Anaplastic large cell lymphoma with the t(2;5)(p23;q35) NPM/ALK chromosomal translocation and duplication of the short arm of the non-translocated chromosome 2 involving the full length of the ALK gene

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Abstract
This report describes a case of anaplastic large cell lymphoma with the canonical t(2;5)(p23;q35) translocation in association with duplication of the short arm of the non-translocated chromosome 2, as demonstrated by two colour fluorescence in situ hybridisation. Because the tumour cells were tetraploid, these abnormalities were in duplicate, with four copies of the full length ALK gene and two copies of the t(2;5)(p23;q35) translocation. Despite multiple copies of the normal ALK gene, immunohistochemical, reverse transcriptase polymerase chain reaction, and western blot analysis demonstrated that only the fusion gene NPM/ALK was expressed and that normal ALK genes remained silent. Although based on a single case, these data indicate that structural rather than numerical abnormalities of the ALK gene are implicated in the pathogenesis of anaplastic large cell lymphomas.

Keywords: ALK gene; chromosomal translocation; duplication; NPM/ALK; fluorescence in situ hybridisation

Results and discussion
The immunomorphological methods confirmed the diagnosis of common type anaplastic large cell lymphoma with the coexpression of CD30 and epithelial membrane antigen (EMA), T cell markers, and nuclear and cytoplasmic expression of the chimaeric NPM/ALK protein specific to the t(2;5)(p23;q35)
translocation. However, in this case the size of the malignant cells was larger than is usually seen in common type anaplastic large cell lymphoma.

The cytogenetic study displayed the following tetraploid karyotype: 94,XXYY,+X,+Y, t(2;5)(p23;q35)×2,ish t(2;5)(ALK+;ALK+)×2, dup(2)(p11;p25)×2,ish dup(2)(ALK++)×2, +5, add (8)(p23)×2, I(17)(q10) (fig 1). The four copies of chromosome 2 were abnormal. Two of them resulted from a standard t(2;5) translocation and had the characteristic aspect of the derivatives 2 generated by t(2;5). The cytogenetic aspect was confirmed by in situ hybridisation with the Vysis LSI ALK probe (Ref 32-190069). This probe contains two fragments located on each side of the breakpoint at 2p23: a 5’ centromeric fragment (green signal) and a 3’ telomeric fragment (red). The 3’ telomeric fragment was translocated on to the derivative 5 at 5q35, whereas the 5’ centromeric fragment remained on the derivatives 2 of t(2;5) (fig 1). The four copies of chromosome 2 were abnormal. Two of them resulted from a standard t(2;5) translocation and had the characteristic aspect of the derivatives 2 generated by t(2;5). The cytogenetic aspect was confirmed by in situ hybridisation with the Vysis LSI ALK probe (Ref 32-190069). This probe contains two fragments located on each side of the breakpoint at 2p23: a 5’ centromeric fragment (green) and a 3’ telomeric fragment (red). The 3’ telomeric fragment was translocated on to the derivative 5 at 5q35, whereas the 5’ centromeric fragment remained on the derivatives 2 of t(2;5) at 2p23. The other two copies of chromosome 2 had a duplication of the short arm between 2p11 and 2p25. Because the ALK gene is located within the duplicated material at 2p23 there were two copies of the ALK gene on each chromosome 2. The tetraploid clone must have resulted from a duplication of an initial pseudodiploid clone, as demonstrated by the similar aspects of the two der(2)(p23;q35) and of the dup(2)(p11;q35) (fig 1).

RT-PCR analysis confirmed the presence of NPM/ALK transcripts in the absence of the full length ALK transcripts (not shown). Similarly, western blot analysis showed that only the chimaeric NPM/ALK (80 kDa) and not the full length ALK protein (200 kDa) was expressed (fig 2).

The process by which the t(2;5)(p23;q35) translocation transforms cells is not known. However, despite the fact that there are some anaplastic large cell lymphoma cases that are negative for this translocation, in vivo and in vitro models have provided evidence that this cytogenetic abnormality is by itself oncogenic. The ALK protein, the normal functions of which have yet to be elucidated, is expressed in rare cases of non-anaplastic large cell lymphoma and in a few normal tissues. Theoretically, this protein functions as a tyrosine kinase receptor but its ligand remains unknown. So far, the activation of the tyrosine kinase domain of ALK has been shown to be the consequence of the gene rearrangement. The chromosomal partners isolated to date, namely NPM and TPM3, are proteins with dimerisation domains, enabling the formation of NPM/ALK and TMP3/ALK homodimers, and thereby allowing the activation/autophosphorylation of the TK domain of ALK. So far, numerical abnormalities of the normal ALK gene have not been demonstrated. We describe herein a case of anaplastic large cell lymphoma with four copies of the normal ALK gene and two copies of the t(2;5)(p23;q35) translocation. The cytogenetic pattern suggests that these structural abnormalities occurred before the cells became tetraploid. Regarding ALK expression, our data show that the normal ALK gene, even in multiple copies, remains silent and that only the NPM/ALK gene is expressed in this case. This implies that downregulation of the normal ALK gene is maintained.
Although based on a single case, these data indicate that structural rather than numerical abnormalities of the ALK gene are implicated in the pathogenesis of anaplastic large cell lymphomas.

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