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A t(4;22) in a Meningioma Points to the Localization of a Putative Tumor-suppressor Gene

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Summary

Cytogenetic analysis of meningioma cells from one particular patient (MN32) displayed the stem-line karyotype 45, XY, −1, 4p+, 22q−, 22q+, which thus had rearrangements of both chromosomes 22. The 22q+ marker appeared as a dicentric: 22 pter→q11::1p11→qter. The reciprocal product of this translocation has presumably been lost because it lacked a centromere. The 22q− chromosome also appeared to have lost sequences distal to band q11. We assumed that this marker could have been the result of a reciprocal translocation between chromosomes 4 and 22. To investigate the 4p+ and 22q− chromosomes in more detail, human-hamster somatic cell hybrids were constructed that segregated the 22q− and 4p+ chromosomes. Southern blot analysis with DNA from these hybrids showed that sequences from 22q were indeed translocated to 4p+ and that reciprocally sequences from 4p were translocated to 22q−, demonstrating a balanced t(4;22)(p16;q11). On the basis of these results we presume that in this tumor a tumor-suppressor gene is deleted in the case of the 22q+ marker and that the t(4;22) disrupts the second allele of this gene. The latter translocation was mapped between D22S1 and D22S15, a distance of 1 cM on the linkage map of this chromosome. The area in which we have located the translocation is within the region where the gene predisposing to neurofibromatosis 2 has been mapped.

Introduction

The involvement of a tumor-suppressor gene in the etiology or pathology of a particular type of cancer is often indicated by loss of a specific chromosome or part of a chromosome (Cavenee et al. 1986). Until now five tumor–suppressor genes have been isolated; these include the retinoblastoma gene (Weinberg 1989), the p53 gene (Eliyahu et al. 1989; Finlay et al. 1989), a gene called "DCC" which is involved in the pathology of colorectal carcinoma (Fearon et al. 1990), a gene involved in Wilms tumor associated with the WAGR syndrome (Call et al. 1990; Gessler et al. 1990; Rose et al. 1990), and the gene which is involved in the predisposition to neurofibromatosis 1 (NF1; Cawthon et al. 1990; Viskochil et al. 1990; Wallace et al. 1990).

Meningioma is a common benign mesenchymal intracranial or intraspinal tumor arising from arachnoidal cells surrounding the brain and spinal cord. Cytogenetic analysis and RFLP analysis of these tumors reveal a specific chromosomal aberration. In about 50% of the tumors one copy of chromosome 22 is lost (Zang 1982). In a few percent of the cases other aberrations of chromosome 22, such as translocations (breakpoints are mapped in region 22q11–22q12) or deletions of the larger part of the long arm, are found (Katsuyama et al. 1986; Al Saadi et al. 1987; Casalone et al. 1987; Dumanski et al. 1987; Meese et al. 1987; Seizinger et al. 1987*a;* Maltby et al. 1988; Rey et al. 1988; Casartelli et al. 1989; Poulsgård et al. 1989). These findings suggest that inactivation of both alleles of a tumor-suppressor gene on chromosome 22 is a causal event in the development of meningioma.

The autosomal dominant disorder neurofibro-

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matosis 2 (NF2) predisposes to acoustic neurinomas, meningiomas, and other tumors of the central nervous system. Loss of sequences on chromosome 22 has also been observed in tumors associated with NF2 (Seizinger et al. 1986; Seizinger et al. 1987*b*; Rouleau et al. 1990). The gene predisposing to NF2 has been located between D22S1 and D22S28 by using linkage analysis (Rouleau et al. 1990). At present it is not clear whether the gene predisposing to NF2 is the same as the gene involved in sporadic meningioma.

In the course of our search for the exact position of the meningioma locus on chromosome 22, we analyzed tumor cells from a patient in whom both copies of chromosome 22 were involved in translocations. One of the marker chromosomes was the result of a reciprocal translocation between chromosomes 4 and 22. We presume that this translocation has inactivated a tumor-suppressor gene. Using hybrid cell lines we mapped the translocation breakpoint to a position between D22S1 and D22S15.

Material and Methods

Patient

The patient, born in 1925, had a history of head injuries: in 1931 he had a car accident resulting in brain damage, and in 1947 he had a concussion of the brain, after which his sense of smell was impaired. In 1968 an olfactorial meningioma was removed surgically. In 1989 a second meningioma (MN32), located on the right temporal side of the brain, was removed. This tumor showed a combination of three histological types: syncytial, transitional, and fibroblastic. These are characteristic of all meningiomas. The localization of the tumor was completely outside the previous operation position, and it was not considered to be a local recurrence.

Tissue Culture, Hybrid Cell Lines, and Cytogenetics

The meningioma specimen was obtained within 30 min after surgery. Both preparation for tissue culture and tissue culture conditions were as described elsewhere (Koper et al. 1990). Chromosome analysis was carried out on cultured cells after one passage using G- (trypsin-Giemsa), R- (acridine orange), and Q- (quinacrine) banding.

The somatic cell hybrids were constructed according to a method described elsewhere (Geurts van Kessel et al. 1981). Interspecies hybrid cell lines were obtained by fusion of thymidine kinase–deficient (TK^-) A3 Chinese hamster cells with cultured meningioma cells. Inactivated Sendai virus was used as the fusogen. Independent hybrid clones were selected in F10 medium supplemented with HAT (hypoxanthine, aminopterin, and thymidine), 2 μM Ouabain, and 15% FCS (Biological Industries). The hybrid cell lines were analyzed by R-banding; at least 10 metaphases/hybrid cell line were studied. This analysis was repeated several times during culture of the hybrid cell lines. Possible rearrangements between hamster and human chromosomes were verified using chromosome painting. This was done by biotinylation of human DNA and by in situ hybridization on metaphase spreads of the hybrid cell lines. The eventual presence of the marker 22q+, which resembles hamster chromosome 6, was also analyzed by using this technique.

DNA Extraction, Southern Blotting, Hybridization, and Densitometric Analysis

High-molecular-weight DNA was isolated from cultured tumor cells (first passage) and from hybrid cell lines according to standard procedures. DNA samples were digested to completion with restriction enzymes (Boehringer Mannheim and Promega) and were separated by electrophoresis, transferred to nylon membranes (Hybond N^+; Amersham), and hybridized to radiolabeled probes according to a method described by Feinberg and Vogelstein (1983). Conditions for hybridization are slightly modified from those described by Amasino et al. (1986). Blots were hybridized at 65°C in 0.25 M sodium phosphate pH 7.2, 0.25 M NaCl, 7% SDS (biochemical 44244; British Drug Houses), 1 mM EDTA, and 10% polyethylene glycol (M_r 6 × 10^3, biochemical 44271; British Drug Houses). After hybridization, blots were washed once in 3 × SSC, 0.1% SDS for 20 min at 65°C; once in 1 × SSC, 0.1% SDS for 20 min at 65°C; and once for 20 min at 65°C in 0.3 × SSC, 0.5% SDS. Membranes were exposed to Kodak XAR-5 film at −70°C with an intensifying screen. The following loci were examined: c-abl (0.6-kb *Eco*RI/*Bam*HI fragment; Heisterkamp et al. 1983), bcr (a third-exon *Pst*I c-DNA probe of 393 bp; Heisterkamp et al. 1985), D22S1 (Barker et al. 1984; Julier et al. 1988), D22S15 (Rouleau et al. 1988), myoglobin (MB, a third-exon PCR probe; Weller et al. 1984), D22S201 (this probe has been localized on 22q13-qter; N. A. van Biezen, unpublished data), D4S62 (Thayer et al. 1987), F5.53 (Bakker et al. 1987), and D4S125 (Nakamura et al. 1988). The sequence of these probes on the long arm of chromosome 22 is centromere–bcr–D22S1–D22S15–MB–c-sis–telomere. The location of these probes on the

short arm of chromosome 4 is centromere–D4S62–F5.53/D4S125–telomere. Quantitative densitometric scanning of the X-ray films was performed with a model 620 video scanning densitometer (Bio-Rad Laboratories).

Results

Cytogenetic analysis of meningiomas after surgery and short-term tissue culture was carried out routinely. In one case (MN32) we observed that both copies of chromosome 22 were altered. Table 1 shows the results of the cytogenetic analysis, indicating that all meningioma cells show an abnormal karyotype. Besides the basic chromosomal aberrations, additional aberrations were found in some of the cells. These additional aberrations probably arose after the tumor had been formed. The basic stem line 45, XY, −1, 4p+, 22q−, 22q+ is shown in figure 1. One marker chromosome (22q+) is involved in a translocation with chromosome 1, creating a dicentric chromosome: 22 pter→q11::1p11→qter. The 22q− marker has also lost sequences distal to band q11. The aberrations of chromosome 22 are not constitutional, because control cells of the patient displayed a normal karyogram.

To investigate the presence of sequences on the long arm of chromosome 22 in the tumor cells, we prepared a Southern blot carrying tumor DNA versus human placental DNA and hybridized it with probes located on chromosome 22. As a control a probe for the c-abl gene, which is located on chromosome 9, was used. The results are illustrated in figure 2, together with the densitometric analysis of the hybridization signals. When the hybridization signals of the meningioma lane and those of the placental control lane are compared, it appears that in the meningioma cells probably two copies of c-abl, bcr, and D22S1 are present and that presumably only one copy each of D22S15 and D22S201 is present. Also, densitometric analysis of the autoradiographs is in agreement with this hypothesis (fig. 2). The placental lane contains about 1.8 times more DNA than does the meningioma lane. Thus, these findings suggest that the chromosome 22 probes located at 22q11 (bcr and D22S1) are still present in two copies and that sequences distal to D22S1 (e.g., D22S15 and D22S201) are definitely present in MN32 but probably in only one copy.

The karyotype of tumor MN32 indicates that both marker chromosomes (22q− and 22q+) have lost sequences distal to band q11. However, hybridization results (fig. 2) showed that these sequences are retained in the tumor cells. Furthermore, the karyotype showed a 4p+ marker suggesting a possible t(4;22). This possibility was investigated using somatic cell hybrids. MN32 tumor cells were fused with a hamster cell line (A3). This resulted in (*a*) five independent human-hamster hybrid cell lines that segregated the aberrant chromosome 22q− and (*b*) three independent hybrid cell lines that segregated the 4p+ marker chromosome. Representatives of this group of hybrid cell lines are hybrids 6A and 14G-10. In 80% of the cells from cell line 6A the 22q− chromosome was found; additional human chromosomes in this line were 3, 6, 9, 11–13, 15–17, 20, and X. Cell line 14G-10 contained the 4p+ marker in 90% of the cells, next to human chromosomes 3, 5, 8, 9, 14, 16, 17, 19–21, and X. No hybrid cell lines which segregated the 22q+ chromosome were obtained.

DNA isolated from the hybrid cell lines and from human and hamster control cells was used for Southern blot analysis with probes from the relevant chromosomal regions. The results obtained with hybrids 6A (22q−) and 14G-10 (4p+) are illustrated in figure 3. DNA derived from hybrid 6A (22q−) hybridized with D22S1 (fig. 3*A*) but not with D22S15 (fig. 3*B*). DNA from hybrid 14G-10 (4p+) hybridized only with D22S15 (fig. 3*B*). Other probes distal to D22S15, e.g., MB, behaved in a manner analogous to that of

Table 1

Cytogenetic Findings in Meningioma 32 (MN32)

No. of Chromosomes	Sex Chromosomes	Missing or Abnormal Chromosomes	No. of Cells
45	XY	−1, 4p+, 22q−, 22q+	8
44	XY	−1, 4p+, 13p+, −14, 22q−, 22q+	2
44	XY	−1, 4p+, −10, +12, 13p+, −14, 22q−, 22q+	6
45	XY	−1, 4p+, −10, +12, 13p+, −14, +16, 22q−, 22q+	12
90	XY	Same, tetraploid	2

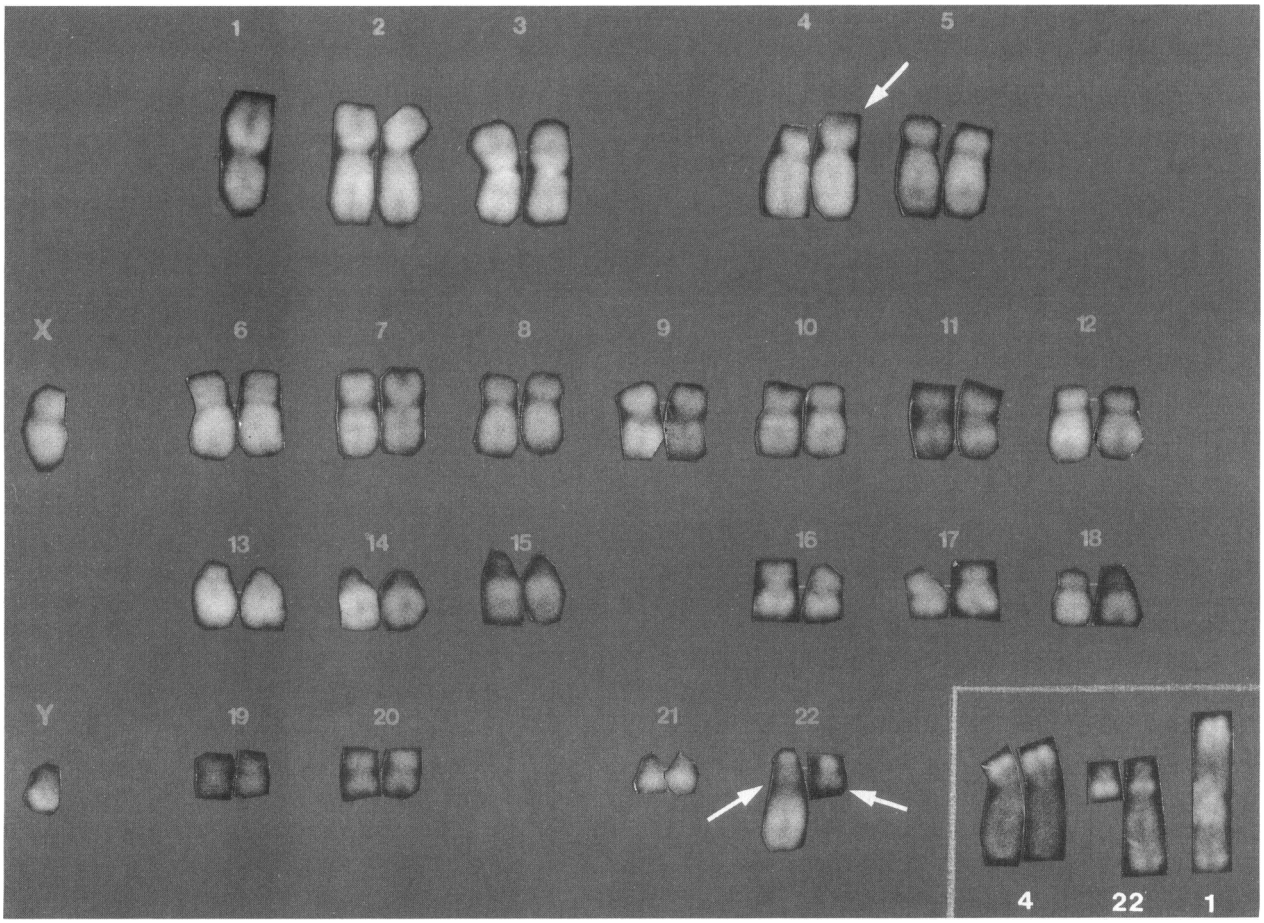

Figure 1 Basic stem-line karyotype of meningioma 32 (MN32): 45, XY, −1, 4p+, 22q−, 22q+. Arrows indicate structural abnormalities (Q-banding). Insert shows the normal and aberrant chromosomes from pairs 4, 22, and 1 (R-banding).

D22S15 (results not shown). This indicates that sequences on 22q distal to D22S1 are indeed present on the 4p+ marker. To investigate the possibility of a balanced translocation t(4;22), we hybridized the same blots with probes located on 4p16. Probe D4S62, which has been located at position 4p16.2-16.1, recognizes a 2.4-kb fragment in both placental and 14G-10 lanes (fig. 3*C*). This suggests that sequences up to position 4p16.2 are still retained on the 4p+ marker chromosome. Probe F5.53 (fig. 3*D*) recognizes in placental DNA the cognate fragment of 6.5 kb and a cross-hybridizing band at 3.4 kb. Both bands were mapped on chromosome 4, at positions 4p16.3 (6.5 kb) and 4p16.1-15.1 (3.4 kb) (G. J. van Ommen, personal communication). The 3.4-kb band is also present in lane 14G-10. Thus, sequences from 4p16.1-15.1 are retained on the 4p+ chromosome, a finding which confirms the results obtained with probe D4S62. The cognate 6.5-kb band is found in hybrid 6A, suggesting that this part of chromosome 4 has been translocated to the 22q− marker chromosome. This conclusion was confirmed by the finding that probe D4S125 (located at 4p16.3) also hybridizes to DNA from hybrid 6A but not to that from 14G-10 (result not shown). The same reciprocal marker segregation pattern was observed with the other hybrid cell lines.

Thus, taken together the hybridization results strongly suggest that the 4p+ and 22q− marker chromosomes are indeed the products of a reciprocal translocation t(4;22) (p16;q11). Figure 4*A* shows an outline of the position of the probes on chromosomes 4 and 22, and in figure 4*B* their position on the translocation products is indicated. Hybridizations with 25 other single-copy probes for chromosome 22 (N. A. van Biezen, unpublished data) showed that all probes

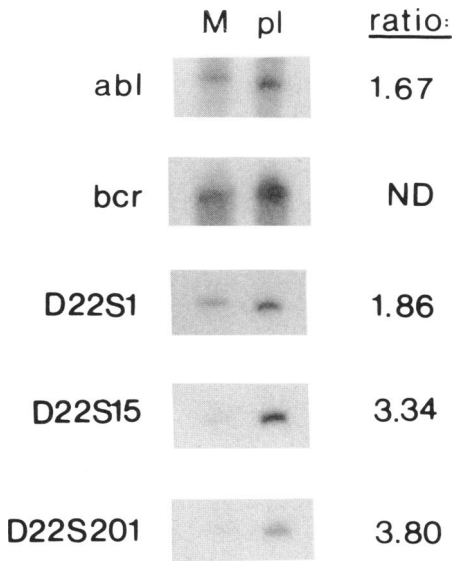

Figure 2 Analysis of copy number of different probes in MN32. Lane M, DNA isolated from MN32. Lane pl, DNA isolated from a human placental control DNA. Also indicated is the ratio of the area under the curve of placental and MN32 signals after densitometric scanning. ND = not done.

could be located either on the 4p+ or on the 22q− chromosomes (results not shown). This finding underlines the conclusion that the t(4;22) is indeed reciprocal and that no substantial parts of chromosome 22 appear to be missing.

Discussion

To localize the putative tumor-suppressor gene involved in meningioma, we selected a tumor (MN32) in which all cells contained the basic chromosomal aberrations −1, 4p+, 22q−, and 22q+, with additional aberrations appearing in some of the cells (table 1). The basic aberrations are probably involved in the etiology of the tumor and included two structurally abnormal chromosomes 22. This is in agreement with the indicated role of chromosome 22 in the development of meningioma (Zang 1982). The meningioma described in our study was the second meningioma in this patient. The second tumor was found far away from the position of the first, which argues against recurrence of the first tumor. To explain the occur-

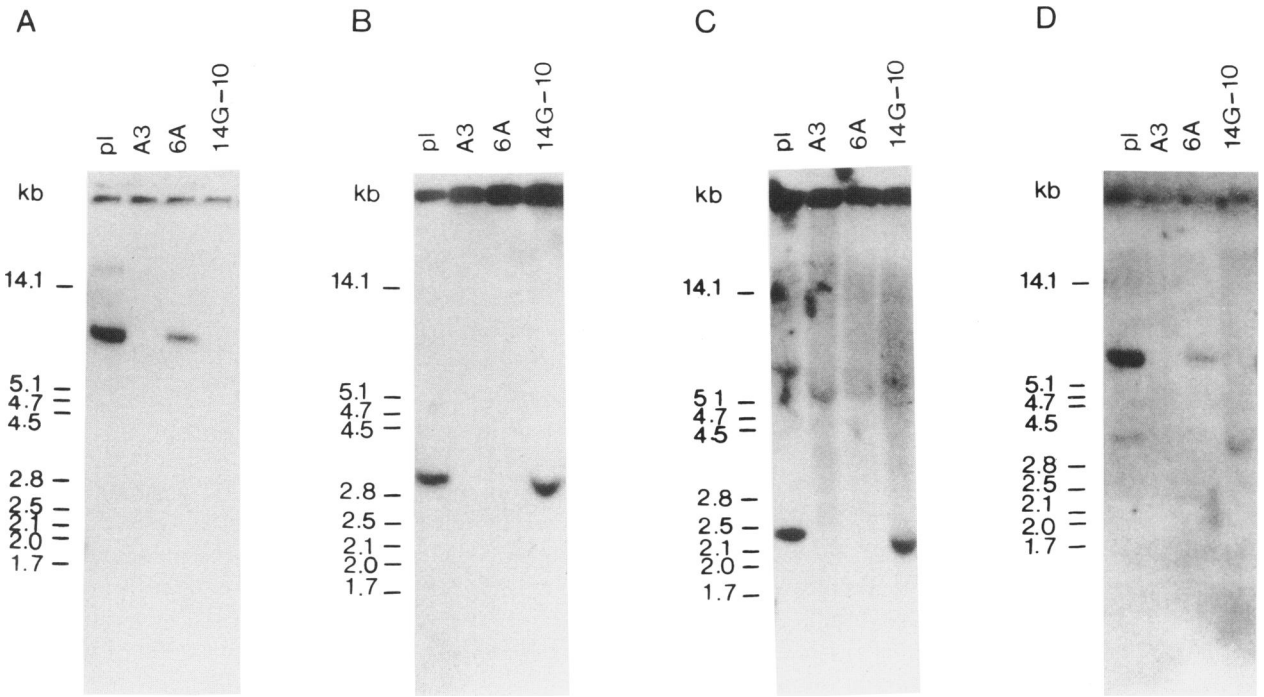

Figure 3 Southern blots of DNA from (hybrid) cell lines. Lane pl, Human placental control DNA. Lane A3, Chinese hamster (A3) DNA. Lane 6A, Hybrid MN32/A3 6A (22q−). Lane 14G-10, Hybrid MN32/A3 14G-10 (4p+). *A*, *Hin*dIII-digested DNA probed with D22S1. *B*, *Eco*RI-digested DNA probed with D22S15. *C*, *Eco*RI-digested DNA probed with D4S62. *D*, *Hin*dIII-digested DNA probed with F5.53. Besides the cognate hybridization signal at 6.5 kb, there is also a cross-hybridizing band (3.4 kb), which is located at 4p15.1-16.1 (G. J. van Ommen, personal communication).

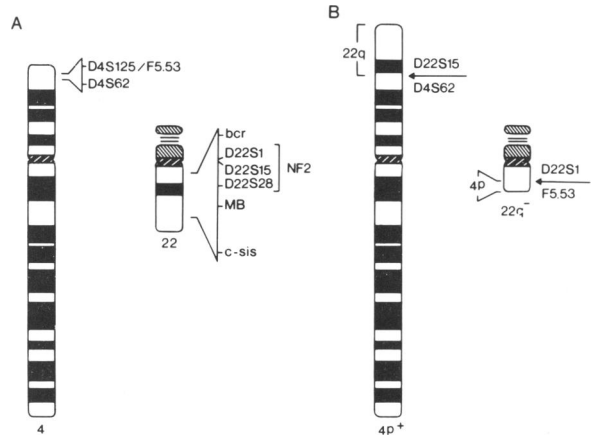

Figure 4 *A*, Regional localization of probes for loci on normal chromosomes 4 and 22. The area in which the NF2 gene has been mapped is indicated. *B*, Schematic representation of reciprocal t(4;22), creating 4p + and 22q − marker chromosomes in meningioma MN32. The breakpoints (indicated with arrows) are shown according to DNA probes for chromosomes 4 and 22.

rence of two meningiomas one could argue that the patient is in fact suffering from NF2. However, this is not very likely, considering the finding that the tumor cells contained two aberrant chromosomes 22 whereas control cells of the patient displayed a normal karyotype. It is known that meningiomas may occur following trauma (Preston-Martin et al. 1980, 1983). This patient suffered from several head injuries, which may explain the occurrence of two meningiomas in this case.

In MN32 both chromosomes 22 are involved in translocations. One copy of chromosome 22 (22q −) is involved in a balanced translocation with chromosome 4: t(4;22) (p16;q11). Both products of the reciprocal translocation are still present in the tumor. In this case, inactivation of a meningioma tumor–suppressor gene at or near the translocation breakpoint represents a very likely hypothesis. If this mechanism of inactivation is operative, we would expect that the exact localization of the breakpoint corresponds to the localization of the gene. Hybridization experiments using hybrid cell lines segregating the reciprocal products of the t(4;22) were performed to map the breakpoints on chromosome 4 and 22. The breakpoint on chromosomes 4 is located between D4S62 (4p16.1) and F5.53 (4p16.3) (fig. 3*C* and *D*). This area has been investigated intensively because it contains the gene presumed to be responsible for Huntington chorea (Bucan et al. 1990). The distance between D4S62 and F5.53 is estimated to be 3 cM, on the basis of multilocus linkage analysis (Cheng et al. 1989). On chromosome 22 the breakpoint was mapped between D22S1 and D22S15 (fig. 3*A* and *B*). The distance between D22S1 and D22S15 is at most 1 cM, and the cumulative lod score between these loci is 5.35 at a recombination fraction of zero (Rouleau et al. 1989; Zhang et al. 1990). This localization agrees with the localization of translocation breakpoints in six other meningiomas, which were all mapped at 22q11 (Casalone et al. 1987; Maltby et al. 1988; Rey et al. 1988). A schematic representation of the reciprocal translocation t(4;22) is indicated in figure 4*B*.

The other copy of chromosome 22 (marker 22q +) is also involved in a translocation, leading to a dicentric chromosome: 22 pter→q11::1p11→qter. The reciprocal product of this dicentric chromosome was not found, probably because it lacks a centromere. As D22S1 is probably still present in two copies in the tumor DNA (fig. 2), it seems that the breakpoint in this marker is distal to D22S1. Sequences distal to D22S1 are presumably present in only one copy in MN32 (fig. 2), and we showed that these sequences were present on marker chromosome 4p + as a result of the reciprocal t(4;22). Therefore it seems that the translocation breakpoint in the dicentric 22q + chromosome is also located between D22S1 and D22S15. This rearranged chromosome could have lost the tumor-suppressor gene together with the reciprocal product of the translocation. If this is the case, we would expect that the translocation in the 22q + marker is closer to the centromere than is the breakpoint in the 22q − chromosome. It is also possible that this translocation, too, disrupts the tumor-suppressor gene.

In MN32 the localization of the t(4;22) between D22S1 and D22S15 on chromosome 22 is identical to the position of the translocation t(11;22) (q24;q12), which is found in most cases of Ewing sarcoma and of neuroepithelioma (McKeon et al. 1988; Turc-Carel et al. 1988; Zhang et al. 1990). However, the balanced translocation in Ewing sarcoma is reminiscent of that observed in chronic myeloid leukemia and suggests that the t(11;22) leads to the activation of a proto-oncogene rather than to the inactivation of a tumor-suppressor gene as is the case in meningioma. Therefore we would expect that the gene involved in meningioma and the one involved in either Ewing sarcoma or neuroepithelioma are different.

So far, two earlier reports have suggested a localization of the meningioma tumor–suppressor gene. Both are in conflict with the localization suggested by our experiments. Dumanski et al. (1987) describe a men-

ingioma in which cytogenetic analysis shows a partial deletion of the q arm of one copy of chromosome 22. RFLP analysis of DNA derived from this tumor shows loss of one copy of the c-sis gene. Although the MB probe in this patient was not informative, the authors claim that densitometric analysis of the autoradiographs shows that the MB gene is still present in two copies. This suggests that localization of the tumor–suppressor gene should be distal to MB. MB is approximately 20 cM distal to D22S1/D22S15 (Julier et al. 1988). On the basis of these data and the mapping of the NF2 gene, it has been suggested that the meningioma tumor–suppressor gene and the gene predisposing to NF2 are different genes. The second report (Zhang et al. 1990) describes a meningioma also with a cytogenetically observed 22q – chromosome. In situ hybridization with probe D22S15 suggests that this fragment is still present on the 22q – chromosome and that, consequently, the suppressor gene is expected to be distal to D22S15. Thus, as of yet there is no consensus on the position of the meningioma tumor–suppressor gene. It could be that there are indeed two genes, with the more distal one being responsible only for sporadic meningioma and with the proximal one being involved in both sporadic meningioma and NF2. However, it is also possible that the t(4;22) induces a position effect, which could alter the expression of a distally located gene.

The present report is the first detailed localization of a reciprocal translocation breakpoint in meningioma. We presume that this translocation interferes with either the structure or the expression of a tumor–suppressor gene. Recently, two translocations that have been described in families with NF1 have led to the successful isolation of the gene predisposing to this disease (Cawthon et al. 1990); Viskochil et al. 1990; Wallace et al. 1990). The area in which we have located the translocation is within the region where the gene predisposing to NF2 has been mapped (Wertelecki et al. 1988; Rouleau et al. 1990). Therefore, on the basis of our results it is possible that the putative meningioma tumor–suppressor gene located at the t(4;22) and the gene predisposing to NF2 are one and the same.

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