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Cytogenetically complex SEC31A-ALK fusions are recurrent in ALK-positive large B-cell lymphomas

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Fusion tyrosine kinases involving anaplastic lymphoma kinase (ALK) are central to the pathogenesis of numerous malignancies, in which they represent important diagnostic and therapeutic targets.1 Van Roosbroeck et al recently reported in this Journal the elegant characterisation of a cytogenetically cryptic SEC31A-ALK fusion arising from complex chromosomal rearrangements in a case of ALK-positive large B-cell lymphoma (LBCL).2 As this fusion had previously been identified only in a single case of inflammatory myofibroblastic tumour,3 the authors proceeded to show that it produced a constitutively active fusion tyrosine kinase able to transform haematopoietic cells in vitro and susceptible to an ALK-selective small molecule kinase inhibitor. However, whether the SEC31A-ALK fusion is a recurrent oncogenic event in lymphoma remained unknown. We now report a second case of ALK-positive LBCL harbouring a complex SEC31A-ALK fusion, confirming it as an important lymphomagenic oncogene and further highlighting difficulties in its cytogenetic identification.
ALK-positive LBCL is a rare tumour of post-germinal centre B-cells which occurs most frequently in adult males, many of whom present with advanced disease and pursue an aggressive clinical course. Most cases carry a t(2;17)(p23;q23)/CLTC-ALK while a minority harbour a t(2;5)(p23;q35)/NPM-ALK. The patient reported here was clinically typical. He was a 66 year-old man with a short history of weight loss, night sweats and dysphagia. Serial imaging showed rapid development of widespread lymphadenopathy, and numerous lymphomatous deposits in the liver and bones. An inguinal lymph node biopsy showed a typical diffuse and sinusoidal infiltrate of large, EBV-negative, immunoblastic/plasmablastic lymphoid cells (Figure 1A) which expressed CD138, IRF4, EMA, CD4, CD45 and perforin, but not several other B or T cell antigens, and showed lambda immunoglobulin light chain restriction. ALK was expressed with a granular cytoplasmic staining pattern (Figure 1B). Bone marrow and duodenal biopsies were similarly involved (Ann Arbor stage 4B). The patient was treated with multi-agent chemotherapy but died three weeks after diagnosis.

Cytogenetic analysis revealed the karyotype 45,XY,der(1;17)(q10;q10),t(2;4)(p2?4;q21) (Figure 1C). Although expression of ALK by the neoplastic cells suggested a translocation involving ALK at 2p23, the breakpoint on chromosome 2 appeared to be at 2p24-25, telomeric to ALK. Fluorescence in situ hybridisation (FISH) using an ALK breakapart probe nevertheless showed a split signal pattern in which the 5' (centromeric) and 3' (telomeric) elements were clearly separated in both interphase and metaphase cells. However, both signals remained nearby in interphase cells and in metaphases they were seen to be in proximity, in the normal orientation, on the p arm of der(2) (Figure 1D-E). These results suggested a complex rearrangement on der(2) involving ALK and a gene at 4q21-qter. Prompted by the report of Van Roosbroeck et al., we investigated the involvement of SEC31A on 4q21 in the formation of a SEC31A-ALK fusion. FISH using an in-house SEC31A breakapart probe showed a split signal in which the 5' (telomeric) element hybridised to der(2) and the 3' (centromeric) element remained on der(4) (Figure 1F). RT-PCR was subsequently performed on RNA isolated from fixed lymphoma tissue using SEC31A exon 24 and ALK exon 20 primers, designed to identify the previously reported SEC31A-ALK fusion. This yielded a correctly-sized PCR product which, when sequenced, confirmed the expected in frame SEC31A-ALK fusion transcript (Figure 1G). In all three SEC31A-ALK translocations now reported, complex rearrangements involving the two partner genes have been observed. These were probably required to generate a functional SEC31A-ALK fusion, as the relative transcriptional orientation of the two genes precludes its formation by a simple reciprocal
translocation. The requirement for a complex rearrangement probably underlies the comparative rarity of SEC31A-ALK amongst ALK fusions. A simple scenario that may be postulated in the present case is t(2;4)(p2?4;q21) followed by inversion of a segment of der(2) including the 3' ends of SEC31A and ALK, bringing together the 5' end of SEC31A and the 3' end of ALK. Unfortunately, whole chromosome painting to further characterise the der(2) gave equivocal results and we were unable to detect the reciprocal ALK-SEC31A transcript by RT-PCR.

This report complements that of Van Roosbroeck et al., confirming SEC31A-ALK as a recurrent event in ALK-positive LBCL. Recognition of this translocation in clinical practice is important for diagnosis of these lymphomas, which are probably under-recognised by histopathology alone, as they often have an aggressive clinical course which may warrant a modified treatment approach and as they may be susceptible to newly developed ALK kinase inhibitors. Cytogeneticists should be aware of the spectrum of complex rearrangements which may underlie SEC31A-ALK fusions. In particular, since ALK breakapart probes may be only minimally separated, vigilance is necessary in the FISH analysis.

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References


Figure 1
Immunohistological, cytogenetic and molecular analysis. (A) Lymph node biopsy (Haematoxylin & eosin stain) showing an immunoblastic/plasmablastic lymphoma typical of ALK-positive LBCL. (B) Granular cytoplasmic expression of ALK by lymphoma cells. Immunostaining was performed with anti-ALK mouse monoclonal antibody clone 5A4 (Leica Biosystems Newcastle Ltd, Newcastle upon Tyne, UK) using heat-mediated epitope retrieval on the Ventana Benchmark automated staining platform. Histology images were captured on an Olympus BX51 microscope with Cell A imaging software, magnification x600. (C) Karyotype derived from lymph node biopsy. (D) FISH using ALK LSI dual-colour breakapart probe (Abbott Molecular, Maidenhead, UK) on interphase cells shows split of one ALK allele with green (5′, centromeric) and red (3′, telomeric) signals remaining in proximity. Arrows: split alleles; stars: intact alleles. (E) FISH using ALK LSI dual-colour breakapart probe on metaphase cells shows subtle separation of one ALK allele on der(2) Arrow: split allele; star: intact allele. (F) FISH using an in-house SEC31A dual-colour breakapart probe (BAC RP11-57B24, Spectrum Green, centromeric to SEC31A; BAC RP11-429022, Spectrum Red, telomeric to SEC31A; labelled probes obtained from Genome Resources Facility, The Centre for Applied Genomics, Hospital for Sick Children, Toronto, Canada) on metaphase cells.
shows split of one SEC31A allele, with the red (5', telomeric) signal translocated to der(2) and the green (3', centromeric) signal remaining in on der(4). Arrows: split allele; star: intact allele. Cytogenetic images were acquired on a Nikon Eclipse 80i microscope with Cytovision 4.5.2 image analysis software (Genetix Europe Ltd, Gateshead, UK). (G) RT-PCR using forward SEC31A exon 24 primers and reverse ALK exon 20 primers, and sequencing of the resulting product, identified a SEC31A-ALK fusion transcript identical to that previously identified. Left panel: mwm, 100 base pair ladder; 1, patient cDNA; 2, negative control (no cDNA); 3, negative control (control cDNA). mwm and column 1 were run non-adjacent on the same gel, and have been juxtaposed during preparation of the figure. RNA was extracted from formalin-fixed paraffin-embedded lymphoma tissue using the Ambion RecoverAll kit (Applied Biosystems, Warrington, UK) and cDNA synthesised using the First-Strand cDNA synthesis kit (GE Healthcare, Little Chalfont, UK) with random hexamers. Forward primers: external SEC31A-F1; 5’ CAGGAGCTCCACCACCATC 3’, internal SEC31A-F2; 5’ GCCTCCTGGAAACACAGGTA 3’; reverse primers: external ALK-R1; 5’ TTGGGGTTGTAGTCGGTCAT 3’, internal ALK-R2; 5’ CGGAGCTTGCTCAGCTTGTA 3’. Right panel: SEC31A-ALK fusion cDNA sequence. Red, SEC31A exon 24; green, ALK exon 20. Primer sequences are underlined. Sequencing of the internal 140 bp PCR product was by Beckman Dye Terminator Cycle Sequencing with primers SEC31A-F2 & ALK-R2 on a Beckman CEQ8000 sequencer.