PIK3CA, PTEN, and ATM. The assay covers 49 hotspot regions in 51 other genes, including BRAF, MET, KRAS, HRAS, NRAS, PTPN11, and MAP2K1, as well as EZH2 and CDKN2A (for a complete list of the target regions, see Supplemental Table 2).

Sequencing data were analyzed according to the bioinformatic pipeline recommended and provided by Swift Biosciences. Quality control and adapter trimming of the FASTQ (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/) was done using FastQC (version 0.11.2) and Trimmomatic (version 0.32).\textsuperscript{30} Raw
reads were mapped to the human reference genome (build GRCh37/hg19) using the BWA-mem algorithm (https://github.com/lh3/bwa; version 0.7.10). Primer soft clipping on aligned SAM files was performed with Primerclip (https://github.com/swiftbiosciences/primerclip; version 0.3.8). Primer-clipped SAM files were converted to BAM format with Picard AddOrReplaceCeGroups (http://broadinstitute.github.io/picard), and an indexed BAM file was generated. To determine quality metrics at the sample and interval level, Picard CollectTargetPcrMetrics (http://broadinstitute.github.io/picard/) was used.

Variant calling was performed with LoFreq\textsuperscript{35} and Mutect2 (Genome Analysis Toolkit [GATK], version 4.1.4.1) after executing the indel realignment and the base quality score recalibration steps defined in the GATK Best Practice recommendations.\textsuperscript{36} Exonic variants and variants affecting splicing sites identified by both variant callers in samples with a coverage more than 50x were selected, and were annotated with Snpeff/SnpSift (version 5.0e).\textsuperscript{37,38} Germlinal variants and polymersome chain reaction artifacts (defined with a variant allele frequency <5%), as well as those variants present in the Single Nucleotide Polymorphism Database (dbSNP150)\textsuperscript{39} with a European population frequency 1% or more (1000 Genomes Project database,\textsuperscript{44} Exome Aggregation Consortium,\textsuperscript{55} or Genome Aggregation Database\textsuperscript{60} [gnomAD version 2.1.1] databases) were excluded. In silico functional impact of the selected variants was assessed with CADD and MutationAssessor,\textsuperscript{70} and only those predicted to be damaging were selected. Aligned reads were manually reviewed with Integrative Genomics Viewer.\textsuperscript{39} TP53 variants were considered as somatic mutations when, in addition to fulfilling the previous criteria, they were identified in COSMIC database release version 92 (http://cancer.sanger.ac.uk/cosmic, accessed December 7, 2020) or the International Agency for Research on Cancer TP53 database release 20 (http://p53.iarc.fr/, accessed December 7, 2020).\textsuperscript{46} A flow chart describing the variant filtering strategy used can be found in the supplemental materials (Supplemental Figure 1).

**Statistical Analysis**

Differences between groups were evaluated by Fisher-Freeman-Halton test for categorical variables and by Student t test for continuous variables. Disease-specific survival was defined as the time between the date of the diagnostic biopsy and the date of death in those cases in which the cause of death was related to the disease. Survival was assessed with the Kaplan-Meier method and groups were compared using the log-rank test. Statistical analyses were carried out using R software, version 3.5.1. Statistical significance was set at \( P < .05 \) (2-sided).

**RESULTS**

**Clinical and Epidemiologic Data**

The series included 16 FDCSs, 6 IPT-like FDCSs, and 8 IPTs. Median age at the time of diagnosis was 60 years for FDCS (range, 36–88), 57 years for IPT-like FDCS (range, 28–74), and 53 years for IPT (range, 28–76). Whereas 56% of the FDCSs were nodal, all the IPT-like FDCSs and the IPT cases were extranodal. All the IPT-like FDCSs were splenic or hepatic. Case 8 was previously published by Parada et al.\textsuperscript{41} Clinical data, as well as treatments for each case and outcomes, are summarized in the Table.

**Morphologic Features, Expression of Antigens of Dendritic Cell Differentiation, and EBV Status**

Pathologic features are shown in Figure 1, A through I. In the FDCS group some cases were composed of large fusiform cells with a moderate amount of pale cytoplasm, arranged in fascicles or in a storiform pattern (Figure 1, A), whereas other cases showed an epithelioid morphology. The degree of atypia was highly heterogeneous, and a variable amount of interspersed mature lymphocytic infiltrate was present. Mean mitotic rate was 6/10 HPF (range, 0–31).

The 6 IPT-like FDCS cases were well-defined lesions formed by loosely aggregated spindle cells with pale eosinophilic cytoplasm and oval or elongated nuclei, without relevant atypia, admixed with a dense, mixed inflammatory infiltrate mainly composed of plasma cells (Figure 1, D). Mean mitotic rate was less than 1/10 HPF (range, 0–6). One previously diagnosed hepatic IPT was reclassified as IPT-like FDCS based on the characteristic morphology and EBER positivity.

The IPT cases were well-defined lesions composed of an interlacing fibroconnective tissue framework with spindle bland-nucleated cells accompanied by a mixed inflammatory infiltrate mainly consisting of lymphocytes and plasma cells (Figure 1, G). No mitosis was seen in any of the IPT cases.

Although all FDCSs and IPT-like FDCS variants were positive for at least 2 follicular dendritic cell markers, differences were observed regarding the expression of CD21 and CD23 between the 2 groups. FDCS had a significantly greater number of cases with intense and diffuse positivity for at least 1 of these 2 antigens (14 versus 2, respectively; \( P = .03 \)). Interestingly, CXCL13 was highly specific for FDCS, being positive in 12 of 15 cases (80%) and negative in all IPT-like FDCSs (Figure 1, B and E). No CD21, CD23, CD35, or CXCL13 expression was observed in any of the IPT cases (Figure 1, H) in which this determination could be performed. As expected, all the IPT-like FDCSs showed positivity for EBER, and it was negative in FDCS and IPT cases (Figures 1, C, F, and I, and 2).
<table>
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<th>Sex</th>
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<th>Specimen Type/Tumor Site</th>
<th>Clinical Presentation</th>
<th>Treatment</th>
<th>Follow-up</th>
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<td>No</td>
<td>SR/cervical node</td>
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<td>Laterocervical and mediastinal lymphadenopathy</td>
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<td>Weight gain, edema of legs and scrotum</td>
<td>Surgical resection (R2) + CT (CHOP ×3) + RT</td>
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<td>Yes</td>
<td>SR/abdomen</td>
<td>Painless abdominal mass</td>
<td>Surgical resection + CT (CHOP ×6)</td>
<td>Alive, NED 14 y after diagnosis</td>
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<td>Yes</td>
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<td>Chest pain, lymphadenopathy</td>
<td>Surgical resection + RT</td>
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<td>NA</td>
<td>NA</td>
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<td>IPT-like FDCS</td>
<td>No</td>
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<td>IPT-like FDCS</td>
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<td>Yes</td>
<td>IPT-like FDCS</td>
<td>No</td>
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<td>Alive, NED 2 y after diagnosis</td>
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<td>IPT-like FDCS</td>
<td>No</td>
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<td>IPT-like FDCS</td>
<td>No</td>
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<td>No</td>
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<tr>
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<td>Surgical resection</td>
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**Abbreviations:** AWD, alive with disease; Bx, biopsy; CHOP, cyclophosphamide, doxorubicin, vincristine, and prednisolone; CMyOP, intermediate dose of nonpegylated liposomal doxorubicin combination; CT, chemotherapy; DOD, dead of disease; EP, etoposide and cisplatin; FDCS, follicular dendritic cell sarcoma; GEMOX, gemcitabine and oxaliplatin; HVCD, hyaline-vascular Castleman disease; IPT, inflammatory pseudotumor; IPT-like FDCS, inflammatory pseudotumor-like follicular dendritic cell sarcoma; M1, metastasis; NA, not available; NED, no evidence of disease; R, rituximab; R2, incomplete resection; R-COP, rituximab, cyclophosphamide, prednisone, and vincristine; RT, radiotherapy; SR, surgical resection.
of the cases (Figure 2). For a complete list of the mutated genes, see Supplemental Table 3.

**TP53 Status**

Expression of p53 was observed in 15 of 16 cases of FDCS (93.8%). Strong expression was observed in 13 cases, whereas p53 expression was low in 2 (cases 2 and 4). The mean percentage of p53-overexpressed neoplastic cells among the p53-positive FDCS cases was 60% (range, 15%–90%). p53 staining was evaluated as negative in 6 of 6 IPT-like FDCSs and in 8 of 8 IPTs with only occasional weak positive cells, always less than 10% of the tumor cells (Figure 4, A). TP53 missense mutations were identified in 4 of 14 FDCSs (28.6%) (Figure 2). All mutations were located at the DNA-binding domain of the protein (Supplemental Figure 2). All TP53-mutated cases displayed diffuse p53 positivity by immunohistochemistry (defined as positivity in >80% of neoplastic cells) (average, 84%; range, 80%–90%) (Figure 4, B through E), whereas nonmutated cases exhibited a heterogeneous range of p53 expression (average, 46%; range, 5%–90%). FISH study of the 17p region could be performed in 11 of 16 FDCSs, identifying a 17p deletion in one case with a TP53 mutation (Figure 4, C inset) and a monosomy in a TP53 wild-type case. No 17p alterations were identified in the other 9 FDCS cases in which FISH could be performed (Figure 2).

**MYC Expression and MYC Status**

MYC (Figure 5, A through F) overexpression was observed in 6 of 16 FDCSs (37.5%) (Figure 2 and Figure 5, A, C, and E). The average percentage of MYC-positive neoplastic cells in MYC-overexpressed cases was 63% (range, 40%–92%), whereas the 10 cases considered negative for MYC protein expression displayed less than 10% positive tumor cells in each case. MYC gene numeric alterations were detected in 6 of 16 FDCSs (42.9%): 5 cases showed amplifications and 1 case showed copy number gain (Figure 2). Amplified cases displayed a heterogeneous degree of amplification, ranging from 4.4 signals/nucleus on average in the case with lowest-grade amplification to 9.5 signals/nucleus on average in the case with the highest grade. MYC rearrangements were observed in 3 of 14 FDCS cases (21.4%), which concomitantly also had MYC amplifications (Figure 2 and Figure 5, B, D, and F). In 2 of these cases, the amplification...
corresponded to the rearranged allele, whereas in the other case, the amplification occurred in the nonrearranged allele. Interestingly, in the 2 cases in which the amplification occurred in the rearranged allele, both alterations were present in most of the nuclei (Figure 5, B and D), whereas in the other case, although the majority of nuclei showed MYC amplification, rearrangement was present in only about 20% of the nuclei (Figure 5, F). We observed a significant association between MYC amplification/rearrangement and MYC overexpression: 5 FDCSs with MYC overexpression in which FISH was performed also displayed MYC structural alterations, whereas no overexpression was found in any of the other cases with no gene alterations or in the case with just an MYC gain (P < .001) (Figure 2). No MYC overexpression or MYC alterations were observed among the IPT-like FDCSs (0 of 6) or the IPT cases (0 of 8) (Figure 2).

**Correlation of Alterations and Clinical Impact**

The relationships among the expression of MYC and p53 and the proliferative index evaluated by immunostaining for Ki-67 in FDCS cases were analyzed. FDCS cases with TP53 mutation displayed significantly higher Ki-67 values than wild-type cases (average Ki-67 values: 49% and 16%, respectively; P < .02). An association between MYC and p53 expression was found (P = .01), with a moderate positive correlation (R² = 0.5181, P = .04). No significant correlation was found between p53 expression and Ki-67 index in nonmutated FDCS (P = .68). MYC expression did not show any association with Ki-67 proliferation index (P = .71), regardless of MYC status.

When assessing the influence of these alterations in the clinical outcome, no significant differences in disease-specific survival were seen between those FDCS cases with MYC overexpression or MYC gene alterations compared with the rest of the FDCS cases (P = .05 and P = .04, respectively). Likewise, no impact on disease-free survival was evidenced for TP53 status or p53 expression.

**DISCUSSION**

FDCS is an uncommon neoplasm presenting with variable morphologic features and clinical behavior. Despite the fact that it has been classically clustered with histiocytic neoplasms and other subtypes of dendritic cell–derived neoplasms, a recent study suggested that molecular alterations underlying the oncogenesis of both neoplasms might be different. In this study, we characterized a series of FDC tumors including FDCS and IPT-like FDCS and investigated the potential role of MAPK pathway activation in their pathogenesis, which has been extensively described in histiocytic neoplasms.

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**Figure 2.** Overview of subgroup-specific alterations identified in the study. The heat map shows the case-specific distribution of EBER, MYC expression, MYC alterations, p53 expression, TP53 mutations, and lack of mutations in genes of the MAPK pathway. Each column represents a case and variables are plotted in rows. Asterisks indicate cases with previous history of hyaline-vascular Castleman disease (HVCD) or cases with HVCD features in the biopsy. White spaces mean that the determination was not performed in that case. Abbreviations: FDCS, follicular dendritic cell sarcoma; FISH, fluorescence in situ hybridization; IHC, immunohistochemistry; IPT, inflammatory pseudotumor; NA, the result was not assessable; NGS, next-generation sequencing.

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Figure 3. MAPK pathway status. EGFR assessment by immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) in follicular dendritic cell sarcoma (FDCS) (A and B), inflammatory pseudotumor (IPT)-like FDCS (C and D), and IPT (E and F) (EGFR IHC, original magnification ×200 [A, C, and E]; EGFR FISH, original magnification ×1000 [B, D, and F]).
Unlike Go et al, who found BRAF V600E mutation in 18.5% of FDCs and other dendritic and histiocytic cell neoplasms, we did not observe BRAF V600E mutation in any FDCS or IPT-like FDCS, with a similar number of cases included in both series. Although some studies in histiocytic neoplasms have suggested that BRAF and MAP2K1 mutations would be mutually exclusive, concurrent mutations of BRAF and MAP2K1 in some cases of LCH have been identified. We have studied multiple points of the MAPK pathway without observing alterations that could suggest a relevant role in the oncogenesis of FDCS. The diffuse expression of EGFR has been suggested to reflect the highly complex and heterogeneous cytogenetics of this locus already described in FDCS.6,8 In our series we found eusomic cases, cases displaying low trisomy, and cases displaying low polysomy. However, cases with high EGFR copy number were not identified, and no correlation was observed between EGFR expression and EGFR copy number status. This is in line with the suggestion by Vermy et al, who postulated that EGFR protein expression in FDCS might be due to a ligand-dependent activation by cognate ligands produced in the tumor microenvironment, and that this activation might contribute to sustain viability and proliferation of neoplastic FDCS cells but does not represent a proper oncogenic mechanism. This idea is further supported in our study by the lack of association between EGFR expression and the expression of downstream elements of the MAPK pathway such as p-ERK1/2.

It has been suggested that cyclin D1 expression might be a surrogate marker of MAPK pathway activation in LCH.23,24 In our series, more than one-third of FDCSs expressed cyclin D1. However, we have not identified MAPK pathway–activating alterations in any of the cases, and the only 2 FDCS cases with p-ERK1/2 overexpression did not coexpress cyclin D1, suggesting that cyclin D1 expression might not be related to MAPK pathway status in FDCS. Other mechanisms may be involved in cyclin D1 overexpression in FDCS.44 These observations are in line with what was reported recently by Massoth et al, who studied a cohort of 44 FDCSs with a comprehensive next-generation sequencing panel and did not find any mutations in MAPK pathway genes. In that study, CDKN2A deletions, TP53 mutations, and PTEN truncating mutations were among the most common molecular alterations, although they were not specific to the FDCS group and were also found in malignant histiocytosis. The gene panel used in our study included 8 genes that were also included in the Massoth et al panel (CDKN2A, TP53, PTEN, BRAF, KRAS, NRAS, MAP2K1, and PTPN11). No alterations were found either in MAPK pathway genes or in PTPN11 in any of the 2 studies. Unlike Massoth et al, we did not find alterations in CDKN2A and PTEN. However, most CDKN2A alterations in this study were DNA copy number losses, and our panel could not assess this type of changes. The low frequency of PTEN mutations, present in only 3 of 44 FDCSs in their series, may explain the differences observed between both studies.

The role of TP53 in the evolution from HVCD to FDCS was suggested long ago based on the identification of p53-positive dysplastic FDCs from HVCD cases that later transformed to FDCS. Since then, although p53 overexpression has been described in many case reports,45 TP53 mutations have been elusive, and have been described only in one study and a handful of case reports. Unlike Massoth et al, we did not find alterations in CDKN2A and PTEN. However, most CDKN2A alterations in this study were DNA copy number losses, and our panel could not assess this type of changes. The low frequency of PTEN mutations, present in only 3 of 44 FDCSs in their series, may explain the differences observed between both studies.

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Figure 4. TP53 expression in follicular dendritic cell sarcoma (FDCS) cases. A, Immunohistochemical (IHC) staining against p53 in an inflammatory pseudotumor–like FDCS case, showing only scattered weakly positive cells. B to E, p53 expression in the 4 mutated FDCS cases. All cases displayed a diffuse and strong expression of p53. In C (case 7), the inset shows 17p deletion by fluorescence in situ hybridization (FISH) (p53 IHC, original magnification ×400 [A], ×40 [B through D], and ×200 [E]; FISH, original magnification ×1000 [C inset]).
Figure 5. MYC expression and MYC structural alterations. Three cases of follicular dendritic cell sarcoma showed concomitant MYC amplification and rearrangement. In case 7 (A and B) (dual-color dual fusion probe with IGH, green signal; MYC, red signal; centromeric chromosome 8 [CEP8] in aqua) and case 32 (C and D) (MYC break-apart probe [BAP]), an amplification of the rearranged allele was observed, whereas in case 30 (E and F) (MYC BAP), the amplified allele was the nonrearranged one. Although in the first 2 cases the rearrangement was present in most of the neoplastic cells, in case 30 only 20% of the neoplastic cells presented MYC rearrangement. All 3 cases displayed intense and diffuse MYC expression (MYC immunohistochemistry, original magnification ×200 [A, C, and E]; MYC fluorescence in situ hybridization, original magnification ×1000 [B, D, and F]).
targeted genomic sequencing.\textsuperscript{4,5} In our series, MYC overexpression was observed only in the FDCS group. Furthermore, MYC overexpression correlated with MYC gene structural alterations, suggesting that these alterations impact the expression of the protein and thus could have a role in the oncogenesis of these neoplasms. The presence of MYC alterations and protein overexpression in a high percentage of tumor cells further supports its role as a primary oncogenic event. Although MYC amplification does not seem to be a frequent oncogenic mechanism in lymphoid neoplasms, it is a key alteration in some soft tissue neoplasms, where an association between gene amplification and protein overexpression has been evidenced.\textsuperscript{46} On the other hand, MYC rearrangements (a well-known oncogenic mechanism in lymphomagenesis) have not been previously described in either histiocytic or dendritic cell neoplasms.

In conclusion, our findings support the recent findings by Massoth et al\textsuperscript{41} suggesting that FDCSs do not share the same pathogenic events involved in histiocytic cell neoplasms with which they are classified, an observation consistent with the different cell of origin of those tumors.\textsuperscript{46} We have described MYC structural alterations (both copy number alterations and translocations) as a potential oncogenic hit in the pathogenesis of around 40\% of FDCSs and have identified TP53 mutations in almost a third of FDCSs. Despite not being a main objective of the study, the FISH techniques carried out have allowed us to glimpse differences between FDCS and IPT-like FDCS in terms of cytogenetic complexity. The lower karyotypic complexity in EBV-associated lymphoid neoplasms has already been described in diffuse large B-cell lymphoma\textsuperscript{51} and plasmablastic lymphoma.\textsuperscript{52} The cytogenetic landscape, together with the constant association of EBV, the lack of CXCL13 expression, and the absence of MYC structural aberrations and p53 overexpression, seems to segregate the IPT-like FDCS from the FDCS entity, and suggests that the oncogenesis of FDCS should be different from that of the IPT-like variant, although further studies aiming to characterize specifically the molecular alterations driving the oncogenesis of the IPT-like FDCS variant should be performed to confirm this hypothesis.

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References