Physical localisation of the breakpoints of a constitutional translocation t(5;6)(q21;q21) in a child with bilateral Wilms' tumour

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Abstract

A 6 month old boy presented with bilateral Wilms' tumour. Cytogenetic analysis of the lymphocytes from the patient showed a de novo balanced translocation t(5;6)(q21;q21), which was also present in the tumour material as the sole cytogenetic abnormality. To facilitate the identification of the translocation breakpoints, we have established a lymphoblastoid cell line (MA214L) from the patient which maintains the translocation in culture. We have used Genethon microsatellite markers as sequence tagged sites (STSs) to isolate yeast artificial chromosome (YAC) clones to 5q and 6q from human genomic libraries. Using fluorescence in situ hybridisation (FISH) on metaphase preparations of MA214L, we have physically defined the translocation breakpoints between YAC clones on each chromosome arm. The genetic distance separating the flanking YACs on 6q21 is 3 cM, while that on 5q21 is 4 cM. To date this is the first report of these chromosomal regions being implicated in Wilms' tumourigenesis.

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Wilms' tumour is a paediatric lesion of the kidney and is one of the most common solid malignancies of childhood. Disease can occur in one or both kidneys, approximately 8% of cases being bilateral. The suggestion of a genetic component in the aetiology of the tumour has come from several observations. Firstly, bilateral disease is associated with an early age of onset. Secondly, there is a high incidence of bilateral tumours in cases with a family history of Wilms' tumour and in patients with associated congenital anomalies.[1] Histological features indicate that the tumour occurs as a result of aberrant embryological development of the kidney. The disease is therefore an ideal model for studying the association between processes involved in tissue development and predisposition to malignancy.[2]

Molecular and cytogenetic analysis of Wilms' tumours has implicated the involvement of several different loci in the development of the disease. These chromosomal regions include 11p13, 11p15, 16q, and chromosome 7.[3 4] At present, only one gene that has been shown to be involved in the disease has been identified. The gene, WT1, was cloned from the 11p13 region which is frequently deleted in Wilms' tumour patients with associated congenital malformations, including aniridia, genitourinary tract anomalies, and mental retardation (collectively called the WAGR syndrome).[5] WT1 encodes a zinc finger DNA binding transcription factor and is mutated or deleted in 10% of non-familial tumours examined. A role for WT1 in the developing kidney and genitourinary tract has been postulated.[2 3] It has been shown by linkage analysis that none of the existing candidate loci encodes the primary genetic defect in familial Wilms' patients.[3] Recently, however, linkage to 17q has been reported in a large Canadian Wilms' tumour family.[6] It is likely, however, that additional loci exist which are involved in Wilms' tumour predisposition. We have identified a patient with early onset bilateral Wilms' tumour. Cytogenetic analysis of patient lymphocytes showed a de novo balanced translocation t(5;6)(q21;q21). As far as we are aware these regions have not previously been described as being important in the development of Wilms' tumour.

Materials and methods

PATIENT MATERIAL/CYTOGENETIC ANALYSIS
Peripheral blood from the patient was collected in heparin, and lymphocytes separated using standard procedures. A lymphoblastoid cell line (MA214L) was established through EBV transformation of the patient's lymphocytes.[7] Karyotype analysis was performed on G banded metaphases from the patient's lymphocytes and MA214L as described by Seabright.[8]

YAC LIBRARY SCREENING
Zeneca and CEPH human YAC libraries were screened directly by PCR with primers specific for microsatellites genetically linked to chromosomes 5q and 6q.[9 10] Isolated clones were grown and DNA was extracted.

FISH ANALYSIS
FISH analysis with YAC clones was carried out as previously detailed.[11] Briefly, YAC DNA (1.5 µg) was labelled with biotin-16-dUTP for single coloured FISH analysis using a nick translation system (BRL). In two colour analysis, DNA from two separate YAC clones was labelled, one with biotin-16-UTP, the other

Figure 1 Partial karyotype of the t(5;6)(q21;q21) in MA214L. Arrows indicate the 5q21 and 6q21 breakpoints.

with digoxygenin-11-dUTP. Labelled DNA was hybridised to denatured metaphase chromosomes for three days at 37°C. Following stringent washing, biotin labelled probes were detected with avidin-Texas red and biotinylated antavidin. The digoxygenin labelled probes were detected with mouse antidigoxygenin and FITC conjugated antibodies. The chromosomes were counterstained with DAPI (0.5 µg/ml) and viewed under a Zeiss Axioskop fluorescence microscope. Images were captured with a Photometrics CCD camera and analysed using a Digital Scientific Smartcapture system.

Results and discussion

We have described a patient with early onset bilateral Wilms' tumour. Cytogenetic analysis of the patient's lymphocytes showed a de novo translocation t(5;6)(q21;q21) as the sole cytogenetic abnormality (fig 1). The translocation was also present in the tumour material. The patient had no other congenital abnormalities and after treatment is alive and developing normally. Parental chromosomes were normal.

The characterisation of cytogenetic aberrations in cancer has led to the identification of genes important in the disease process.[12] It is possible that the association between the observed translocation and the presence of a tumour in the patient may be fortuitous. However, it is equally likely, given the patient's history, that either 5q21 or 6q21 or both contain sequences involved in predisposition to Wilms' tumour. As far as we are aware these regions have not previously been described as being involved in the development of the malignancy. However loss of heterozygosity (LOH) of the long arm of chromosome 6 involving the 6q21 region has been reported in a number of other cancers including renal, breast, and ovarian carcinomas.[13-15] In addition, cytogenetic deletions involving 6q21 have been reported in non-Hodgkin's lymphoma and acute lymphocytic leukaemia.[16] The region 6q21 is also the site of a gene(s) involved in cellular senescence.[17] LOH at chromosome 5q21 has been shown in malignancies including familial adenomatous polyposis, colorectal and gastric carcinoma, and human small cell lung cancer. The tumour suppressor genes APC and MCC isolated from this region have been implicated in the development of these tumours.[18-20]

We aimed to localise the translocation breakpoints in this patient with a view to cloning the region and identifying sequences which
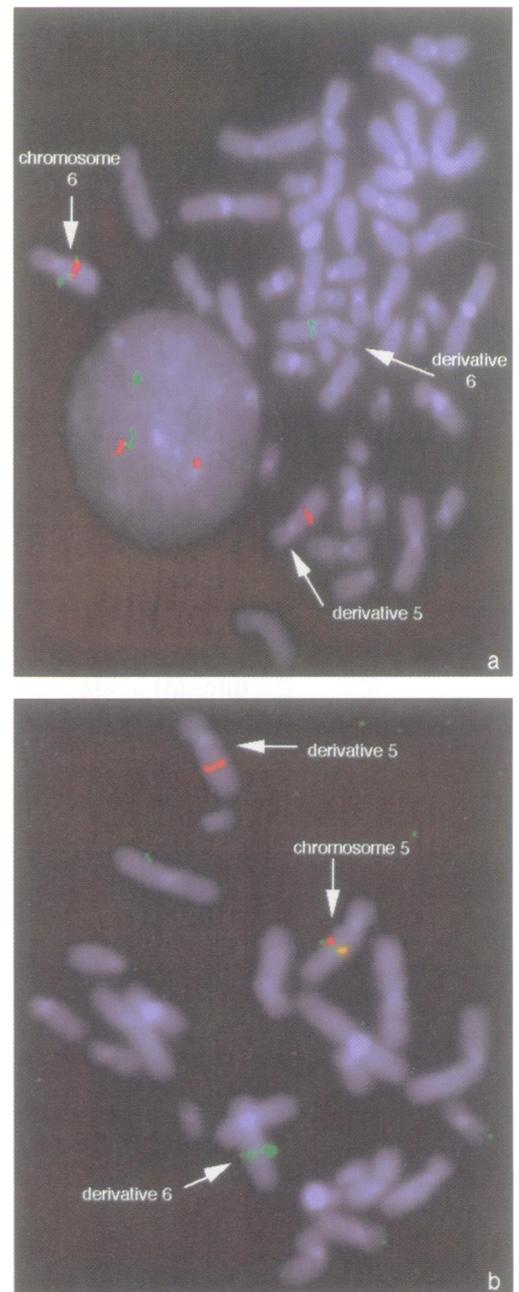

Figure 2 Localisation of the translocation breakpoints by dual colour FISH. (a) Hybridisation of the YACs corresponding to D6S301 and D6S447 onto metaphase chromosomes from MA214L. D6S301 labelled with digoxygenin-11-dUTP was detected with fluorescein conjugated antibodies and is visualised as a green signal. D6S447, labelled with biotin-16-dUTP, is detected with Texas Red and visualised as a red signal. D6S301 remains on the derivative chromosome 6 while D6S447 is moved by the translocation to the derivative chromosome 5. (b) Hybridisation of the YACs corresponding to D5S495 (visualised as a red signal) and D5S433 (visualised as a green signal) onto metaphase chromosomes from MA214L. D5S495 remains on the derivative chromosome 5 while D5S433 is moved by the translocation to the derivative chromosome 6.

predispose to bilateral Wilms' tumour. To facilitate the study we have established a lymphoblastoid cell line (MA214L). Karyotype analysis indicated that the reciprocal translocation was the sole abnormality in MA214L (fig 1). We used YACs isolated to Genethon microsatellites genetically linked to chromosome 5q and 6q in FISH analysis of MA214L metaphases. The localisation and

Table 1 Physical localisation of YAC clones isolated from the Zeneca and CEPH human YAC libraries by Genethon microsatellites[10] from (A) 6q21 and (B) 5q21

Locus symbol	YAC coordinate	Physical localisation
(A)		
D6S445	16HH2	6q14
D6S462	16IG10	6q16.1
D6S450	17FC11	6q16.1
D6S275	38GE5	6q16.1
D6S283	966-b-10	6q16.3
D6S301	9DB4	6q21
D6S447	830-d-9	6q21
D6S268	6GG5	6q21
D6S404	17DA11	6q21
(B)		
D5S647	27HB9	5q12
D5S424	24FC2	5q13
D5S618	31EG12	5q15
D5S495	31AG3	5q21
D5S433	38DG2	5q21
D5S492	4DB6	5q22

degree of chimaerism of the newly isolated YAC clones was first determined through single colour FISH analysis of metaphase preparations of normal lymphocytes.[11] The designation of microsatellites and cytogenetic location of isolated YAC clones is shown in table 1. To identify the YAC clones flanking the translocation breakpoints, YACs localised to 5q21 and 6q21 were then used in two colour FISH analysis of metaphase preparations from MA214L. The breakpoints on chromosome 6q21 were identified as lying between YACs isolated to microsatellites D6S301 and D6S447 (fig 2a), which are separated by a genetic distance of 3 cM.[10] The breakpoints on chromosome 5q21 were identified as lying between YACs isolated to D5S495 and D5S433 (fig 2b), which are separated by a genetic distance of 4 cM.[10]

Recently, in a separate cytogenetic study investigating trisomy 12 in Wilms' tumour, we identified a translocation t(2;6)(p25;q21) in the tumour material from a patient with sporadic malignancy; normal kidney cells from the same preparation had a normal karyotype, 46,XY (Mitchell *et al*, manuscript in preparation). Collectively, this may suggest 6q21 as being the more important of the two regions in the development of Wilms' tumour. Now that the breakpoints of the translocation on each chromosome arm have been physically defined, we are in a position to select intervening microsatellite markers in an attempt to isolate YAC clones that span the translocation break-

points. Such a YAC will facilitate the identification of the target gene(s) involved in the translocation, in addition to investigating the role of this region in the development of Wilms' tumour and malignancy in general.

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