Short reports

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

Anaplastic large cell lymphomas of T cell or null cell phenotype are particularly frequent in children.1 These tumours have a good prognosis and are frequently associated with a specific chromosomal translocation t(2;5)(p23;q35) involving the ALK gene (2p23), which encodes a tyrosine kinase that is not expressed in normal lymphoid cells.2 3 This abnormality has been shown to be oncogenic in vivo and in vitro.4 5 In addition, a few cases with cytogenetic variants have been characterised and all involve a rearrangement of the ALK gene.6 7 All patients with 2p23 rearrangements produced a chimaeric protein containing the C-terminal domain (tyrosine kinase domain) of the ALK protein. In the t(2;5)(p23;q35)7 and the t(11;2)(q23;p23)8 translocations the chimaeric proteins are able to dimerise and thus induce autophosphorylation of the tyrosine kinase domain of ALK, which becomes activated in the cytoplasm of the tumour cells. To date, neither the pathway by which ALK transforms the cells nor the function of the normal ALK gene is known.9 Here, we report a case of anaplastic large cell lymphoma with structural as well as numerical abnormalities of the ALK gene.

Methods

The material tested in our study was an inguinal lymph node removed surgically from a 10 year old boy who presented with an isolated lymphadenopathy without clinical and biological abnormalities. The tissue was studied using standard protocols for routine pathology (fixed in Duboscq-Brasil fluid), molecular techniques (frozen in liquid nitrogen), and cytogenetic studies (soaked in RPMI). Immunohistochemical and the reverse transcriptase polymerase chain reaction (RT-PCR) were performed as described previously.3 Western blot analysis was performed with standard protocols7 using the ALKC monoclonal antibody (kindly provided by Dr B Falini, University of Perugia, Italy). Standard cytogenetic analysis3 7 was carried out and combined with a fluorescence in situ hybridisation (FISH) method using a two colour ALK probe (VysisTM LSIR ALK dual colour rearrangement probe; Vysis, Voisins le Bretonneux, France).9

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) x 2, t(2;5)(alk+;alk+), dup(2)(p11;p25) x 2, add(8)(p23)x 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 differently coloured probes labelling opposite sides of the breakpoint in the ALK gene found in the t(2;5) translocation. The telomeric fragment (red) is translocated on to the der(5) and the centromeric fragment (green signal) remains on the der(2): derivatives resulting from the t(2;5) translocation. Two copies of the ALK gene (indicated as dup ALK) are present on the two copies of dup(2)(p11p25). Two copies of the der(2), of the der(5), and of the dup ALK are seen because of tetraploidy.

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 forms cells is not known. However, despite the fact that there are some anaplastic large cell lymphoma cases that are negative for this translocation,1 in vivo and in vitro models have provided evidence that this cytogenetic abnormality is by itself oncogenic.45 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.8 Theoretically, this protein functions as a tyrosine kinase receptor but its ligand remains unknown.8 So far, the activation of the tyrosine kinase domain of ALK has been shown to be the consequence of the gene rearrangement.7 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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