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Isolation of anonymous, polymorphic DNA fragments from human chromosome 22q12–qter

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Summary. A series of 195 random chromosome 22-specific probes, equivalent to approximately 1% of the size of this chromosome, have been isolated from a chromosome 22-specific bacteriophage lambda genomic library. These probes were mapped to four different regions of chromosome 22 on a panel of five somatic cell hybrids. Restriction fragment length polymorphisms were detected by 28 of the probes mapping to 22q12–qter. Evolutionarily conserved sequences in human, mouse, and Chinese hamster DNA were detected by 12% of the isolated probes.

Introduction

Chromosome-specific DNA markers that recognize restriction fragment length polymorphisms (RFLPs) have become an important instrument for analysis of the human genome in health and disease. So far, relatively few such markers have been isolated on chromosome 22, which therefore contains substantial gaps in the genetic linkage map (Kaplan and Emanuel 1988; Julier et al. 1988; Rouleau et al. 1989).

We have previously localized the tentative meningioma locus to chromosome region 22q12.3–qter by deletion mapping of tumor DNA (Dumanski et al. 1987). Furthermore, the locus for bilateral acoustic neurofibromatosis (NF-2) has been assigned to chromosome 22 by linkage to the anonymous marker D22S1 (Rouleau et al. 1987). Since these findings include an overlapping region on chromosome 22 and these diseases are associated in the sense that meningioma occurs as a secondary tumor in patients with NF-2, a single locus might be involved in the genesis of both disorders. To further define the position of these loci, we have generated a series of polymorphic DNA markers specific for the distal part of the q arm of chromosome 22.

In the present paper we report 195 single-copy DNA probes mapping to four different regions of chromosome 22. Of these, 28 mapping to 22q12–qter were found to detect restriction fragment length polymorphisms. These probes will permit the construction of a tight genetic linkage map as well as a long-range physical map of that region.

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Materials and methods

Chromosome 22-specific library

A bacteriophage lambda chromosome 22-specific genomic library constructed at the Lawrence Livermore National Laboratory from flow-sorted chromosome 22 was used (ATCC no. 57714). This library contains the chromosome 22 fraction digested to completion with *Hin*dIII and ligated into the phage Charon 21A. An aliquot from the library was diluted with phage dilution medium (SM) and plated onto *E. coli* LE392 to produce single bacteriophage lambda plaques (Maniatis et al. 1982).

Mapping of new probes

A panel consisting of five human-rodent somatic cell hybrids was used: WEGROTH D2; WESP-2A-TG8; PgMo 22; 1/22 AM 27, and A_3EW_2-6A (Table 1). Extracted DNA from each of the cell lines (14 μg), from Chinese hamster and mouse liver (7 μg), and from human placenta (7 μg) was digested to completion with *Eco*RI or *Hin*dIII, separated on 0.8% agarose gel, and blotted onto a nylon filter (Gene Screen Plus, New England Nuclear) (Bergerheim et al. 1989). The human chromosome 22 DNA content in the hybrids was confirmed by hybridizing the filter with known chromosome 22-specific probes (Table 1) labeled with ^{32}P by random oligonucleotide priming (Feinberg and Vogelstein 1984). A series of such blots were then used for the mapping of isolated inserts from the chromosome 22-specific library. Filters were treated with alkali to remove the hybridized probe and rehybridized with new probes up to 20 times.

Cloning and characterization strategy

The chromosome 22-specific probes were isolated from the library using two different procedures, A and B, as described by Dumanski (1990). Procedure A is based on the conventional methods of preparation of phage insert DNA (Maniatis et al. 1982; Grossberger 1987; Dumanski et al. 1988) and includes a step for identifying inserts that contain repetitive sequences prior to their hybridization to the cell hybrid panel DNA. This permits the adjustment of the hybridization conditions to evaluate the information derived from the single-copy

sequences of the insert more easily. Radioactively labeled inserts, free from repetitive DNA elements, were hybridized to the filters with the somatic cell hybrid panel DNA in the presence of sonicated single-strand salmon sperm DNA (Sigma) (Bergerheim et al. 1989). Inserts containing repetitive DNA elements were hybridized in the presence of sonicated single-strand human placental DNA (mean size 0.5–1.0 kb, 100 μg/ml of hybridization solution) to filters prehybridized with sonicated, single-strand human placental DNA (100 μg/ml). The isolation of phage insert DNA in the rapid procedure B is entirely based on the PCR technique, and competitive hybridization conditions are used for all inserts hybridized for the first time to the somatic cell hybrid panel. The labeled insert DNA was hybridized in the presence of human DNA, and the filters had been prehybridized with salmon sperm DNA (see above). Clones showing a single-copy signal on chromosome 22 were rehybridzed to the filters in the presence of salmon sperm DNA in the hybridization solution to identify those containing repetitive DNA elements.

RFLP screening

Cloned DNA fragments were screened for RFLPs on Southern blots containing DNA samples from five to nine unrelated individuals, the samples having been digested with 10–34 restriction endonucleases (New England Biolabs). In an initial screening, the probes were tested on a panel of DNA from nine unrelated individuals, the DNA having been digested with ten restriction endonucleases (*Msp*I, *Taq*I, *Rsa*I, *Pst*I, *Bam*HI, *Eco*RI, *Pvu*II, *Hin*dIII, *Bgl*II, *Eco*RV). Further testing for RFLPs was undertaken for some of the probes on a panel in which DNA from five unrelated individuals had been digested with 24 additional restriction endonucleases. The criteria for selecting probes for this additional test were: (1) the chromosomal localization (probes mapping to 22q12–q13 were preferentially tested); (2) detection of evolutionarily conserved sequences, (3) good quality of the autoradiographic signal, and (4) evidence for RFLPs with low allele frequencies detected with the enzymes used in the initial screening.

Results

Somatic cell hybrid panel

Prior to its use for mapping of the new probes, the somatic cell hybrid panel was characterized with the known human chromosome 22-specific probes D22S9, D22S1, MB, PDGFB, and CYP2D. It was of particular importance to define the location of the human chromosome 22 breakpoints in the hybrids 1/22AM27 and A_3EW_2-6A with respect to these markers since they involve the q12–q13 region. With use of these probes, the breakpoints and chromosomal constitution of the hybrids could be confirmed (see references in Table 1). Table 1 shows that this panel of cell hybrids subsequently allowed us to assign new probes to four different regions of human chromosome 22. The strength of the autoradiographic signal derived from human chromosome 22 varied significantly among the hybrids (being strongest in 1/22AM27 and A_3EW_2-6A and weakest in PgMo22) (data not shown), consistent with a loss of the human chromosome 22 sequences in a large subpopulation of the latter culture.

Characterization of inserts

The two strategies for the development of new chromosome 22 probes permitted us to characterize inserts containing exclusively single-copy sequences as well as inserts containing single-copy sequences adjacent to repetitive DNA elements. This was accomplished using competitive filter hybridization of a radioactive probe containing reiterated sequences in the presence of a large excess of nonlabeled, sonicated, single-strand human DNA (Litt and White 1985; Nakamura et al. 1987). The aim was to isolate chromosome 22-specific probes within the entire range of cloned inserts (0–9 kb) in the library, as it was considered that this should give the most representative sample.

Approximately 40% of all inserts analyzed contained single-copy sequences that could be assigned to chromosome 22. However, a number of probes also detected additional loci on other chromosomes (Table 2). A total of 195 single-copy probes specific for human chromosome 22 were generated with a total insert length of 605 kb, which is equivalent to approximately 1% of this chromosome. The number of inserts assigned to the four different regions of chromosome 22 was proportional to the estimated genetic length of these parts of the chromosome (Julier et al. 1988; Rouleau et al. 1989), suggesting that the isolated probes were evenly scattered along the entire chromosome 22.

The distribution of inserts detecting single-copy loci on chromosome 22 was as follows: pter–q11, 49 inserts (25%); q11–q12, 41 inserts (21%); q12–q13, 72 inserts (37%); and q13–qter, 33 inserts (17%). The number of inserts listed includes the inserts detecting exclusively single-copy loci on chromosome 22 as well as 16 inserts detecting other loci in ad-

Table 1. The characterization of human chromosome 22 in the somatic cell hybrids used

Cell line	Cytogenetic analysis		Molecular genetic analysis (Southern blot hybridization signal)				
	Human chromosome 22 content	Reference	D22S9	D22S1	MB	PDGFB	CYP2D
WEGROTH-D2[a]	pter–qter[b]		+	+	+	+	+
WESP-2A-TG8[a]	pter–q11[c]	de Klein et al. (1982), Geurts van Kessel et al. (1983)	+	–	–	–	–
PgMo22[a]	q11–qter[c]	Geurts van Kessel et al. (1981a, b)	–	+	+	+	+
1/22AM27[d]	pter–q13[c]	Geurts van Kessel et al. (1980)	+	+	+	+	–
A_3EW_2-6A[d]	q12–qter[c]	Geurts van Kessel et al. (1985)	–	–	+	+	+

[a] Mouse chromosomal background
[b] WEGROTH-D2 contains chromosome 22 as the only human chromosome (unpublished)
[c] Other human chromosomes were present
[d] Chinese hamster chromosomal background

Table 2. Summary of chromosomal assignment of inserts as established by hybridization to the Southern blots with somatic cell hybrid panel DNA

Procedure	No. of inserts analyzed	Inserts detecting single-copy chromosome 22 loci	Inserts detecting single-copy chromosome 22 loci and other loci	Inserts detecting single-copy loci *not* on chromosome 22	Other inserts[a]
A	228	97	11	59	61
B	265	82	5	75	103
A and B	493	179	16	134	164

[a] This category encompasses three groups ordered in descending frequency with which such clones were found: (a) inserts showing a single- or a multiband pattern that was present in all somatic cell hybrids as well as in control human DNA; (b) inserts that repeatedly gave a weak autoradiographic signal, making the analysis of films impossible; and (c) inserts that repeatedly gave a very high background, making the analysis of films impossible

Table 3. Chromosome 22 probes disclosing restriction fragment length polymorphisms

D no.	Probe name	Insert size (kb)	Enzyme	Constant bands (kb)	Allele sizes (kb)	Frequency (%)
22q12–q13						
D22S85	KI-106	4.8	*Taq*I	2.5, 1.2	4.2 / 4.7	61 / 39
D22S86	KI-117	5.5	*Msp*I	1.5	9.5 / 6.8	78 / 22
D22S87	KI-120	0.73	*Msp*I	–	4.5 / 4.2	89 / 11
D22S90	KI-185	6.2	*Taq*I	–	7.7 / 6.3, 1.4	50 / 50
D22S91	KI-211	6.7	*Msp*I	2.5, 1.4, 1.3, 0.9, 0.7	1.1 / 0.95	44 / 56
D22S92	KI-218	1.1	*Msp*I	–	0.7 / 0.6	44 / 56
D22S93	KI-474	4	*Msp*I	2.5, 0.5	3.3 / 2.45	72 / 28
D22S98	KI-1149	2.1	*Msp*I	1.85, 0.9	4.2 / 1.6	17 / 83
			*Eco*RV	20, 10, 9.5, 6.5	21 / 22	11 / 89
D22S99	KI-1522	0.5	*Pvu*II	–	6 / 4.2	17 / 83
			*Eco*RI	–	9.3 / 8.5	17 / 83
D22S100	KI-1545	3.3	*Msp*I	2.7	12 / 2.6	17 / 83
D22S102	KI-436	2.1	*Taq*I	5	4.1 / 3.8	50 / 50
			*Apa*I	–	10 / 9.5	30 / 70
D22S103	KI-1546	3.9	*Pst*I	4.3, 2.3, 0.8	3.8 / 1	22 / 78
			*Eco*RV	6.8	16–20, 4 alleles 20% heterozygosity	
D22S104	KI-149	9	*Hinc*II	–	12 / 8 / 4.2, 3.8	80 / 10 / 10
D22S105	KI-778	2.2	*Stu*I	–	4.2 / 3.6	90 / 10
D22S106	KI-1543	0.6	*Eco*RV	–	21 / 20	11 / 89
D22S158	KI-261	3.8	*Kpn*I	–	18 / 7.5	50 / 50
D11S159	KI-844	1.4	*Xba*I	–	16 / 15	46 / 54
D11S160	KI-711	3.8	*Msp*I	13	4.0 / 2.8	17 / 83
22q13–qter						
D22S82	KI-63	2.1	*Taq*I	3.0, 2.1, 1.2	3.2 / 1.6	61 / 39
D22S83	KI-94	4.8	*Pst*I	2.0, 1.45	1.3 / 1.35 / 1.4	90 / 5 / 5
D22S84	KI-216	3	*Pvu*II	–	3.9 / 2.2, 1.7	28 / 72
D22S94	KI-1105	1.95	*Taq*I	–	6.6 / 5.5	22 / 78
D22S95	KI-839	2	*Eco*RV	–	20 / 15	33 / 67
D22S96	KI-262	4.8	*Eco*RV	–	23 / 18	50 / 50
D22S97	KI-260	4.1	*Taq*I	2.3	2.6 / 2.4	45 / 55
			*Bgl*II	5	3.3 / 1.6	17 / 83
D11S157	KI-536	2.2	*Eco*RV	–	6.5 / 5.4, 1.1	22 / 78
D11S161	KI-424.1	3.8	*Pst*I	–	14 / 13	89 / 11
D11S162	KI-445	6.5	*Bam*HI	4.3	15 / 14	42 / 58

dition to the single-copy loci on chromosome 22. The average insert size of the new probes was 3.8 kb in procedure A and 2.3 kb in procedure B. Approximately 53% of the clones isolated by procedure A and 49% by procedure B contained sequences reiterated to various degrees in the human genome.

Twenty-three (12%) of the cloned DNA segments specific for chromosome 22 detected sequences evolutionarily conserved in human, mouse, and Chinese hamster DNA (data not shown). Thirteen of the clones were isolated by procedure A and ten by procedure B, with average insert sizes of 4.0 and 2.4 kb, respectively. In 12 of these inserts repetitive DNA elements were identified, and the distribution on chromosome 22 was as follows: pter–q11, five clones; q11–q12, five clones; q12q13, eight clones; and q13–qter, five clones. The 13 clones

containing conserved sequences assigned to 22q12–qter were screened for RFLPs with 34 restriction endonucleases, and 6 of them identified polymorphisms (D22S83, D22S100, D22S102-105, Table 3). In addition, 6 clones detecting evolutionarily conserved sequences on other human chromosomes were identified.

RFLP screening

The screening for RFLPs was limited to the probes mapped to the region 22q12–qter. Of the 56 inserts within 22q12–q13 and the 28 inserts within 22q13–qter, 28 polymorphic markers were identified. Of these, 22 detected two-allele polymorphisms consistent with a single change of the recognition site for a restriction endonuclease. One insertion/deletion polymorphism, one three-allele polymorphism, and one polymorphism showing multiple alleles were found (D22S83, D22S104, and D22S103, respectively). As expected, RFLPs were detected most frequently with *Msp*I and *Taq*I (Barker et al. 1984). The details of these RFLPs are summarized in Table 3.

Discussion

The 28 polymorphic probes[1] presented more than double the number of polymorphic markers reported on the second smallest of the human autosomes (Kaplan and Emanuel 1988). These probes are likely to fill the present gaps in the genetic linkage map of the distal part of this chromosome. In addition, the nonpolymorphic markers will be important tools for the construction of a long-range physical map based on pulsed-field gel electrophoresis. Surprisingly many of the isolated probes (12%) detected sequences that are evolutionarily conserved in the human, mouse, and Chinese hamster DNA. It is likely that most of these clones represent functional genes on chromosome 22. Seven of these, which were found to detect RFLPs, will be possible to locate more precisely by linkage analysis in reference families.

One observation that is difficult to explain was the significant number of clones containing inserts that on hybridization to the somatic cell panel and control human DNA gave a similar single or multiband pattern in all cases (Table 2).

The PCR-based strategy was efficiently applied for the rapid isolation of chromosome 22-specific DNA markers. Construction of genomic, chromosome-specific libraries (preferably in plasmids), where the insert size range does not exceed the limitations of the PCR technique, and use of the procedure B approach for the characterization of clones should permit a rapid increase in the number of recombinant DNA probes specific for different human chromosomes.

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[1] The polymorphic probes will be submitted to the American Type Culture Collection

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