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Identification of Two Cosmids Derived from within Chromosomal Band 3p21.1 That Contain Clusters of Rare Restriction Sites and Evolutionarily Conserved Sequences

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Summary

We have isolated large numbers of human recombinants from a cosmid library constructed from an interspecific (hamster/human) somatic cell hybrid whose only human component is an intact chromosome 3. Unique sequence probes were isolated from these recombinants and were used to localize them along the length of chromosome 3 by hybridization to a somatic cell hybrid deletion panel. We identified two cosmids, cA84 (D3S92) and cA199 (D3S93), derived from within chromosomal band 3p21.1. Both cosmids contained multiple rare restriction sites that were tightly clustered within the cosmids. We have therefore identified, in a region consistently deleted in a variety of lung cancers, two cosmids that may contain genes that are candidates for involvement in lung cancer.

Introduction

Consistent rearrangements and deletions involving the short arm of chromosome 3 have been observed in a variety of human malignancies. Karyotypes of small-cell lung cancer (SCLC) demonstrate a deletion that commonly encompasses the 3p14-p23 region (Whang-Peng et al. 1982; Falor et al. 1985). Other SCLC tumor cells have various larger deletions or translocations involving this region (Whang-Peng et al. 1982). Deletion of DNA sequences at the chromosomal region 3p21 has been found in all major types of lung cancer (Kok et al. 1987). Both spontaneous and hereditary renal cell carcinomas have been found to exhibit alterations in the short arm of chromosome 3 (Cohen et al. 1979; Pathak et al. 1982; Wang and Perkins 1984). In an attempt to further characterize deletions associated with these tumors, several groups have used physically localized probes that detect polymorphisms, to show that these tumors are associated with loss of 3p sequences (Brauch et al. 1987; Naylor et al. 1987; Zbar et al. 1987; Johnson et al. 1988; Kovacs et al. 1988).

For the past 4 years we have been isolating probes from chromosome 3. These probes will aid in the construction of a physical map and, in addition, may identify candidate genes involved in the development of SCLC. Our strategy was to isolate thousands of recombinants from somatic cell hybrids (hamster/human) whose only human component is an intact chromosome 3 (Carlock et al. 1986; Smith et al. 1987). The probes could then be localized along the length of the chromosome by using either a somatic cell hybrid deletion panel (Gerber et al. 1988) or in situ hybridization.

During the course of this work we identified two recombinants that were particularly interesting because both recombinants map within chromosomal band 3p21.1, and because both contain multiple rare restriction sites, they are potential candidate genes involved in the generation of SCLC.

Material and Methods

Hybrid Cell Lines

We have previously described a human chromosome 3-specific hybrid cell deletion mapping panel that was produced using selectable genetic markers on the long

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arms of both chromosomes 3 and 8 (Gerber et al. 1988). The Chinese Hamster ovary (CHO) parent cell line Urd$^-$C is an auxotrophic mutant requiring exogenous uridine or complementation with a gene on the long arm of chromosome 3 for growth (Patterson et al. 1983). UCTP-2A-3 is a hybrid containing a normal human chromosome 3 as its only nonhamster genetic material (Firnhaber et al. 1985). The Urd$^-$C hybrids were initially screened using Cellogel electrophoresis for the presence of human Acylase 1 (Acyl) activity (Voss et al. 1980), which has previously been localized to 3p21 (Miller et al. 1985). One hybrid (R10-4) was selected after human parental cells were fused with the mouse HGPRT$^-$ cell line, RAG. HGPRT$^+$ cells were then screened for the human transferrin receptor located at 3q26-qter (Kidd and Gusella 1985) by using a human-specific antibody provided by Y. Miller. Hybrids were analyzed by sequential Giemsa-11/GTG banding (Morse et al. 1982) and repeatedly were subcloned and analyzed cytogenetically prior to DNA preparation. Detailed molecular analysis of two hybrids (R10-4 [3p21.1-3qter] and 3;7/UC2E-1 [3p21.1-3qter]) has revealed that the breakpoint in the 3;7 hybrid is distal to the breakpoint of R10-4, thus allowing the regional localization of probes within the 3p21.1 band (Drabkin et al. 1989).

Isolation of Human Recombinants and DNA Preparation

Recombinants containing human inserts were identified by hybridization to total human genomic DNA (Gusella et al. 1980). DNA was isolated from these recombinants according to a method described elsewhere (Smith et al. 1987). Genomic DNA was isolated from the somatic cell hybrids according to a method described elsewhere (Gerber et al. 1988).

Restriction-Enzyme Digestion of DNA

With the exception of *Not*I and *Sfi*I, all restriction enzymes used in the present study were obtained from Bethesda Research Laboratories (BRL). *Not*I and *Sfi*I were obtained from Pharmacia. DNAs were digested in enzyme buffers according to the manufacturers specifications, except that we typically used 4 units restriction endonuclease/μg input DNA. Restriction-endonuclease maps of the two cosmids were derived using a combination of techniques, including (1) indirect end-labeling and (2) hybridizing fragments produced with one restriction endonuclease to fragments generated with another restriction endonuclease.

Hybridization Conditions

Isolated fragments were purified from LMP agarose (BRL) and were radiolabeled using random hexanucleotide primers (Feinberg and Vogelstein 1984). Hybridization conditions were according to a method described elsewhere (Smith et al. 1987).

Results

Restriction Maps of the Two Cosmids

Cosmids cA84 (D3S92) and cA199 (D3S93) were two of eight cosmids that we identified in a sample of 200 cosmids with human inserts isolated from a chromosome 3–specific cosmid library and tested for the presence of the rare restriction sites *Not*I, *Mlu*I, *Nru*I, *Sac*II, *Bss*HII, and *Sfi*I (Smith et al. 1987). Restriction maps of these two cosmids are shown in figure 1. Both cosmids contain several islands of clustered rare restriction sites. Many of the restriction fragments derived from these cosmids hybridized to both hamster and human DNA, suggesting that they contain conserved sequences. Fragments that hybridized to both hamster and human DNA are shown above the restriction map in figure 1.

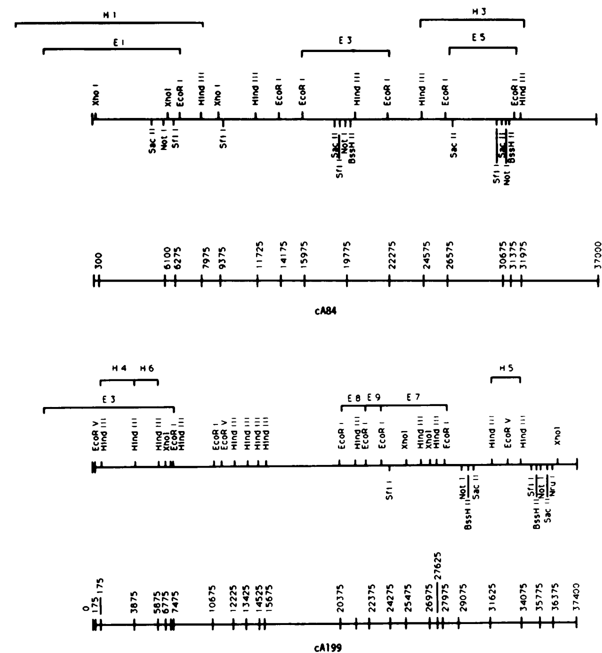

Figure 1 Restriction maps of cA84 and cA199. The rare restriction sites are drawn on the bottom and the common restriction sites are drawn on the top of the respective maps. Fragments that were found to contain evolutionarily conserved sequences are shown on the top of each map. The largest *Eco*RI and *Hin*dIII fragments are E1 and H1, respectively, and fragments are numbered sequentially from largest to smallest.

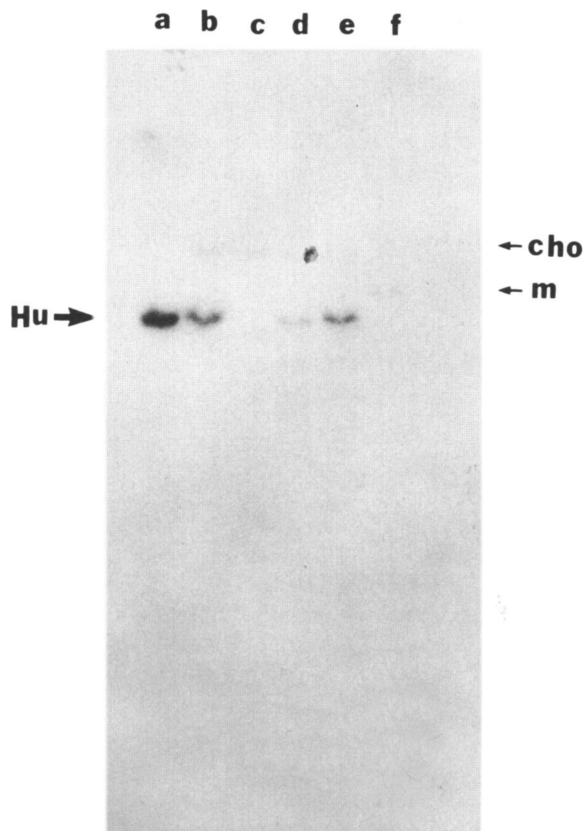

Figure 2 Human and hybrid DNAs digested with *Eco*RI and hybridized with the probe cA84E3. The human (Hu), hamster (cho), and mouse (m) bands are indicated by arrows. Lane a, human placenta; lane b, hybrid H3-4 (3pter-q21:::3q26-3qter); lane C, TL9542/UC12-8 (3p14.2-3qter); lane d, 3;8/4-1 (3p14.2-3pter); lane e, 3;7/UC2E-1 (3p21.1-3qter); lane f, R10-4 (3p21.1-3qter). The chromosomal 3 content of the hybrids is indicated in parentheses.

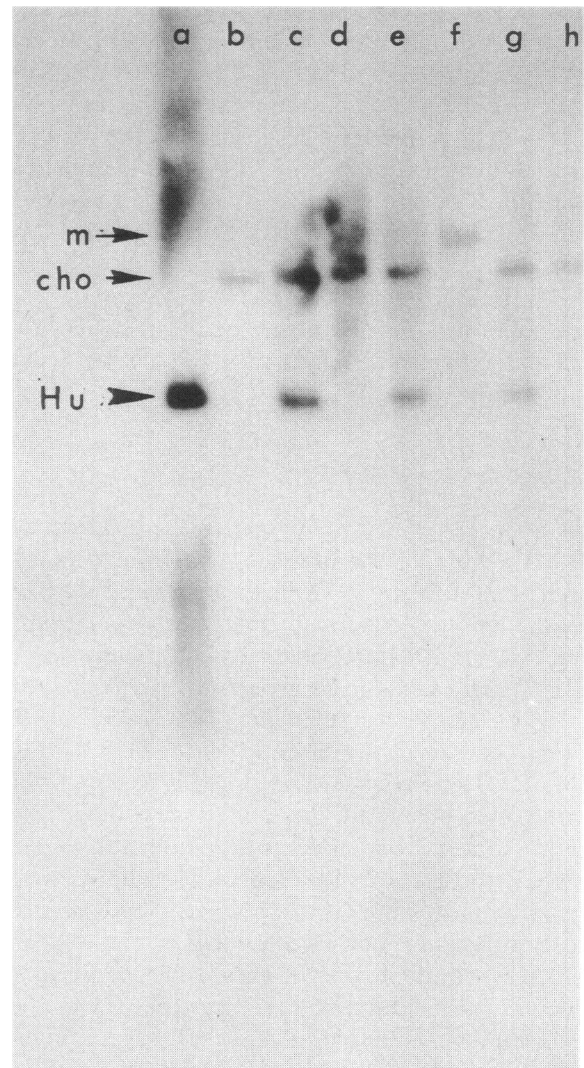

Figure 3 Human and hybrid DNAs digested with *Eco*RI and hybridized with the probe cA199H4. The human (Hu), hamster (cho), and mouse (m) bands are indicated by arrows. Lane a, human placenta; lane b, cho; lane C, hybrid H3-4 (3pter-3q21:::3q26-3qter); lane d, TL9542/UC12-8 (3p14.2-3qter); lane e, 3;8/4-1 (3p14.2-3pter); lane f, R10-4 (3p21.1-3qter); lane g, 3;7/UC2E-1 (3p21.1-3qter); lane h, UCH12 (3cen-qter). The chromosomal 3 content of the hybrids is indicated in parentheses.

Subchromosomal Localization of cA84 and cA199

Fragments derived from the cosmids were first tested for the presence of highly repetitive DNA sequences by hybridization to labeled human DNA according to a method described elsewhere (Smith et al. 1987). Fragments that did not hybridize to repetitive sequences were then tested as unique-sequence chromosome 3–specific hybridization probes.

Several unique-sequence hybridization probes derived from the two cosmids were hybridized to a somatic cell hybrid deletion panel. Figure 2 shows the result of hybridizing the third largest *Eco*RI fragment from cA84 to several of the hybrids from the deletion panel. This fragment contains sequences conserved between mouse, hamster, and human DNA and thus detects additional bands in each hybrid. This fragment fails to detect a human band in two of the hybrids (TL9542/UC12-8 [3p14.2-qter] and R10-4 [3p21.1-qter]). The fragment does detect a human band to hybrid 3;7/UC2E-1 [3p21.1-qter], which was derived from the Greig polysyndactyly 3;7 chromosome translocation, t(3;7)(p21.1; p13). Figure 3 shows the result of hybridizing the fourth largest *Hin*dIII fragment derived from cA199 to the chro-

mosome 3 deletion panel. This fragment also contains evolutionarily conserved sequences and thus hybridizes to mouse, hamster, and human DNA. This fragment does not detect a human band from hybrids TL9542/UC12-8, R10-4, and UCH12 [3cen-qter] but does detect such a band from hybrid 3;7/UC2E-1. Both cosmids can therefore be localized within chromosome band 3p21.1 in the region distal to the breakpoint of hybrid R10-4 but proximal to the breakpoint of hybrid 3;7/UC2E-1. This subchromosomal assignment has been confirmed with two additional fragments from each of the cosmids (cA84E1, cA84E5, cA199E8, and cA199E7).

Discussion

Whang-Peng et al. (1982) carried out a detailed analysis of the chromosomes present in SCLC and reported a consistent deletion in the region spanning 3p14-p23. This finding has been verified using polymorphic probes from this region (Naylor et al. 1987). Recently Kok et al. (1987) reported a deletion of band 3p21 in all types of lung cancer. Thus, the finding that a specific region of chromosome 3 is consistently deleted in SCLC suggests that deletion of a specific gene may be responsible for the development of SCLC and possibly other types of lung cancer. This model is based on studies with retinoblastoma, in which molecular deletions (Cavenee et al. 1983) led to the identification of the Rb gene locus as a recessive "anti-oncogene" (Lee et al. 1987).

To help identify the gene(s) responsible for the development of these cancers, it will be necessary to construct a physical map of the region. To date, the only gene precisely localized to this region is aminoacylase 1 (Miller et al. 1985), although other genes—including cholecystokinin [3p21-pter] (Takahashi et al. 1986), beta-1 galactosidase [3cen-p21] (Bootsma and McAlpine 1979), and antigen genes msk9, 10, 32, and 33 [3p21-pter] (Rettig et al. 1984)—have been localized to larger regions. In addition, there are three anonymous DNA segments mapped to 3p21—DNF15S2 (Goode et al. 1986), D3S2 (Gerber et al. 1988), and D3S28 (Cannizzaro et al. 1988).

The two cosmids we have identified should also be useful in the characterization of this region. Both cosmids contain fragments that detect conserved sequences in rodents and humans. Using these fragments, we detected mRNAs from several human tissues, which supports the hypothesis that identification of CpG dinucleotide islands can be used to localize new genes (Lindsay and Bird 1987). In addition, because both contain multiple rare restriction sites, they will be useful as junction or linking clones in the construction of long-range restriction maps.

These two cosmids were identified by screening 200 chromosome 3–specific cosmids for rare restriction sites (Smith et al. 1987). This analysis identified eight cosmids that contained clusters of rare sites; six of these eight cosmids were localized to other regions of the chromosome (Smith et al. Manuscript in preparation). To identify other cluster cosmids, we have to date screened more than 2,000 chromosome 3–specific cosmids and identified 72 additional cluster cosmids. These should include quite a number from the region of interest.

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