Complex Variant Translocation t(1;2) With TPM3-ALK Fusion Due to Cryptic ALK Gene Rearrangement in Anaplastic Large-Cell Lymphoma

To the Editor:

Anaplastic large-cell lymphoma (ALCL) is frequently associated with the recurrent translocation t(2;5)(p23;q35) that results in activation of the anaplastic lymphoma kinase (ALK) gene at 2p23 by fusion to the ubiquitously expressed gene encoding the nucleolar phosphoprotein nucleophosmin (NPM) at 5q35. This translocation leads to aberrant nuclear (and cytoplasmic) expression of ALK, which is normally silent in hematopoietic tissues.\(^1\)\(^2\)

Approximately 20% of ALK-positive ALCLs do not express ALK in
the nucleus but show aberrant expression of \textit{ALK} restricted to the cytoplasm.\textsuperscript{3,4} These “cytoplasmic \textit{ALK} only” ALCL do not contain the t(2;5), suggesting that other genetic abnormalities can result in aberrant \textit{ALK} expression. So far, only very limited data on cytogenetic alterations of this subtype of ALCL are available. Nevertheless, in very recent issues of \textit{Blood}, “cytoplasmic \textit{ALK} only” ALCL cases have been described to contain variant rearrangements of the chromosomal region 2p23. Wlodarska et al\textsuperscript{5} reported 3 cases with cryptic inv(2)(p23q35). Rosenwald et al\textsuperscript{6} described 1 case each with t(1;2)(q21; p23) and t(2;3)(p23;q21). Finally, Lamant et al\textsuperscript{7} reported a t(1;2)(q25; p23) in 1 patient. Cloning of the translocation breakpoint showed a fusion of \textit{ALK} to the \textit{TPM3} gene in 1q25 encoding a nonmuscle tropomyosin. A \textit{TPM3-ALK} fusion transcript was detectable by reverse transcriptase-polymerase chain reaction (RT-PCR) in 2 of 3 additional ALCL, with \textit{ALK} staining restricted to the cytoplasm.\textsuperscript{7}

We recently investigated 3 cases of “cytoplasmic \textit{ALK} only” ALCL

![Fig 1. Metaphase FISH with LSI ALK probe (A) and subsequent R-banding analysis (B) shows insertion of genomic material into the ALK locus indicated by spatial separation of the red signal for 3 \textit{ALK} (telomeric) and the green signal for 5 \textit{ALK} (centromeric) on the short arms of both der(2) chromosomes (arrows) as compared with the intact chromosome 2 (arrowhead). (C) In interphase nuclei of tumor cells, insertion into the ALK locus on both derivative chromosome 2 leads to separation of 3 and 5 \textit{ALK} signals in addition to each 1 colocalized, indicating the intact ALK locus. (D) In contrast to the larger nucleus of the tumor cells, the smaller nuclei of nonneoplastic cells show 2 colocalized signals for 3 and 5 \textit{ALK}, which is the regular signal constellation for 2 intact chromosomes 2. (B, small picture) FISH with a chromosome 1 painting probe on R-banded chromosomes shows a major part of the short arm of the der(2) chromosomes to derive from chromosome 1. (E) RT-PCR amplifies a PCR product of approximately 300 bp specific for \textit{TPM3-ALK} fusion (Lane 2). Lane 1, 100-bp ladder; lane 3: H2O control.](image-url)
by means of fluorescence in situ hybridization (FISH). Whereas 2 cases turned out to contain the previously described inv(2)(p23q35), we report here a cryptic insertion into the ALK gene observed in the third patient. The lymph node biopsy specimen was obtained from a 5-year-old male patient with stage IVB disease. Histological evaluation showed a CD30(+)Kil)-positive ALCL of null cell phenotype. Using the ALK1 antibody (Dako, Glostrup, Denmark), only 20% of the tumor cells stained positive, with strong cytoplasmic ALK protein expression but lack of any nuclear ALK expression.

Chromosome analysis of R-banded metaphases derived from unstimulated short-term lymph node cultures showed clonal aberrations in 6 of 16 metaphases. The karyotype was 50 − 51, XY, +X, del(1)(q21), +2, der(2)dup(2)(p25p21)dup(2)(p11p25) × 2, +6, +7, +17 [c(p6)46, XY] [10]. To investigate the case for a cryptic ALK rearrangement, we performed FISH with the LSI ALK assay (Vysis, Downers Grove, IL) according to the manufacturer’s instructions. The LSI ALK assay consists of 2 differentially labeled probes for the centromeric (5′) and telomeric (3′) regions of the ALK gene that showed to be separated in cases with t(2;5) and inv(2). Using this assay, 38 of 100 interphase cells each displayed 2 isolated signals for the centromeric (green) and telomeric (red) ALK probes, indicating 2 rearranged copies of the ALK gene in addition to 1 colocalization of each 1 signal derived from an intact ALK locus. FISH on metaphases followed by subsequent R-banding analysis showed the short arms of the intact chromosome 2 and both der(2) chromosomes each to contain 1 signal for the centromeric and the telomeric ALK probe. Nevertheless, the spatial separation of the 2 ALK probes on the der(2) chromosome suggests an insertion of genomic material into the ALK gene on the der(2) (Fig 1A through D).

As for R-banding analysis, there was no evidence for material from chromosomes other than chromosome 2 to be contained in the der(2) chromosomes, suggesting that a complex inv(2) might lead to insertion of 2q35 material into the ALK locus. To test this hypothesis, FISH with YAC probes 884F10, 770F5, and 914E7 flanking and spanning the 2q35 breakpoint of the inv(2), respectively, was applied. These hybridizations did not provide evidence for the genomic material inserted into the ALK locus to be derived from a complex inv(2).

Considering the loss of chromosome 1 material due to the del(1)(q21) in the tumor cells, it was also tempting to speculate that the material inserted into the ALK locus might be derived from the long arm of chromosome 1. Consequently, FISH on archived R-banded slides was performed with a whole chromosome 1 painting probe (AGS, Heidelberg, Germany). Surprisingly, this analysis showed a major part of the short arms of the der(2) chromosomes to be derived from chromosome 1 (Fig 1B, small picture), thus the present case contains a complex translocation t(1;2)(q12;p23) leading to a TPM3-ALK fusion, we aimed to investigate the case for this hybrid transcript. No fresh material for molecular analyses was available from initial diagnosis and the patient is in continuous complete remission for 2.5 years now after initial treatment according to the German NHL-BFM95 trial (group III-K3). 3 Thus, RNA was extracted from cells in Carnoy’s fixative by means of RNeasy (QIAGEN, Hilden, Germany), and first-strand synthesis was performed with pooled ALK-specific primers (5′-AGC ACA CTT CAG GCA GCC GCT TCT TCA CAG CCC-3′ and 5′-CAT TCC GGA CAC CTG GCC TTA ACA CAC CCC-3′) using the SuperScript III System (Promega, Madison, WI). Nested PCR according to Lamant et al 4 amplified the characteristic PCR product of approximately 300 bp (Fig 1E). Sequencing of this PCR product confirmed the presence of the TPM3-ALK fusion transcript.

In summary, we identified a cryptic ALK gene rearrangement due to an insertion that, by molecular cytogenetics and RT-PCR analysis, turned out to be a complex variant translocation t(1;2) leading to TPM3-ALK fusion. On the one hand, the present case confirms TPM3-ALK fusion to be a recurrent mode of ALK activation in ALCL with ALK expression restricted to the cytoplasm. 3 On the other hand, this case again indicates that ALK gene rearrangements might be cytogenetically hidden, eg, due to complex breakage events. Indeed, in the present case as well as in the cases with inv(2), the ALK rearrangements would not have been cytogenetically detected without applying molecular cytogenetics. Thus, in our opinion, FISH with an ALK-specific probe should be generally integrated into the cytogenetic analysis of “cryptic ALK only”-ALCL so that cryptic ALK rearrangements are not missed.

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REFERENCES


Detection of TT Virus DNA in Plasma-Derived Clotting Factor Concentrates

To The Editor:

Clotting factor concentrates such as factor VIII and factor IX have been used as replacement therapy in patients with hemophilia to control hemorrhagic episodes. Since around 1980, when the delayed announcement of possible human immunodeficiency virus (HIV) contamination in such products became a serious social problem in Japan, patients with hemophilia have been concerned about various viral contamination of blood products.

Recently, an unenveloped, single-stranded DNA virus, named the TT virus (TTV), was identified as a transfusion-transmissible agent reported to be associated with non–A-G hepatitis.1 The reported prevalence rates of TTV have varied widely, depending on the primers used in the polymerase chain reaction (PCR) detection system. The primer set used by Takahashi et al2 is 10 to 100 times more sensitive than the one designed for selection of targeted materials using monoclonal antibodies, is likely to achieve complete elimination of TTV DNA.

Unenveloped viruses tend to be more difficult to inactivate than enveloped viruses. Enveloped viruses, such as vesicular stomatitis virus (VSV) and Sindbis virus, are easily inactivated, whereas unenveloped viruses, such as ECHO virus and poliovirus, are not inactivated by solvent/detergent treatment. Some viruses, such as parvovirus, are thought to be resistant to heat.4 TTV, an unenveloped and putative parvovirus-like virus,5 may be resistant to methods commonly used for viral inactivation. Immunoaffinity chromatography may be recommended for the elimination of viruses resistant to inactivation.

Table 1. Characteristics of 3 Clotting Factor Concentrates

<table>
<thead>
<tr>
<th>Brand</th>
<th>Clotting Factor</th>
<th>Production Steps After Source (viral inactivation)</th>
<th>TTV DNA</th>
<th>Total Investigated Lots (%)</th>
<th>Production</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Factor VIII</td>
<td>(1) Ion-exchange chromatography</td>
<td>28/28 (100%)</td>
<td>28/28 (100%)</td>
<td>Cryoprecipitation</td>
</tr>
<tr>
<td>B</td>
<td>Factor VIII</td>
<td>(2) Nano-filtration</td>
<td>0/24 (0%)</td>
<td>0/24 (0%)</td>
<td>Dry heat 65°C, 96 h</td>
</tr>
<tr>
<td>C</td>
<td>Factor IX</td>
<td>(1) Immunoaffinity chromatography</td>
<td>0/25 (0%)</td>
<td>0/25 (0%)</td>
<td>Immunoaffinity chromatography</td>
</tr>
</tbody>
</table>

*All products are made in Japan from plasma source taken from domestic, screened volunteers. Individual donor plasma is screened using serologic tests for syphilis, HIV1, HIV2, HTLV-1, HBsAg, HBeAb, HCVAb, and ALT. Abnormal plasma is excluded. Brand A and C are made by the same company.
†Tri-(n-butyl)-phosphate (TNBP)/Triton X-100.

REFERENCES
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