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Original Paper

Analysis of 1;17 Translocation Breakpoints in Neuroblastoma: Implications for Mapping of Neuroblastoma Genes

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Deletions and translocations resulting in loss of distal 1p-material are known to occur frequently in advanced neuroblastomas. Fluorescence *in situ* hybridisation (FISH) showed that 17q was most frequently involved in chromosome 1p translocations. A review of the literature shows that 10 of 27 cell lines carry 1;17 translocations. Similar translocations were also observed in primary tumours. Together with the occurrence of a constitutional 1;17 translocation in a neuroblastoma patient, these observations suggest a particular role for these chromosome re-arrangements in the development of neuroblastoma. Apart from the loss of distal 1p-material, these translocations invariably lead to extra copies of 17q. This also suggested a possible role for genes on 17q in neuroblastoma tumorigenesis. Further support for this hypothesis comes from the observation that in those cell lines without 1;17 translocations, other chromosome 17q translocations were present. These too lead to extra chromosome 17q material. Molecular analysis of 1;17 translocation breakpoints revealed breakpoint heterogeneity both on 1p and 17q, which suggests the involvement of more than 2 single genes on 1p and 17q. The localisation of the different 1p-breakpoints occurring in 1;17 translocations in neuroblastoma are discussed with respect to the recently identified candidate tumour suppressor regions and genes on 1p. In this study, we focused on the molecular analysis of the 17q breakpoints in 1;17 translocations. Detailed physical mapping of the constitutional 17q breakpoint allowed for the construction of a YAC contig covering the breakpoint. Furthermore, a refined position was determined for a number of 17q breakpoints of 1;17 translocations found in neuroblastoma cell lines. The most distal 17q breakpoint was identified in cell line UHG-NP and mapped telomeric to cosmid cCI17–1049 (17q21). This suggests that genes involved in a dosage-dependent manner in the development of neuroblastoma map in the distal segment 17q22-qter. Future studies aim at the molecular cloning of 1;17 translocation breakpoints and at deciphering the mechanisms leading to 1;17 translocations and possibly to the identification of neuroblastoma genes at or in the vicinity of these breakpoints. © 1997 Published by Elsevier Science Ltd.

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INTRODUCTION

Loss of distal 1p-material is one of the most frequent genetic changes observed in advanced neuroblastomas [1]. They are the result of either simple deletions or unbalanced translocations involving another chromosome than chromosome 1. Recent studies using FISH have shown that 10 of 27 neuroblastoma cell lines carried unbalanced 1;17 translocations [2–4]. Similar chromosome re-arrangements have also been observed in primary neuroblastomas [5]. Unbalanced 1;17

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translocations lead to loss of distal 1p material and extra copies of 17q. Interestingly, in almost all cell lines without 1;17 translocations, other unbalanced 17q re-arrangements have been observed which also lead to 17q over-representation [2–4]. We therefore proposed that genes on 17q could be involved in neuroblastoma, possibly through a gene dosage effect. Molecular analysis of 1p- and 17q-breakpoints of these translocations revealed breakpoint heterogeneity. As no neuroblastoma genes have been cloned thus far, the involvement of multiple tumour (suppressor) genes in these translocations cannot be excluded.

In this study, we compared the mapping data of the 1p breakpoints with the available data on candidate tumour suppressor regions and genes on the short arm of chromosome 1 in neuroblastoma. Also, 17q breakpoints in 1;17 translocations, including the constitutional 17q breakpoint, were studied in greater detail.

MATERIALS AND METHODS

Molecular cytogenetic analysis of 1;17 *translocation breakpoints in neuroblastoma cell lines*

The 17q breakpoints of cell lines GI-ME-N [6], IMR32 [7], TR14 [8], SJNB-8, SJNB-12 [9] and UHG-NP [4] were analysed in detail using fluorescence *in situ* hybridisation (FISH). For this purpose the following panel of 17q-region specific probes were used: cCI17-698, cCI17-473, cCI17-1049, cCI17-1707 [10], 7G4 (NF1) [11], 397F9 (THRA1)(GDB 1d GOO-195-323) and SCYA7 [12]. Chromosome 17q region specific probes were cohybridised with a chromosome 17 centromere specific probe (p17H8) [13] in dual-colour FISH experiments according to Van Roy and associates [14].

Physical mapping of the constitutional 17*q breakpoint*

Regional mapping of the constitutional 17*q breakpoint using FISH on hybrid cell metaphases and interphase nuclei.* Mapping of 17q probes relative to the translocation breakpoint was done on metaphases from hybrid cell lines containing the derivative chromosomes of the constitutional 1;17 translocation [15, 16]. Interphase distance measurements were used in order to estimate the physical distance between selected breakpoint flanking markers. For this purpose, dual-colour FISH was performed on interphase nuclei using A12M2 (proximal 1p36.2 constitutional breakpoint flanking marker) [15–17] in combination with SCYA7 (distal 17q11.2 constitutional breakpoint flanking marker) [15, 16]. Interphase distance measurements were also used in order to select additional breakpoint flanking markers from a total of 67 cosmids which were previously assigned to 17q11.2–12 [10]. Interphase distance measurements were performed according to Trask and associates [18]. Images were recorded with a black and white CCD camera (Sony IMAC-CCD S30). Distances were measured in at least 50 nuclei using dedicated software (ISIS, MetaSystems, Germany).

Construction of a YAC contig covering the constitutional 17*q breakpoint.* The CEPH mega YAC library was screened following standard procedures [19] for probes cCI17–1079 [10] and cMCP-3 (SCYA7) [12]. For probe cCI17–1079, two screening rounds of the CEPH mega YAC library were performed. In the first experiment, the filters of the CEPH mega YAC library were screened with the entire cosmid clone, while the second screening was performed with the isolated insert from the cosmid clone. This insert was also used to screen the ICI YAC library [20]. All positive YAC clones were provided by the YAC Screening Centre, Leiden (YSCL), supported by EU grant PL930088.

Analysis of YAC clones was achieved by different methods. The insert size of the YAC clones was determined using pulsed field gel electrophoresis (PFGE). Sizes of YAC inserts were determined by comparison with Yeast DNA PFGE marker (Pharmacia).

YAC clones were tested by FISH on normal metaphase chromosomes to determine their map position and possible chimerism. For localisation of the chromosome 17 specific YAC clones on the hybrid cell lines, the probe of interest was cohybridised with probes allowing identification of the derivative 1 and 17 chromosomes: pUC1.77 (*D1Z1*) for the heterochromatic region of chromosome 1 [21] and p17H8 (*D17Z1*) for the centromeric region of chromosome 17 [13].

Polymerase chain reaction (PCR) analysis was used to determine the STS content of the YAC clones. STS sequence information was obtained from the Whitehead Institute/MIT and CEPH-Généthon databases.

Fibre FISH

Fibre FISH slides were prepared using a modified procedure which combines the protocols of Parra and Windle [22] and Fidlerova and associates [23]. Briefly, methanol–acetic acid fixed nuclei from normal human lymphocytes were placed on silan-coated slides (2% 3-aminopropyl-trimethoxysilan, Merck). Slides were immediately immersed (without drying) in 70 ml of lysis buffer (0.5% SDS, 50 mM EDTA, 200 mM Tris pH 7.4) for 7 min, 70 ml of 94% ethanol was added dropwise and slides were kept in this solution for 7 min. After this initial fixation, slides were removed gently and fixed in 70% ethanol for 30 min. Evaluation of the slides was done by counterstaining with DAPI prior to hybridisation. The use of ethanol instead of methanol greatly improved the quality of the preparations.

RESULTS

The localisation of 1p breakpoints in 1;17 translocations and localisation of candidate tumour suppressor regions and genes are depicted in Figure 1. Figure 2 summarises the mapping data for FISH experiments using region-specific chromosome 17q probes. A summary of the physical mapping of the constitutional 17q breakpoint is represented in Figure 3.

DISCUSSION

1p breakpoints in 1;17 *translocations and* 1*p-tumour suppressor candidate regions and genes*

The 1p breakpoints observed in cell line IMR32, SJNB-8 and SJNB-12 were mapped proximal to *MYCL* (1p34.3) [24] and might coincide with the most proximal tumour suppressor region described by Schleiermacher and associates [25] (NB-R2) and Takeda and associates [26].

The 1p breakpoints observed in GI-ME-N and UHG-NP map within the region which is supposed to contain a neuroblastoma suppressor gene associated with *MYC*N amplified tumours [27]. Finally, a third and most distal region on 1p was proposed by Caron and associates [28]. At present, the smallest deletion in this region is defined by the interstitial deletion in cell line SK-N-AS [29]. The smallest region of deletion was further narrowed down by Martinsson and associates (pp. 1966–1970), and is defined proximally by

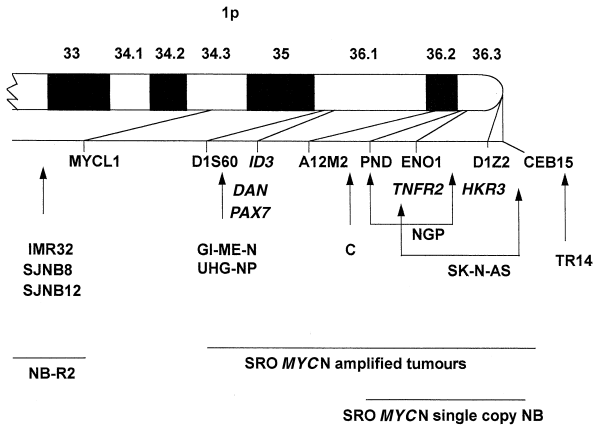

Figure 1. Schematic representation of 1p breakpoints in 1;17 translocations and localisation of candidate tumour suppressor regions and genes on chromosome 1p. The chromosomal position of relevant DNA markers is indicated. The position of the 1p breakpoints is indicated by arrows. Candidate genes are indicated in italics. The position of the constitutional 1p36 breakpoint is indicated by 'c'. Horizontal lines represent the regions supposed to harbour a tumour suppressor gene: the most proximal region NB-R2 [25], and the two more distal located regions associated with *MYCN* amplified tumours and with non-amplified tumours [27, 28].

marker *D1S244* and distally by marker *D1S80*. The constitutional 1p breakpoint maps within a 2 Mb cluster containing multiple tRNA and snRNA genes [15–17]. None of the 1;17 translocation breakpoints coincide with the constitutional 1p36.2 breakpoint. This constitutional 1p36.2 breakpoint maps proximal to the SK-N-AS deletion [29]. Interestingly, a reciprocal 1;15 translocation was described in neuroblastoma cell line NGP [4, 30, 31]. Further molecular analysis revealed that a 2 Mb region around the breakpoint was duplicated on the derivative 15 chromosome in this cell line [30]. The proximal breakpoint of this translocation might be in close proximity and distal to the constitutional 1p36.2 breakpoint. At present, a few candidate neuroblastoma suppressor genes have been mapped to the distal short arm of chromosome 1, these include: *ID3* [32], *TNFR2* [33], *CDC2L1* [34], *DAN* [35], *E2F2* [36], *PAX*7 [37] and *HKR3* (Maris and associates, pp. 1991–1996). The *CDC2L1* gene was shown to map distal to the consensus region of deletion, whereas *ID3*, *TNFR2*, *DAN*, *E2F2* and *PAX7* were mapped proximal to the consensus region as proposed by White and associates [29]. The *HKR3* gene maps within the consensus region of deletion, and is therefore regarded as a good candidate gene (Maris and associates, pp. 1991–1996). It remains to be determined whether one of these candidate genes was disrupted by the unbalanced 1;17 translocations in the analysed neuroblastoma cell lines. Finally, one 1p breakpoint was located at the very distal end of the chromosome 1 short arm in cell line TR14 [3, 4]. Further analysis with near-telomeric probes is in progress in order to determine whether distal 1p material is lost.

Molecular cytogenetic analysis of 17*q breakpoints of* 1;17 *translocations in neuroblastoma cell lines*

Figure 2 summarises the mapping data for FISH experiments using region-specific chromosome 17q probes. The most distal breakpoint was observed in cell line UHG-NP. This breakpoint was found to be telomeric from cosmid clone cCI17-1049 previously assigned to 17q21 [10]. YAC clones located distal to cCI17-1049 are being analysed in order to obtain a further delineation of this breakpoint. Furthermore, additional cell lines are now being analysed in order to identify 17q rearrangements involving smaller 17q segments. This will allow us to define a minimal critical region for 17q duplications in neuroblastoma. Presently, this segment is defined by UHG-NP as 17q22-qter, a region which is estimated to be 29 Mb in size.

Physical mapping of the constitutional 17*q breakpoint*

The closest proximal and distal flanking markers for the constitutional 1;17 translocation breakpoints have been determined in a previous study as A12M2 and PND for the 1p36 breakpoint and NF1 and SCYA7 for the 17q11.2 breakpoint, respectively [15, 16]. In order to estimate the maximum physical distances of A12M2 to the 1p36 breakpoint and SCYA7 to the 17q11.2 breakpoint, interphase measurements were performed between cHE2.6 (A12M2) and cMCP-3 (SCYA7) on interphase nuclei from a hybrid cell line containing the derivative chromosome 1. The distance between these two markers was 0.62 μm (S.D. = 0.23). According to previous studies, this interphase distance

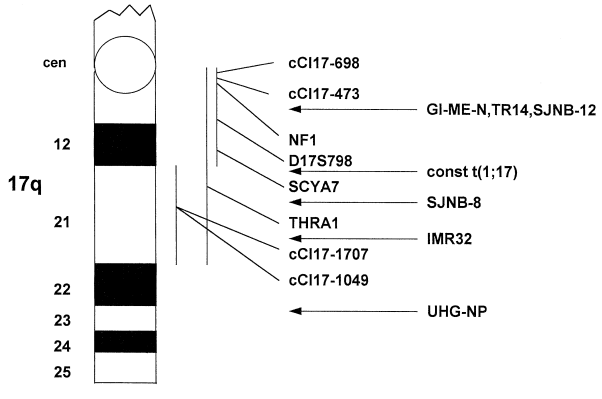

Figure 2. Schematic representation of 17q breakpoints in 1;17 translocations in neuroblastoma cell lines on chromosome 17. The chromosomal position of relevant DNA markers is indicated. The breakpoints of the cell lines are indicated by arrows.

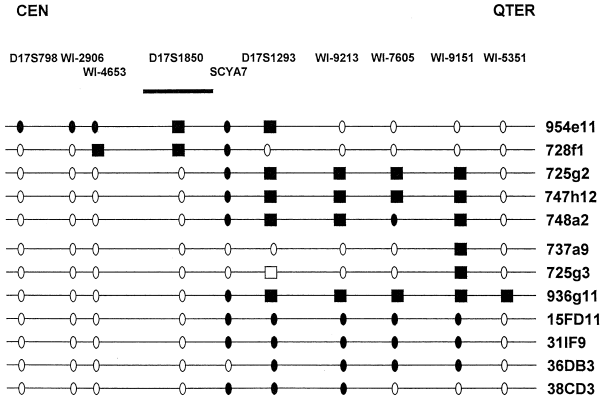

Figure 3. STS content mapping of YAC clones. STSs are indicated at the top. The bold line represents the current location of the constitutional 17q breakpoint. Closed and open circles represent STSs that are present or absent on the YAC clones, respectively. YAC clones are shown as horizontal lines.

correlates with a physical distance of 500 kb to 1 Mb [18]. Using the cMCP-3 probe, a 1380 kb YAC clone (748-a-2) was isolated from the CEPH mega YAC library [38]. FISH on control metaphases and YAC data showed that this YAC clone was chimeric. No evidence was found by FISH analysis that this YAC clone was breakpoint overlapping.

We looked for additional markers located distal to the translocation breakpoint but proximal to SCYA7. For this purpose, 67 cosmids which were previously assigned to 17q11.2–12 by FISH [10] were tested. Cosmids were co-hybridised with SCYA7 on normal metaphase chromosomes to determine their relative position to SCYA7. Cosmids located distal to SCYA7 were excluded for further analysis. The remaining cosmids were hybridised to the derivative chromosomes 1 and 17. Cosmids located proximal to the 17q breakpoint, thus hybridising to the derivative chromosome 17, were also excluded. Finally, interphase distances on hybrid nuclei containing the derivative chromosome 1 were measured between cHE2.6 (A12M2) and the selected cosmids. Five out of 19 cosmids showed interphase distances which were within the same range as the distance between A12M2 and SCYA7. No cosmid was found which was located clearly more closely to A12M2.

Simultaneous to the search for markers in the SCYA7 region, we also looked for a more closely proximal breakpoint flanking marker, this is between NF1 and the 17q breakpoint. From all available linkage maps, the Cooperative Human Linkage Center (CHLC) map was the only map which included the genetic position of the *NF1* gene [39]. Locus *D17S798* was the next marker distal to *NF1* at a genetic distance of 6.2 cM. No conclusions could be drawn from this map for the position of *D17S798* relative to SCYA7. Therefore, 14 YAC clones from a MIT-YAC contig, which included *D17S798*, were analysed. Detailed information on these YACs will be published elsewhere. All YACs which hybridised to 17q on normal chromosomes mapped to the derivative chromosome 17 and not to the derivative chromosome 1. This indicates that *D17S798* is proximal to the 17q breakpoint. Consequently, further refinement of the position of the 17q breakpoint was obtained.

According to the MIT database and in accordance with other results [38], the SCYA7-positive YAC clone 748-a-2 was part of a YAC contig. These YACs, together with those showing overlap by fingerprint data from CEPH/Généthon, were analysed. Details of FISH results on normal chromosomes and hybrid cell line analysis will be published elsewhere. YAC clone 936-g-11 was found to overlap the 17q translocation breakpoint, as hybridisation signals were observed on both the derivative chromosomes 1 and 17 (data not shown).

In order to construct a physical map of the 17q translocation breakpoint region, the sequence tagged site (STS) content mapping of most of the selected YACs was done (Figure 3). According to the available data from MIT and CEPH, one particular YAC clone, 954-e-11, links the STS *D17S798* to the SCYA7 contig. However, our STS analysis could not confirm the presence of STS *D17S1293* on YAC clone 954-e-11, while this STS is present on all YAC clones from the SCYA7 region. Further molecular analysis of this YAC clone, including PFGIE analysis, is underway. The STS content map showed that the ICI YACs screened with cosmid cCI17–1079 clearly overlap with the YACs from the SCYA7 contig. This observation thus validates the reliability of the interphase nuclei measurements used for selecting additional markers in the SCYA7 region. These data were further extended by fibre FISH analysis. Cohybridisation of YAC clone 748-a-2 with the cMCP-3 cosmid (SCYA7)(dual colour) on fibres from normal DNA showed that in almost all observed fibres the cMCP-3 cosmid was located on one end of the YAC clone (not shown). This observation could explain why this YAC did not cross the breakpoint. Overlap of the YACs of the SCYA7 contig with the ICI YACs was also visualised by fibre FISH analysis (data not shown). Furthermore, fibre FISH experiments demonstrated that cosmid clones cMCP-3 and cCI17-1079 were partially overlapping (data not shown).

Currently, we are performing further physical mapping in order to clone the constitutional 17q breakpoint and to establish a transcript map of the region. Similar approaches can be envisaged for the 1;17 translocation breakpoints in neuroblastoma cell lines. The cloning of a number of these 1;17 translocation breakpoints will hopefully clarify the mechanisms involved in the generation of these translocations.

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