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REFINED MAPPING OF EIGHT COSMID MARKERS ON HUMAN CHROMOSOME 22

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Summary Eight cosmid clones were regionally assigned to small subregions of chromosome 22 by hybridization with a total of 22 somatic cell hybrids. One cosmid was localized to the proximal part of 22q which contained the region commonly deleted in the DiGeorge syndrome. Seven cosmids showing restriction fragment length polymorphisms were localized to the telomeric region distal to the MB locus, which was reported to be frequently deleted in sporadic meningioma. These cosmids, when finely mapped and ordered, are considered useful for the identification of genetic alterations on this chromosome arm.

Key Words DNA marker, chromosome 22, gene mapping, hybrid cell, DiGeorge syndrome

INTRODUCTION

Chromosome 22, one of the shortest chromosomes among human genomes, is thought to contain several genes responsible for tumorigenic disorders or congenital malformation syndromes. Loss of heterozygosity (LOH) on 22q has been reported in meningiomas, colorectal cancers, pheochromocytomas, and breast cancers, indicating the possible existence of tumor suppressor(s) on the chromosome arm (Dumanski *et al.*, 1987; Okamoto *et al.*, 1988; Tanaka *et al.*, 1992; Shin *et al.*, 1993; Chen *et al.*, 1991). Moreover, in the case of the DiGeorge syndrome (DGS), deletion of a certain region of 22q11 is thought to be closely related with

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this disease (Driscoll *et al.*, 1992). A previous report of ours described how a total of 108 cosmid markers on human chromosome 22 were newly isolated and roughly mapped with five somatic cell hybrids into four different regions of chromosome 22 (Kurahashi *et al.*, 1994). Of these, 64 detected restriction fragment length polymorphism (RFLP) systems that should be very useful for linkage mapping of the chromosome and for detection of LOH in several human tumors. In this study, eight of the 108 cosmid clones, including seven RFLP markers, were selected for the usefulness of their location and were localized to small subregions by mapping on a panel of 22 rodent/human somatic cell hybrids containing a veriety of partial segment of human chromosome 22.

MATERIALS AND METHODS

Of 108 cosmids on chromosome 22, eight cosmids, seven of which had been roughly mapped on 22q12.1-qter and one to 22pter-q11.2, were selected (Kurahashi *et al.*, 1994). A total of 22 rodent/human somatic cell hybrids were used for the mapping procedure, most of which had been developed by fusion of human cell lines, each carrying a different chromosome 22 translocation, with a Chinese hamster ovary cell line deficient in adenylosuccinate lyase activity (Delattre *et al.*, 1991). References for the origin of the other hybrids are as follows: X/22-33-11TG, 1/22AM27 (Geurts van Kessel *et al.*, 1980); CERCH-h, -s (Hors-Cayla *et al.*, 1981); WESP2A (de Klein *et al.*, 1982); KAG, DAG (Boyd *et al.*, 1988); NF13 (Ledbetter *et al.*, 1989).

Southern hybridization was performed with these cosmids as probes to a panel of the 22 hybrids. High molecular weight DNAs were completely digested with *Hin*dIII, separated on agarose gel, and then blotted onto nylon membranes. Cosmid DNA was radiolabeled with [α-^{32}P]dCTP by means of the random-primer method. The radiolabeled cosmids and the membranes were then prehybridized with 200 μg/ml of sonicated human placental DNA in a hybridization solution at 65°C for 24 h to reduce the background effects of human repetitive sequences. Hybridization was performed overnight at 65°C in a solution containing 10% SDS, 7% polyethylene glycol 8000, and 200 μg/ml of human placental DNA. After hybridization, the blots were washed twice at room temperature with 2 × SSC, then twice at 65°C with 0.1 × SSC and 0.1% SDS for 15 min each, and exposed to Kodak XAR films at −80°C for 1–3 days.

RESULTS

The panel of rodent/human somatic cell hybrids allowed us to assign eight cosmids to small subregions of chromosome 22. Regional localization of the cosmids is schematically shown in Fig. 1. Interestingly, cHKAD-26 contained

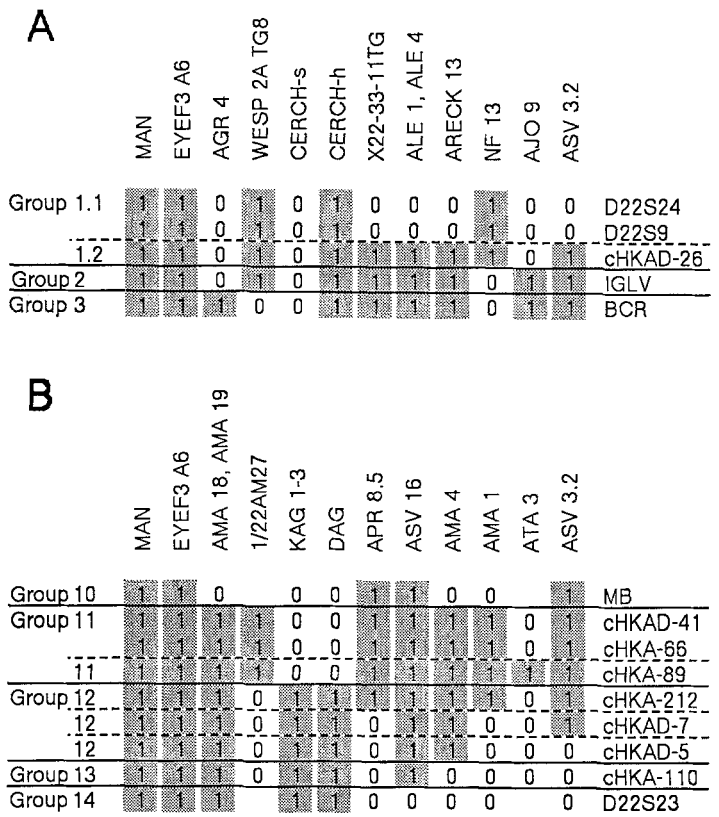

Fig. 1. Mapping of eight cosmids as deduced from the characterization of the panel of somatic cell hybrids. Rectangles indicate the portion of the chromosome 22 present in the hybrids. For a posiitve Southern blot signal, "1" is entered, and for a negative one, "0." The name of the hybrids are indicated at the top, while the nem of the cosmid clones are listed on the right. The data for D22S24, D22S9, IGLV, BCR, MB, and D22S23 were derived from the previous study (Delattre *et al.*, 1991). The order in which cHKAD-41, cHKA-66, and cHKA-89 are listed is arbitrary. On the left are indicated the groups that are explained in the reference, and the subgroups that were deduced in this study. A. Mapping of cHKAD-26 near the centromeric region of 22q. B. Mapping of seven polymorphic clones to the telomeric region of 22q.

multicopy sequences specific to this small subregion.

Some of the cosmids detected new subregions which had not been defined in the previous report (Delattre *et al.*, 1991): Group 11 could be divided into two and Group 12 into three subregions in this study.

In addition, cHKAD-26, cHKA110, and cHKA212 cross-hybridized to mouse and/or Chinese hamster DNAs, indicating that these cosmids contain evolutionarily conserved sequences (data not shown).

DISCUSSION

Region-specific DNA fragments have been powerful tools for the investigation of genetic alterations underlying a large number of human diseases. Although a large set of polymorphic or non-polymorphic markers on chromosome 22 has been developed in recent years, additional markers are required for construction of more detailed linkage map of the chromosome. The eight cosmid markers that were finely mapped to small subregions of chromosome 22 should be a valuable source for genetic analysis of the chromosome. In the panel of hybrids used for this study, some of the cosmids detected new subregions which had not been defined in the previous report (Delattre *et al.*, 1991). This panel thus proved to be very useful for mapping procedures to divide chromosome 22 into more refined subregions. Detailed mapping of the remaining cosmids with the aid of the same panel of hybrids is in progress.

DGS is a developmental field defect that involves the third and fourth pharyngeal pouches, causing absence of thymus and parathyroid glands, cardiac conotruncal abnormalities, and facial dysmorphism. A high-resolution banding technique has recently revealed that deletion of 22q11.21-q11.23 is closely related with the syndrome (Wilson *et al.*, 1992). Dosage analysis of cHKAD-26, which had been mapped near the centromeric region of 22q, proved it to be located in the submicroscopically deleted region of two DGS patients (Kurahashi *et al.*, 1994). The present study localized cHKAD-26 within the region between D22S9 and IGLV, where the region commonly deleted in DGS and identified in a previous report lies (Fibson *et al.*, 1990). Therefore, it was physically confirmed that cHKAD-26 locates in the region commonly deleted in DGS. Interestingly, this cosmid contains unique sequences cross-hybridizing to rodent DNAs and multi-copy sequences specific to this small subregion of chromosome 22 (data not shown). A detailed analysis of the locus, including cosmid walking and cDNA isolation, is now in progress.

LOH on 22q is frequently observed in meningioma, pheochromocytoma, colorectal cancer, and breat cancer. Although the neurofibromatosis 2 (NF2) gene has recently been isolated (Trofatter *et al.*, 1993; Rouleau *et al.*, 1993), there still remains the possibility that additional tumor suppressors associated with development of these tumors exist on the chromosome. Seven clones that were able to detect RFLPs were finely mapped and ordered in the telomeric region of 22q distal to the MB locus, which was reported to be frequently deleted in sporadic meningioma. A recent investigation of ours showed that several cases of sporadic meningioma had lost the distal part of 22q, while LOH was more frequently observed in the telomeric region of 22q in colorectal cancer (unpublished data), indicating that other tumor suppressor gene(s) than the NF2 gene may exist in this chromosomal region. The cosmids used in this study should aid the construction of a deletion map to localize the tumor suppressor gene(s) responsible for colorectal cancer and/

or sporadic meningioma.

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Note added in proof. The cosmid markers obtained in this study have been submitted to the Japanese Cancer Research Resources Bank (National Institute of Health, 23–1, Toyama, 1-chome, Shinjuku-ku, Tokyo 162, Japan) and will be available upon request.

REFERENCES

Boyd Y, Cockburn D, Holt S, Munro E, van Ommen GJ, Gillard B, Affara N, Ferguson-Smith M, Craig I (1988): Mapping of 12 translocation breakpoints in the Xp21 region with respect to the locus for Duchenne muscular dystrophy. Cytogenet Cell Genet **48**: 28–34

Chen LC, Kurisu W, Ngo J, Moore D, Smith HS (1991): Loss of heterozygosity on chromosome 18p and 22q in primary breast carcinomas. Proc Am Assoc Cancer Res **32**: 300

Delattre O, Azambuja CJ, Aurias A, Zucman J, Peter M, Zhang F, Hors-Cayla MC, Roulear G, Thomas G (1991): Mapping of human chromosome 22 with a panel of somatic cell hybrids. Genomics **9**: 721–727

Driscoll DA, Budarf ML, Emanuel BS (1992): A genetic etiology for DiGeorge syndrome: consistent deletions and microdeletions of 22q11. Am J Hum Genet **50**: 924–933

Dumanski JP, Carlbom E, Collins VP, Nordenskjold M (1987): Deletion mapping of a locus on human chromosome 22 involved in the oncogenesis of meningioma. Proc Natl Acad Sci USA **84**: 9275–9279

Fibson WJ, Budarf M, McDermid H, Greenberg F, Emanuel BS (1990): Molecular studies of DiGeorge syndrome. Am J Hum Genet **46**: 888–895

Geurts van Kessel AHM, Westerveld A, de Groot PG, Meera KP, Hagemeijer A (1980): Regional localization of the genes coding for human ACO2, ARSA, and NAGA on chromosome 22. Cytogenet Cell Genet **28**: 169–172

Hors-Cayla MC, Junien C, Heuertz S, Mattei JF, Frézal J (1981): Regional assignment of arysulfatase A, mitochondrial aconitase and NADH-cytochrome b_5 reductase by somatic cell hybridization. Hum Genet **58**: 140–143

de Klein A, Geuerts van Kessel A, Grosveld G, Bartram CR, Hagemeijer A, Bootsma D, Spurr NK, Heisterkamp N, Groffen J, Stephenson JR (1982): A cellular oncogene is translocated to the Philadelphia chromosome in chronic myelocytic leukaemia. Nature **300**: 765–767

Kurahashi H, Akagi K, Karakawa K, Nakamura T, Dumanski JP, Sano T, Okada S, Takai S, Nishisho I (1994): Isolation and mapping of cosmid markers on human chromosome 22, including one within the submicroscopically deleted region of DiGeorge syndrome. Hum Genet **93**: 248–254

Ledbetter DH, Rich DC, O'Connell P, Leppert M, Carey JC (1989): Precise licalization of NF1 to 17q11.2 by balanced translocation. Am J Hum Genet **44**: 20–24

Okamoto M, Sasaki M, Sugio K, Sato C, Iwama T, Ikeuchi T, Tonomura A, Sasazuki T, Miyaki M (1988): Loss of constitutional heterozygosity in colon carcinoma from patients with familial polyposis coli. Nature **331**: 273–277

Rouleau GA, Merel P, Lutchman M, Sanson M, Zucman J, Marineau C, Hoang-Xuan K, Demczuk S, Desmaze C, Plougastel B, Pulst SM, Lenoir G, Bijlsma E, Fashold R, Dumanski J, de Jong

P, Parry D, Eldrige R, Aurias A, Delattre O, Thomas G (1993): Alteration in a new gene encoding a putative membrane-organizing protein causes neuro-fibromatosis type 2. Nature **363**: 515–521

Shin E, Fujita S, Takami K, Kurahashi H, Kurita Y, Kobayashi T, Mori T, Nishisho I, Takai S (1993): Deletion mapping of chromosome 1p and 22q in pheochromocytoma. Jpn J Cancer Res **84**: 402–408

Tanaka N, Nishisho I, Yamamoto M, Miya A, Shin E, Karakawa K, Fujita S, Kobayashi T, Rouleau G, Mori T, Takai S (1992): Loss of heterozygosity on the long arm of chromosome 22 in pheochromocytoma. Genes Chromosomes Cancer **5**: 399–403

Trofatter JA, MacCollin MM, Rutter JL, Murrell JR, Duyao MP, Parry DM, Eldridge R, Kley N, Menon AG, Pulaski K, Haase VH, Ambrose CM, Munroe D, Bove C, Haines JL, Martuza RL, MacDonald ME, Seizinger BR, Short MP, Buckler AJ, Gusella JF (1993): A novel moesin-, ezrin-, radixin-like gene is a candidate for the neurofibromatosis 2 tumor suppressor. Cell **72**: 791–800

Wilson DI, Cross IE, Goodship JA, Brown J, Scambler PJ, Bain HH, Taylor JFN, Walsh K, Bankier A, Burn J, Wolstenholme J (1992): A prospective cytogenetic study of 36 cases of DiGeorge syndrome. Am J Hum Genet **51**: 957–963