ALK kinase.

Oligomerization motif involved in the constitutive activation of the ALK receptor by an unknown molecular mechanism. The aberrant expression of ALK in these tumors results from a rearrangement of the ALK gene with various partner genes including ATIC, TFG, CLTC, CLTC-ALK, TPM3, ALO17, MSN, 1q25, 5q35, Xq12, 2p23, 3q21, 17q23, 5q35, and 3q21. The common molecular feature of all 2p23/ALK-related aberrations is the fusion of the ALK tyrosine kinase domain to the 5′ region of partners, which provides a promotor and most likely an oligomerization motif involved in the constitutive activation of the ALK kinase.

The possible involvement of ALK in B-cell lymphomagenesis also has been hypothesized by Delsol et al. Based upon a series of ALK-positive large B-cell lymphomas (LBCLs) assumed to express full-length ALK receptor by an unknown molecular mechanism.

Here, we report 3 LBCL cases characterized by a granular ALK cytoplasmic immunostaining found to result from an underlying t(2;17)(p23;q23)/CLTC-ALK rearrangement.

Study design

Patients

Cases 1, 2, and 3 are from Ghent, Nijmegen, and Leuven, respectively.

Polymerase chain reaction (PCR) and Southern blot

PCR and Southern blot analysis of IGH/K/L and TCR were carried out as described elsewhere.

Cytogenetics and fluorescent in situ hybridization (FISH)

G-banding analysis and FISH were performed according to previously described protocols. Applied probes included locus-specific identifier (LSI) ALK, whole chromosome paint (WCP) 2, and WCP17 (Vysis, Bergish-Gladbach, Germany), the P1 clone 1111H1 (ALK), and bacterial artificial chromosome (BAC) 758H9 (CLTC).

Combined immunophenotyping and interphase cytogenetic analysis (FICTION) using LSI ALK with either an ALK1 monoclonal antibody (MoAb) or anti-immunoglobulin A (IgA), or anti-IgG (DAKO, Glostrup, Denmark) antibodies were performed on tissue imprints of frozen material and frozen tissue sections (10 μm).

Reverse transcription (RT)–PCR

RNA isolation, cDNA synthesis, nested PCR, and sequencing were carried out to identify ALK fusion transcripts as described elsewhere. To analyze the possible presence of full-length ALK transcripts, 2 RT-PCR experiments were designed amplifying the 5′ end of ALK, primers ALK-ex2f (5′-GCAACATCACGCTTGAAGAACA-3′) and ALK-ex2r (5′-GCTGTT-GAGGAGCCAGGAG-3′), or the 3′ end of ALK, ALK-ex1f (5′-CTCGGC-GAGCAGTGTCAGT-3′) and ALK-ex2r (5′-GGAGAAGGCGATCTGTTGAG-3′). As a positive control for both 5′- and 3′-ALK, cDNA derived from human fetal poly(A)+ RNA (Clontech, Palo Alto, CA) was used. mRNA (1 μg) was reverse transcribed with random hexamer primers in 20 μL of buffer provided by manufacturer; 3 μL cDNA was used for each RT-PCR. With an annealing temperature at 59°C, 35 cycles were performed. The identity of the PCR products was confirmed by sequencing.

Results and discussion

Clinical, pathologic, (cyto)genetic, and molecular features of the cases are summarized in Table 1. Of interest is that the B-cell

From the Departments of Pathology, Clinical Chemistry, Microbiology and Immunology, and Molecular Diagnostics, Center of Medical Genetics and Pediatric Oncology, Ghent University Hospital, Belgium; the Department of Human Genetics and Flanders Interuniversity Institute for Biotechnology, Division for Morphology and Molecular Pathology, Catholic University of Leuven, Belgium; and the Departments of Pathology, Human Genetics, and Pediatric Oncology, University Medical Center Nijmegen, the Netherlands.

Table 1. Characteristics of 3 patients with ALK-positive LBCL

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Case 1</th>
<th>Case 2</th>
<th>Case 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>10</td>
<td>13</td>
<td>26</td>
</tr>
<tr>
<td>Sex</td>
<td>M</td>
<td>F</td>
<td>M</td>
</tr>
<tr>
<td>Clinical presentation</td>
<td>Cervical mass</td>
<td>Cervical mass</td>
<td>Cervical lymph nodes, mediastinal mass, spleen, liver</td>
</tr>
<tr>
<td>Stage</td>
<td>2</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Phenotype</td>
<td>CD3+, CD4+, CD5+, CD45+, CD15+, CD30+, EMA+, CD68+, ALK1+, CD20+, CD79a+ (w), CD138+ (s), Igκ-, Igλ-, IgA+, IgG-</td>
<td>Initial: CD2-, CD3-, CD4-, CD5-, CD45-, CD15-, CD30-, EMA-, ALK1-, CD20-, CD22-, CD23-, CD79a-(w), CD138-, Igκ-, Igλ-, IgA-, IgG-</td>
<td>Initial: CD2-, CD3-, CD5-, CD20-, Igκ-, Igλ-, IgA-; CD3+, CD4+, CD5+, CD45+, CD15+, CD30+, EMA+, ALK1+</td>
</tr>
<tr>
<td>Genotype</td>
<td>PCR: IGH(FR3)-R; TCRγ- R</td>
<td>PCR: IGH(FR1)-R; TCRγ-R</td>
<td>PCR: IGH-R (FR1/2/3); Kε-R, IGL-R</td>
</tr>
<tr>
<td>Diagnosis</td>
<td>ALK-positive LBCL</td>
<td>Initial: ALK-positive null-type ALCL</td>
<td>ALK-positive LBCL</td>
</tr>
<tr>
<td>Therapy</td>
<td>SFOP-LMB 96, group B</td>
<td>ALCL-99 HR</td>
<td>NHL-BFM ALCL99 with ALCL relapse, ABMT</td>
</tr>
<tr>
<td>Outcome</td>
<td>CR</td>
<td>CR</td>
<td>PR</td>
</tr>
<tr>
<td>Follow-up, mo</td>
<td>6</td>
<td>12</td>
<td>3†</td>
</tr>
<tr>
<td>Cytogenetics</td>
<td>Not successful</td>
<td>Not successful</td>
<td>46-47,XX(1q10)(10),del(2)add(2)(p13)(23)(q37;q21), der(5)(3;5)(p25;p34), der(6)(q10), +der(6)(t(6;14)(p21;q11); +del(10)(p12), del(11)(q12)(q15)p24), +14, add(17)(p11),add(17)(q15), +19;inc[p7]; +14, add(17)(q12),add(17)(q21), (q23)</td>
</tr>
<tr>
<td>RT-PCR</td>
<td>ALK-EC mRNA-</td>
<td>ALK-EC mRNA-</td>
<td>nd</td>
</tr>
<tr>
<td></td>
<td>ALK-C mRNA+</td>
<td>ALK-C mRNA+</td>
<td>nd</td>
</tr>
<tr>
<td>FISH† (LSI ALK)</td>
<td>1GO/20/1G+ALK rearrangement</td>
<td>1GO/10/1G+ALK rearrangement</td>
<td>1GO/10/1G=ALK rearrangement+der(2)add(2)(p13)(23)(q37;q21);ish der(2)(l;27)(p23;q23);del(17)(q15);ish der(17)(q21); (p23;q23)</td>
</tr>
</tbody>
</table>

All cases showed monomorphic proliferation of large cells and a granular cytoplasmic ALK1 immunostaining pattern.

EMA indicates epithelial membrane antigen; EBV, Epstein-Barr virus; PCR, polymerase chain reaction; M, male; F, female; s, strong; w, weak; SB, Southern blot; SFOP-LMB, Société Française d’Oncologie Pédiatrique, étude lymphomes B; HR, high risk; NHL-BFM, non-Hodgkin lymphoma-Berlin-Frankfurt-Munster; ABMT, autologous bone marrow transplantation; CHOP, cyclophosphamide, doxorubicin, vincristine, and prednisone; VIM, etoposide, ifosfamide, and mitoxantrone; DHAP, dexamethasone, cytosine, arabinoside, and cisplatin; NR, no remission; RT, radiation therapy; PD, progressive disease; BEAM, BCNU (1,3-bis(2-chloroethyl)-1-nitrosourea), etoposide, cytosine, arabinoside, and methotrexate; CR, complete remission; PR, partial remission; ALK-EC, extracellular portion of ALK; ALK-C, cytoplasmic portion of ALK; nd, not done; G, green signal; and O, orange signal.

* Analyzed using anti-latent membrane protein 1 antibody and EBV-encoded RNA in situ hybridization.
† Interphase and metaphase FISH experiments were performed on tissue imprints (case 1), cytogenetic specimen (case 2 [at diagnosis and relapse]), case 3, and frozen section (case 2 [at relapse]).
‡ Death.
§ Chromosomal aberrations are presented in accordance with the International System for Human Cytogenetic Nomenclature.13

phenotype of case 2, originally diagnosed as null-type ALCL and included in our previous study,1 became evident only at recurrence. Since monoclonal IGH/KL rearrangements were found at relapse (Table 1), diagnostic and relapse biopsies were reanalyzed with a wider spectrum of B-cell markers resulting in a final diagnosis of ALK-positive LBCL. Case 3 was initially diagnosed as diffuse LBCL (DLBCL) with plasmablastic differentiation. ALK expression in this case was found after revision.

All 3 cases showed a granular cytoplasmic ALK expression using the ALK1 MoAb12 (Figure 1B), and the molecular events underlying this expression pattern were investigated. Dual-color FISH with LSI ALK revealed a prominent population of cells with
split signals indicative of ALK rearrangement in all analyzed samples (Table 1). The direct association of ALK rearrangements with the aberrant ALK expression and B-cell immunophenotype of the affected cells was demonstrated by FICTION (fluorescence immunophenotyping and interphase cytogenetics as a tool for the investigation of neoplasm) where LSI ALK was combined with antibodies for ALK or IgA (case 1) (Figure 1C-D), or IgG (case 2) (Figure 1E).

The diagnostic sample of case 2 was already included in our previous study of ALK-positive tumors and shown to contain the CLTC-ALK rearrangement. The same CLTC-ALK fusion product (confirmed by direct sequencing) was detected in the sample at relapse (Figure 2B/case 2). To identify the fusion product of case 1, RT-PCR analysis for some of the known ALK fusions (TPM3-ALK, ATIC-ALK, CLTC-ALK, ALO17-ALK) was carried out. In this case also a CLTC-ALK fusion transcript was present. However, the RT-PCR product was about 100 nucleotides (nt) longer than the product obtained in case 2. Sequencing of the RT-PCR product revealed a fusion of exon 31 of CLTC to exon 20 of ALK, separated by an additional 111 nt (Figure 2D). Sequence analysis suggests that this is the result of the presence of the genomic fusion site 48 nt upstream of ALK exon 20 and the use of a cryptic splice signal in the CLTC intron following exon 31, 63 nt upstream of this genomic breakpoint (Figure 2E). The AG acceptor sequence of this cryptic site is preceded by a 17 nt polypyrrimidine stretch, probably generating a stronger splice signal than the one preceding ALK exon 20. The open reading frame is conserved by this rearrangement. For case 3, again a CLTC-ALK fusion transcript was detected by RT-PCR (Figure 2C) and sequence analysis indicated the same fusion as reported for case 2.

The results of the molecular analysis were further confirmed by metaphase FISH on cases 2 and 3 using LSI ALK, clones covering ALK (1111H1) and CLTC (758H9), and painting...
probes (Figure 1F-G). The FISH patterns were in line with the t(2;17)(p23;q32)/CLTC-ALK.

So far, only very few data on ALK-positive B-cell lymphomas are available in literature.14-17 Of interest is the report of Gascoyne et al18 who found among 70 adult ALCLs, 5 B-cell ALCL cases with ALK expression in both the nucleus and cytoplasm. Only one of these cases displayed a phenotype similar to our cases; in this particular case a t(2;5) was documented by cytogenetics. These data are in contrast with the findings of Delsol et al6 who reported 7 cases of ALK-positive LBCL showing morphologic and phenotypic features of a plasmablastic differentiation. All of these cases were characterized by a granular cytoplasmic staining for ALK1 and a membranous staining for ALK-EC (serum reactive with the extracellular ALK region), assumed to represent expression of full-length ALK receptor through an unknown molecular mechanism. Interestingly, by morphology and phenotypic analysis our 3 cases are very similar to those reported by Delsol et al.6 However, RT-PCR analysis of the present cases with primer sets designed to detected the presence of 3'-ALK but not of 5'-ALK sequences showing that in these cases the ALK reactivity is due to the exclusive expression of CLTC-ALK fusion (Figure 2A).

Occurrence of the same CLTC-ALK rearrangement in all 3 LBCL cases analyzed is intriguing. Although this variant aberration was already found in T-cell ALCL19 and in the inflammatory myofibroblastic tumor,20 it could be a specific event in ALK-positive LBCL. Considering the peculiar morphology and phenotype of these lymphoma cells (CD20+, CD138+, ALK1+, CD30+/-) one might wonder how many similar cases are hidden in the null-type ALCL and DLBLCL groups.

Of interest, the finding of ALK expression/rearrangement in T-/null cell ALCL as well as in LBCL is in line with results published by Kuefer et al,21 Lange et al,22 and Chiarel e et al23 who showed development of either B- or T- and B-cell lymphoma in mice with experimentally overexpressed NPM-ALK.

In conclusion, presented data indicate that ALK activation by CLTC-ALK also plays a role in the pathogenesis of large B-cell lymphomas. The clear correlation of CLTC rearrangement with LBCL is of particular interest and warrants further investigations.

Acknowledgments

The authors thank Ursula Plays, Gerard Merkx, and Femmy Stellink for skillful technical assistance in FISH and cytogenetic analysis; Elisabeth Moreau and Philip Kluin (chairman of the pathology panel of the SNWLK) for pathologic data; and Rita Logist for editorial help.

P.V. is a senior clinical investigator of Fonds voor Wetenschappelijk Onderzoek Vlaanderen.

This text presents research results of the Belgian Programme of Interuniversity Poles of Attraction initiated by the Belgian State, Prime Minister’s Office, Science Policy Programming. The scientific responsibility is assumed by the authors.

References