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Reciprocal Translocation at 1p36.2/*D1S160* in a Neuroblastoma Cell Line: Isolation of a YAC Clone at the Break

L.C. Amler, R. Corvi, C. Praml, L. Savelyeva, D. Le Paslier and M. Schwab

Band 1p36.1-1p36.2 is frequently involved in chromosomal aberrations of neuroblastoma cells, and therefore thought to harbour genetic information which may be involved in tumorigenesis. To map this putative neuroblastoma locus, we screened neuroblastoma cell lines for reciprocal translocations at 1p36.1-2 which may signal the site of an affected gene. We identified a reciprocal 1;15 translocation in cell line NGP by fluorescence *in situ* hybridisation (FISH). As a strategy to clone the translocation breakpoint, we isolated yeast artificial chromosomes (YACs) specific for loci at 1p36. Screening of cell line NGP by FISH identified a YAC, 1050 kbp in size, which hybridised to both derivative 1;15 and 15;1 chromosomes. We conclude that this YAC, which maps to *D1S160*, covers the break. This chromosomal position is within the smallest region of overlap (SRO) found in neuroblastoma tumours and within the region of a constitutional interstitial deletion of a neuroblastoma patient. The YAC we describe here should serve as a DNA source for gene cloning approaches towards the isolation of candidates for the putative neuroblastoma suppressor gene.

Key words: paediatric cancer, tumour suppressor gene, yeast artificial chromosome, positional cloning
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INTRODUCTION

CYTOGENETIC inspection of neuroblastoma cells has revealed deletions and alterations of 1p32-pter in a significant number of patients [1–3]. This finding indicates the presence of genetic material which, if inactivated, can contribute to the tumorigenesis of neuroblastoma. Molecular studies of neuroblastoma DNA have established the smallest region of overlap deletion (SRO) that is localised within 1p36 in up to 89% of tumours [4–7]. Loss of heterozygosity (LOH) studies involving microsatellite loci and fluorescence *in situ* hybridisation (FISH) defined the alterations to sub-bands 1p36.1-2 [8–10]. The significance of alterations in 1p36.1-2 is further supported by the identification of constitutional changes in patients with neuroblastoma, a reciprocal translocation at 1p36 [11] and an interstitial deletion involving the same region [12]. These findings support the idea that a tumour suppressor gene involved in neuroblastoma is located at 1p36.1-2. Attempts to isolate this gene have not been successful as yet, which is mainly due to our lack of information about its precise location within the deleted region. The identification of a chromosomal alteration which directly involves the suppressor locus could overcome this limitation.

To identify such an alteration, we have surveyed neuroblastoma cell lines by FISH for the presence of a reciprocal or balanced translocation at 1p36 which might affect the postulated suppressor gene. Here we show that the cell line NGP has a reciprocal translocation (1;15) (p36.2;q24). Screening of the cell line with yeast artificial chromosomes (YACs) of the 1p36 region identified a non-chimaeric 1050 kbp YAC at *D1S160* which covers the translocation.

MATERIALS AND METHODS

Cell lines and preparation of metaphases

Neuroblastoma (NG-P) cells were cultured in RPMI supplemented with 10% fetal calf serum (FCS) and were arrested in metaphase with colcemid. Fixing of cells was performed according to routine procedures.

DNA labelling

For FISH, the *p1-79/D1Z2* plasmid [13], total chromosome 1 and chromosome 15 libraries (a gift of Joe Gray, University of California, San Francisco, U.S.A.), YACs were either labelled with biotin-16-dUTP or digoxigenin-11-dUTP (Boehringer Mannheim) according to the method of Langer and associates [14].

Fluorescence in situ *hybridisation*

FISH was done as previously described [9, 15]. Probe concentrations were 3 ng/μl for *1p79*, 6 ng/μl for YACs, and 100 ng/μl for the chromosome 1 and 15 libraries. Repetitive sequences in the chromosome libraries were suppressed with a 10-fold excess

Correspondence to L.C. Amler.
L.C. Amler, R. Corvi, C. Praml, L. Savelyeva and M. Schwab are at the Department of Cytogenetics, German Cancer Research Center, Im Neuenheimer Feld 280, D-69120 Heidelberg, Germany; and D. Le Paslier is at the Centre d'Etude du Polymorphisme Humain, Paris, France.

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of *Cot-1* DNA (Bethesda Research Laboratories, Life Technologies, U.S.A.), in case of the YACs with a 100-fold excess.

YAC screening and characterisation

The CEPH YAC library [9] and the CEPH Mega YAC library [16] were screened by PCR. The sequences of the primers for microsatellite loci *D1S160* and PCR conditions are published [17]. DNA from yeast colonies containing YACs was prepared in agarose plugs (Seaplaque; FMC Corp.) as described [18]. Pulsed field gel electrophoresis was performed in a Rotaphor apparatus (Biometra, Version 5.1) according to published methods [19]. After electrophoresis, the separated DNA was transferred to nylon membranes (Amersham, Hybond N^+) and hybridised with ^{32}P-labelled total human DNA [19]. Sizes were determined and compared with yeast or lambda size standards (Biometra). YACs were judged to be non-chimaeric from the FISH analysis on normal chromosomes.

RESULTS

Neuroblastoma cell line NGP has a reciprocal 1;15 (*p*36.2;*q*14) *translocation*

To identify alterations that would signal the position of the postulated neuroblastoma suppressor-locus, we analysed the status of distal 1p in human neuroblastoma cell lines. Metaphases of chromosomes were probed by FISH with DNA from a total chromosome 1 library together with probe *D1Z2*, which recognises the telomeric relgion of 1p [13]. All lines, except one (NGP), showed a deletion of the distal 1p, often associated with an unbalanced translocation by which the distal 1p was replaced by a portion of another chromosome, in the majority of cases from 17q [10, 20] (data not shown). In cell line NGP [21], we detected the distal portion of chromosome 1p to be translocated to the derivative chromosome 15. Additionally, NGP carries one apparently normal copy of chromosome 1. To confirm this observation, we employed dual colour FISH using both chromosome 1 and 15 specific DNA libraries as probes. Inspection of metaphase chromosomes revealed that the distal part of chromosome 15 was translocated to the der(1) (Figure 1a). This observation clearly demonstrated that the translocation is reciprocal. In addition, we detected the presence of 1q material translocated to chromosome 10.

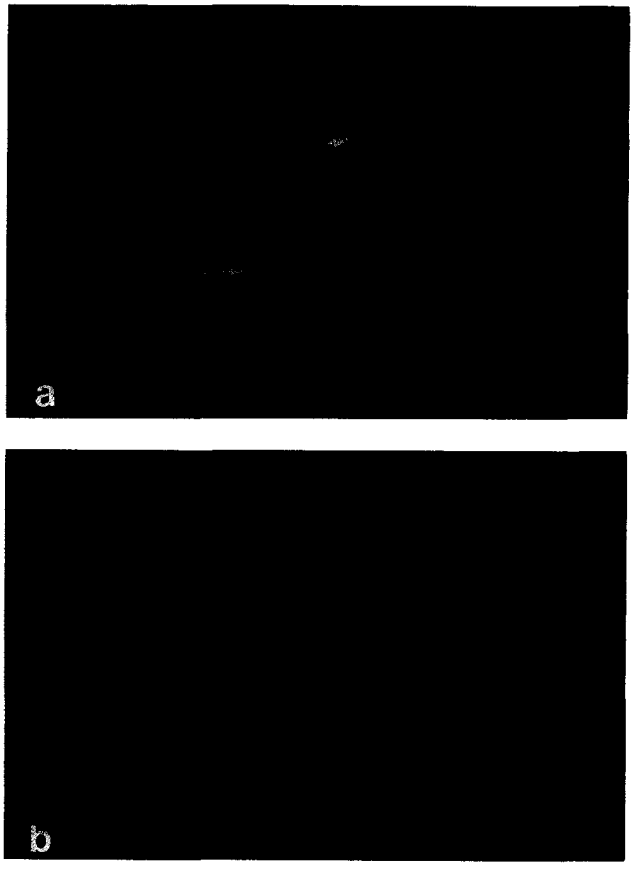

Figure 1. Fluorescence *in situ* hybridisation (FISH) analysis of metaphase chromosomes and interphase nuclei of NGP. (a) Identification of the reciprocal translocation. Total chromosome 1 library labelled with biotin and total chromosome 15 library labelled with digoxigenin were hybridised to NGP metaphases and detected with FITC (green) and rhodamine (red). The chromosomes were counterstained with DAPI. Short arrow: 1p material translocated to chromosome 15; long arrow: 15q material translocated to chromosome 1. A yeast artificial chromosome covers the translocation. (b) *D1S160*: YAC 974G4 defining the position of the break. Three signals are detectable: one on the normal chromosome 1, one on the der(1) and one on the der(15).

YAC 974G4 specific for D1S160 *covers the translocation breakpoint*

To clone the translocation breakpoint in NGP, we employed FISH, and used as the probes YACs which were isolated from the CEPH YAC libraries [16, 18] by PCR screening, based on the sequences of clones from a microdissected 1p36 specific library [5], sequence tagged sites (STSs) that are genetically mapped [22], and gene sequences which were already known to be located at 1p36. YAC clones 927G4 and 924D8, which have been proven to be specific for *D1S160* [17] by PCR (Figure 2b), yielded three signals on metaphases of NGP as if they would identify a region involving the translocation break. These three signals identified the der(1) and der(15) markers, in addition to the normal chromosome 1 (Figure 1b). YAC 927G4 was judged to be non-chimaeric by FISH on metaphases of normal cells. The size of this YAC was determined to be 1050 kbp by PFGE (Figure 2a). YAC50 specific for *D1S149* could be mapped proximal to the translocation (data not shown).

In this study, we demonstrated by FISH that cell line NGP contains a reciprocal 1;15 translocation involving 1p36.2. A YAC specific for the STS *D1S160* was shown to hybridise to both der(1) and der(15) marker chromosomes and, therefore, defines the genetic region of the translocation. The insert of YAC 974G4, 1050 kbp in size, can serve as the DNA source for future studies on the reciprocal translocation in neuroblastoma cell line NGP.

DISCUSSION

Cytogenetic and molecular studies of neuroblastoma cells in the past years have established a high frequency of alterations within band 1p36. The isolation of genetic information specifically contributing to tumorigenesis has not yet been successful due to the large size and genetic complexity of the target region in 1p36. We have, for the first time, identified a neuroblastoma cell line carrying a reciprocal translocation involving 1p36.1-2. The fact that alteration of a copy of chromosome 1 has been described in the original report on line NGP [21], would indicate that the translocation is not a tissue culture artefact, but was present in the original tumour cells. Although unbalanced translocations involving the short arm of chromosome 1 are quite frequent [10, 20], the positions of the breakpoints seem to be

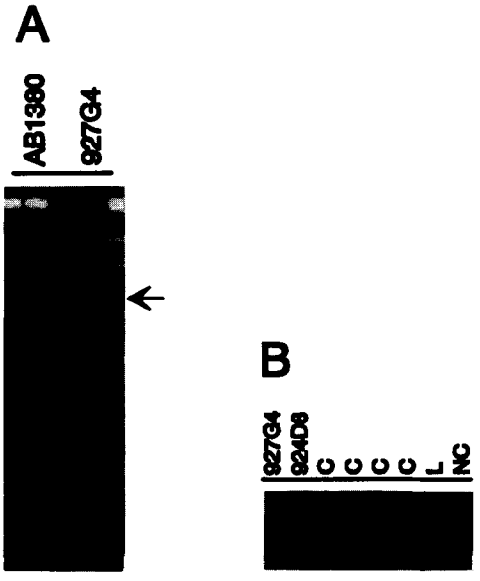

Figure 2. YAC 974G4 is 1050 kbp in size and specific for *D1S160*. (a) Size of YAC974G4 (arrow) was determined by PFGE in comparison with normal yeast chromosomes (strain AB1380). (b) PCR analysis with primers specific for *D1S160*; 974G4 and 924D8, positive for *D1S160*. C, negative controls, unrelated YACs; L, 20 ng normal human DNA; NC, no template control.

irrelevant for neuroblastoma as these alterations are accompanied by deletions of chromosome 1 material. It is possible that the receiprocal translocation we describe here could signal the position of the postulated neuroblastoma suppressor gene.

The reciprocal translocation in NGP is located within the SRO in neuroblastoma cells

Our study by FISH has shown that neuroblastoma cell line NGP carries a reciprocal (1;15) (p36.2;q24) translocation. Attempts to localise the exact position of the translocation break on 1p identified *D1S160* as the locus closest to the translocated region. This was demonstrated through experiments with a YAC which hybridised to both der(1) and der(15) chromosomes involved in the translocation.

How does the available definition of the translocation break compare to the information which has accrued so far? There are three independent settings which have provided relevant information:

(i) The "smallest overlapping region of deletion" (SRO), also referred to as consensus deletion has been identified in neuroblastoma tumour cells. Three independent LOH studies have shown *D1S214* and *D1S96* to be located within the SROs [5, 8, 20]. The genetic distance from *D1S214* and *D1S96*, to the translocation in NGP at *D1S160* is 7 cM [23, 24]. *D1S228*, which is 9 cM proximal to the NGP break, represented the proximal non-deleted border of the SRO in the study of Schleiermacher and associates [8]. Collectively, we can conclude that the translocation is localised within the SROs of neuroblastoma.

(ii) A constitutional deletion of 1p36 in a neuroblastoma patient could be identified. The deleted region has been mapped to *D1S47* [12]. This marker is located in the same region as the SROs for neuroblastoma, and was mapped genetically 9.2 cM distal to *D1S160* [17], which is in very close vicinity to the translocation of NGP. These data show that the constitutional deletion maps to the region which is affected by the translocation in NGP.

(iii) A constitutional reciprocal translocation (1;17) (p36.31-36.21;q12-21) was detected cytogenetically in fibroblasts of a neuroblastoma patient [11]. The break was mapped recently to *D1S149* [25]. In our study, this marker clearly mapped proximal to the translocation in NGP. It is, therefore, unlikely that both translocations involve the same region or affect the same genetic information. This would support the assumption that either more than one locus at 1p36 could play a role in neuroblastoma [26], or alternatively that either the translocation in NGP, or the constitutional translocation are innocuous bystanders unrelated to neuroblastoma.

Currently, the reciprocal translocation in cell line NGP represents the smallest chromosomal alteration at 1p36 described in cells of neuroblastoma. Assuming that the 1p36.2 rearrangement in NGP cells has a role in tumorigenesis, the search for genes affected by this alteration could be an important step towards the isolation of the neuroblastoma suppressor gene.

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Molecular Cytogenetic Analysis of 1;17 Translocations in Neuroblastoma

N. Van Roy, N.C. Cheng, G. Laureys, G. Opdenakker, R. Versteeg and F. Speleman

Loss of chromosome 1 short arm material, resulting from terminal deletions or unbalanced translocations, is a frequent finding in advanced neuroblastoma. In translocations, often relatively small portions of a second chromosome are translocated to the chromosome 1 short arm. The chromosomal origin of this translocated material could often not be identified using banding analysis only. Recent studies, applying fluorescent *in situ* hybridisation, showed that in the majority of these translocations, chromosome 17 is involved. In this study, the nonrandom occurrence of unbalanced 1;17 translocations is further supported by their presence in 3/7 neuroblastoma cell lines. Analysis of the 1p breakpoints extends our earlier observation of breakpoint heterogeneity. A similar scattering of 17q breakpoints was observed. The 1p and 17q breakpoints of the constitutional 1;17 translocation did not coincide with any of the 1;17 translocation breakpoints found in neuroblastoma cell lines. Cell lines, not containing 1;17 translocations, contained other chromosome 17 rearrangements. As a result, extra copies of 17q are found in all cell lines, suggesting a role for genes on 17q in neuroblastoma development. The possible significance of 1;17 translocations in neuroblastoma is discussed.

Key words: 1;17 translocation, chromosome 17 rearrangement, neuroblastoma cell line
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INTRODUCTION

IN ADVANCED neuroblastomas and neuroblastoma cell lines cytogenetic analyses have revealed frequent occurrence of chromosome 1 rearrangements resulting in loss of distal 1p material [1]. The sizes of the lost chromosome 1p segments have varied considerably [2–4]. Molecular studies on larger series of primary tumours have confirmed the high incidence of allelic loss for 1p loci in neuroblastomas [5–7]. This high incidence of deletions has suggested the presence of a gene with tumour suppressor activity [8]. Recent loss of heterozygosity studies on primary tumours and deletion mapping data in cell lines suggest that at least three distinct loci on the chromosome 1 short arm are involved in different subsets of neuroblastoma: an imprinted distal suppressor locus on 1p36.23-31 in *MYCN* single copy