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Amplified DNA in Y1 mouse adrenal tumor cells: isolation of cDNAs complementary to an amplified c-Ki-*ras* gene and localization of homologous sequences to mouse chromosome 6

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ABSTRACT

We have isolated cDNA clones complementary to a c-Ki-ras cellular oncogene that is amplified in Y1 mouse adrenal tumor cells, with the amplified sequences located on double-minute chromatin bodies (DMs) and homogeneously staining chromosomal regions (HSRs). Characterization of the cDNAs included the isolation of corresponding genomic clones, Northern blot analysis of RNA, and DNA sequence analysis. Our studies demonstrate that the c-Ki-ras gene amplified in the Y1 cells is homologous to the human c-Ki-ras2 gene. We have also obtained evidence that, in addition to c-Ki-ras, at least one other transcription unit has been amplified in the mouse adrenal tumor cells. Moreover, by Southern blot analysis of Chinese hamster-mouse somatic cell hybrids, we have determined that the amplified DNA sequences associated with DMs and HSRs, including the c-Ki-ras gene, are present in normal mouse cells on chromosome 6.

INTRODUCTION

Recent studies have demonstrated that the alteration or enhanced expression of specific cellular sequences, referred to as cellular oncogenes, is directly involved in the neoplastic transformation of some mammalian cells. A number of such oncogenes represent "activated" cellular homologues of previously identified retroviral oncogenes (1,2). One mechanism which would result in the enhanced expression of cellular sequences is gene amplification, although the exact nature of the relationship between gene amplification and tumorigenesis remains to be defined.

Atypical cytologic entities, termed DMs and HSRs, which result from a process of gene amplification (3), exist in a variety of mammalian tumor cells but rarely have been described in normal cells. We have demonstrated previously that, among other sequences, a cellular gene (c-Ki-ras) homologous to the transforming gene (v-Ki-ras) of the Kirsten murine sarcoma virus (KiMuSV) is amplified in Y1 mouse adrenal tumor

cells, with the amplified sequences located on HSRs or DMs (4-6). Accompanying the 30-60 fold amplification of the c-Ki-*ras* gene in the Y1 cells is a corresponding increase in the level of c-Ki-*ras* mRNAs and of the 21,000 dalton guanine nucleotide-binding protein ($p21^{c\text{-}Ki\text{-}ras}$) encoded by this gene (6). There are other examples of tumor cells in which cellular DNA sequences having homology to a retroviral oncogene are amplified and located on DMs or HSRs. These include Colo320 human neuroendocrine tumor cells (7), as well as a number of human neuroblastomas (8). Together these observations strongly implicate oncogene amplification in the genesis of some tumors.

We have engaged in studies to clarify the origins, expression and structural organization of the amplified genetic material in the Y1 cells. In this report, we describe the construction of a cDNA library in pBR322 using $polyA^+$-RNA from Y1 cells and the isolation of cDNA clones complementary to c-Ki-*ras* transcripts. From DNA sequence analysis of one of these cDNA clones, we have determined that the c-Ki-*ras* gene amplified in the adrenal tumor cells is homologous to the human c-Ki-*ras*2 gene, which has been implicated as a transforming gene in a number of human tumors (9-12). We have also obtained evidence that, in addition to the c-Ki-*ras* gene, at least one other transcription unit has been amplified in the Y1 cells. Lastly, by Southern blot analysis of DNAs from Chinese hamster-mouse somatic cell hybrids, we have demonstrated that the DM- and HSR-associated amplified DNA sequences in these cells originated from mouse chromosome 6.

MATERIALS AND METHODS

Cell Lines and Mouse Strains

The culture conditions and detailed cytogenetic analyses of Y1 cell lines (Y1-DM, Y1-HSR and Y1-DM Clone 3b) have been published (4,5,13). LAF_1 mice, the parental strain from which the Y1 tumor was isolated, were obtained from the Jackson Laboratory. LAF_1 fibroblasts were established in culture from minced kidneys. Somatic cell hybrids were derived from the fusion of normal mouse cells with established Chinese hamster cells in four separate experiments (14,15). Their chromosome content was determined by trypsin-Giemsa banding and isozyme analysis (14). Chromosome analysis of the hybrids was repeated at the time DNA was extracted.

DNA Blot Hybridizations and Restriction Endonuclease Analysis

Restriction-endonuclease digested DNA samples were subjected to

electrophoresis in 0.7-1% agarose gels and transferred to nitrocellulose as described (4,5,16). Prehybridization, hybridization and washing of filters under stringent conditions of 65°C in 0.1% SDS, 0.1 x SSC (1 x SSC is 0.15 M NaCl, 15 mM sodium citrate), and the ^{32}P-labeling of DNA have been detailed (4,5,16).

RNA Isolation and Blot Hybridization

Total cellular RNA was prepared with guanidine-hydrochloride (17). PolyA$^+$-RNA was recovered from total cellular RNA by affinity chromatography on oligo(dT) cellulose (18). RNA samples were denatured and subjected to electrophoresis through 1.2% agarose, 6% formaldehyde gels as described (19), and transferred to nitrocellulose in 10 x SSC. After hybridization, RNA filters were washed at 55°C in 0.3 x SSC, 0.1% SDS.

Construction of cDNA Library

PolyA$^+$-RNA from Y1-DM Clone 3b was used as a template for synthesis of double-strand cDNA with avian myeloblastosis virus reverse transcriptase (provided by J. Beard) according to published procedures (20). After S1 nuclease treatment to remove single-strand tails, the cDNA molecules were size-fractionated on a 5-20% sucrose gradient (10 mM Tris-hydrochloride, pH 7.4, 0.1 M NaCl, 1 mM EDTA) at 25,000 rpm for 24 hr in a Beckman SW28 rotor. Molecules smaller than 300 nucleotides were discarded. The larger cDNA molecules were recovered by ethanol precipitation and tailed with deoxycytidine at 14°C for 1 hr using terminal deoxynucleotidyl transferase (21). The tailed cDNAs were annealed to PstI-digested pBR322 molecules tailed with deoxyguanosine. The annealing mixture, containing approximately 10:1 (wt/wt) plasmid:cDNA, was incubated at 65°C for 10 min, transferred to 42°C for 4 hr, then allowed to cool to room temperature over a period of 2-3 hr. The resulting recombinant molecules were used to transform E. coli strain HB101 (22). Tetracycline-resistant (15 μg/ml) transformants were grown on duplicate nitrocellulose filters and prepared for hybridization to ^{32}P-labeled probes by the method of Grunstein and Hogness (23).

Recombinant Plasmids and Bacteriophage

Construction of a recombinant library of Y1 DM-DNA fragments in the lambda vector Charon 4A has been published (4). For subcloning of EcoRI inserts of recombinant bacteriophage into the EcoRI site of alkaline phosphatase-treated pBR322, we followed the protocol of Lacy et al. (24). Plasmid DNA was prepared from a cleared lysate of bacteria (25) and

purified by centrifugation through a 5-20% sucrose gradient. Recombinant plasmid pHiHi3 contains a 1 kilobase (kb) insert of v-Ki-ras sequences in the EcoRI site of pBR322 (26).

M13 Cloning and Sequencing

PstI and HincII fragments of plasmid cYKi2 were subcloned into phage m13 (27) and sequenced by the enzymatic method of Sanger (28). A sequence was confirmed by the determination of both strands or of one strand from multiple clones.

RESULTS

Isolation of cDNA Clones

A DM-containing Y1 cell line (Clone 3b) was chosen as the source of polyA$^+$-RNA used in construction of a cDNA library. In order to identify cDNAs complementary to abundantly expressed mRNAs derived from amplified genomic DNA, we used a differential hybridization protocol. Approximately 2,000 transformants from the cDNA library were grown on duplicate nitrocellulose filters and hybridized to ^{32}P-labeled cDNAs synthesized from polyA$^+$-RNAs of either a Y1 or a 3T3 mouse cell line. Seven colonies, representing 0.35% of those screened, produced a hybridization signal only with the Y1 probe. Southern blot analyses revealed that these seven clones represent sequences amplified in the Y1 cells (data not shown).

Initial characterization of these cDNAs included: (1) isolation of the corresponding genomic clones from a recombinant library of Y1 DM-DNA fragments (Ref. 4); (2) Northern blot analyses of RNAs; (3) DNA sequence analyses. The results of these studies, which will be presented in detail elsewhere (George et al., manuscript in preparation), provided evidence that the seven cDNAs represent segments of the 3' non-translated regions of the c-Ki-ras mRNAs. The mouse c-Ki-ras gene encodes three mRNA species which are approximately 5.2, 2.0 and 1.2 kb in size (Fig. 1; Refs. 6 and 29). Two of the seven cDNAs we isolated, represented by clone pVPk4 (k4), hybridize to RNAs 5.2 and 2.0 kb in size (Fig. 1, lane 3), and are complementary to sequences contained within the 3' non-translated region of the 2 kb mRNA species. Five other cDNAs, represented by clone pVPk1 (k1), apparently were derived from the 3' non-translated region of the 5.2 kb mRNA and hybridize only to this species (Fig. 1, lane 4). For example, clone k1 has an insert fragment 1 kb in size, with no long open reading frame. It does have a

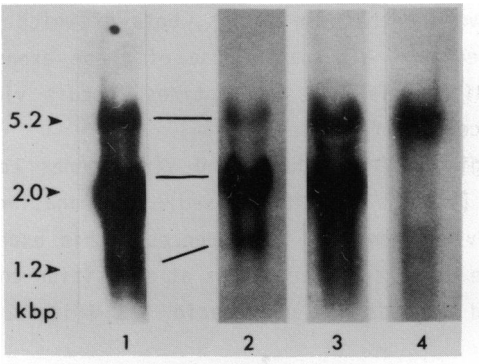

Fig. 1. Hybridization of RNAs to cDNA clones and v-Ki-ras. Total cellular RNA samples (20 μg) from Y1-DM cells were probed with ^{32}P-labeled DNA from the following: (1) v-Ki-ras clone pHiHi3; (2) cDNA cYKi2; (3) cDNA k4; (4) cDNA k1.

poly(A)-addition signal, AATAAA (30), 21 base pairs (bp) 5' of a terminal poly(A) tract (data not shown).

The Amplified Mouse Gene is Homologous to Human c-Ki-ras2.

These results demonstrated that the differential hybridization screening protocol had allowed us to identify cDNAs complementary to amplified DNA sequences. However, in an attempt to identiy cDNAs derived from the coding region of the c-Ki-ras gene, we rescreened the Y1 cDNA library with a probe (pHiHi3) containing the v-Ki-ras gene. Of approximately 2000 transformants screened, we identified one clone homologous to the v-Ki-ras probe. This cDNA contains an insert fragment about 200 bp in size, and was designated cYKi2. Like the v-Ki-ras probe, cYKi2 hybridizes to RNAs 5.2, 2.0 and 1.2 kb in size (Fig. 1), which are expressed at 30-60 fold higher levels in Y1 cells than in control mouse cells (data not shown; but see Fig. 3). Also, in the c-Ki-ras gene, sequences encoding cYKi2 are located about 600 bp 5' of the region encoding cDNA k4 (our unpublished observations).

Human cells contain a v-Ki-ras related gene, termed c-Ki-ras2, that has been implicated as a transforming gene in a number of human tumors (9-12). To determine if the gene amplified in the Y1 mouse cells is homologous to this human gene, we sequenced the cYKi2 cDNA and compared its nucleotide composition to the published sequences of the v-Ki-ras (31) and human c-Ki-ras2 (32-34) genes. The human gene contains five

coding exons. Two of these, designated exon 4A and exon 4B, apparently represent alternative fourth coding exons, both of which are expressed. It has been proposed that alternative use of these exons, which would give rise to two distinguishable polypeptides, occurs via differential utilization of splice acceptor sites (32-34).

The coding regions of the human and viral genes comprise an 189 amino acid product (188 if exon 4B is used). Examination of the cYKi2 sequence reveals obvious homology to sequences within exons 3 and 4B of the human gene. The cYKi2 sequence begins at a position corresponding to amino acid 108 and extends to amino acid 173 (Fig. 2). The cYKi2

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                                             *
    Codon:          108      +               +       +       +       *
    cYKi2:               AT GTG CCT ATG GTC CTG GTA GGG AAT AAG TGT GAT TTG CCT TCT AGA ACA
                            Val Pro Met Val Leu Val Gly Asn Lys Cys Asp Leu Pro Ser Arg Thr

Hu-Ki-ras-2:        ....GAT GTA CCT ATG GTC CTA GTA GGA AAT AAA TGT GAT TTG CCT TCT AGA ACA

   v-Ki-ras:        ....GAT GTG CCT ATG GTC CTA GTA GGG AAT AAG TGT GAC TTG CCT TCT AGA ACA


                                                                         *
    Codon:          125          +       +           +       +   +       +   +
    cYKi2:              GTA GAC ACG AAA CAG GCT CAG GAG TTA GCA AGG AGT TAC GGG ATT CCG TTC
                        Val Asp Thr Lys Gln Ala Gln Glu Leu Ala Arg Ser Tyr Gly Ile Pro Phe

Hu-Ki-ras-2:            GTA GAC ACA AAA CAG GCT CAG GAC TTA GCA AGA AGT TAT GGA ATT CCT TTT
                                        Asp

   v-Ki-ras:            GTA GAC ACG AAA CAG GCT CAG GAG TTA GCA AGG AGT TAT GGG ATT CCA TTC


    Codon:          142      +   +       *                           +
    cYKi2:              ATT GAG ACC TCA GCA AAG ACA AGA CAG GGT GTT GAC GAT GCC TTC TAT ACA
                        Ile Glu Thr Ser Ala Lys Thr Arg Gln Gly Val Asp Asp Ala Phe Tyr Thr

Hu-Ki-ras-2:            ATT GAA ACA TCA GCA AAG ACA AGA CAG GGT GTT GAT GAT GCC TTC TAT ACA
                                                EXON 4B ->|
                                                           aga gtg gag gat gct ttt tat aca
                                                EXON 4A ->|

   v-Ki-ras:            ATT GAG ACC TCA GCG AAG ACA AGA CAG aga gtg gag gat gct ttt tat aca
                                                            Arg     Glu


    Codon:          159      +
    cYKi2:              TTA GTC CGA GAA ATT CGA AAA CAT AAA GAA AAG ATG AGC AAA GAT
                        Leu Val Arg Glu Ile Arg Lys His Lys Glu Lys Met Ser Lys Asp

Hu-Ki-ras-2 (4B):       TTA GTT CGA GAA ATT CGA AAA CAT AAA GAA AAG ATG AGC AAA GAT ......

Hu-Ki-ras-2 (4A):       ttg gtg aga gag atc cga caa tac aga ttg aaa aaa atc agc aaa ......

   v-Ki-ras:            ttg gtg aga gag atc cga cag tac aga tta aaa aaa atc agc aaa ......
                                                Gln Tyr Arg Leu Lys Lys Ser Lys Lys
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Fig. 2. The nucleotide sequence and predicted amino acid sequence of cYKi2 and comparison to the corresponding portions of the viral Ki-ras and human c-Ki-ras2 genes. Nucleotide differences between cYKi2 and the viral sequence are indicated by (*). Differences between the mouse and human sequences are indicated by (+). Amino acids predicted by the viral or human sequence that differ from the cYKi2 mouse sequence are indicated below the appropriate nucleotides. Sequences encoded by human exon 4A and the homologous portion of the viral sequence are indicated by lower case letters.

sequence is homologous to the v-Ki-ras sequence up to the splice site between exons 3 and 4, but diverges beyond that point. As noted previously (32,33) and illustrated in Fig. 2, the v-Ki-ras sequence is more closely related to sequences within human exon 4A than 4B. On the other hand, cYKi2 is clearly homologous to sequences contained within exons 3 and 4B of the human gene. Only 15 of the 197 nucleotides and only 1 of 65 amino acids encoded in this region (at amino acid 129) differ between the human and mouse products.

Amplification of Another Transcription Unit

The results described above indicate that the clones we isolated from the Y1 cDNA library are derived from the c-Ki-ras gene. We explored the possibility that additional transcription units might also be amplified in these cells, but whose level of expression, although increased, was not high enough to be detected with our differential hybridization protocol. We, therefore, re-examined a DNA fragment previously isolated from a recombinant phage that was isolated at random from a Y1 DM-DNA library (4). This 3.8 kb EcoRI fragment, that was subcloned into pBR322 and termed pYd1-1, was chosen because it behaves as "unique sequence" DNA in normal mouse (and human) cells, is amplified in Y1 cells, and is located on DMs and HSRs (5). Furthermore, it is homologous to polyA$^+$-RNA species expressed at higher levels in Y1 cells than in control mouse cells, but does not cross-react with sequences in the v-Ki-ras gene (our unpublished observations). We used Northern blot analysis to ascertain whether the pYd1-1 related transcripts were similar to or distinct in size from the c-Ki-ras transcripts. As shown in Fig. 3, two RNAs, approximately 2.9 and 2.4 kb in size, are detected with the pYd1-1 probe; they are distinct in size from the c-Ki-ras RNAs. The results indicate that, in addition to a c-Ki-ras gene, at least one other transcription unit has been amplified and exhibits enhanced expression in the Y1 cells. We do not yet know if the pYd1-1 transcripts are translated. Also, although the intensity of the RNA bands seen in Fig. 3 suggest that the pYd1-1 related transcripts are not as abundant as the c-Ki-ras RNAs, an accurate determination of the relative abundance of these two RNA classes within the Y1 cells has not yet been made.

Chromosomal Origin of Amplified DNA Sequences

Any model proposed to explain the mechanisms of gene amplification in the Y1 cells and other tumor cells requires information on whether the amplified material originated from one or more than one chromosome. In

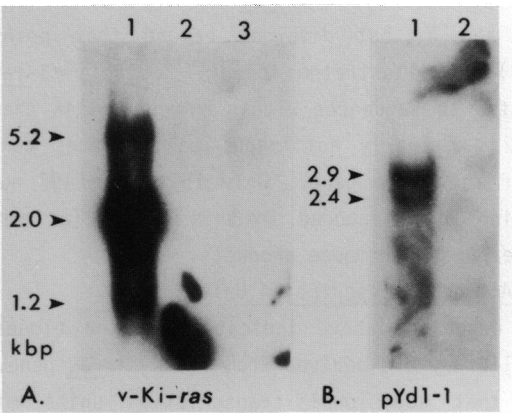

Fig. 3. Filter hybridization analyses of RNA samples. Total cellular RNA (20 μg) from Y1-DM cells (lane 1), 3T3 mouse cells (lane 2), or Chinese hamster clone 380-6 cells (lane 3) were transferred to nitrocellulose and hybridized to ^{32}P-labeled DNA from: (A) pHiHi3 (v-Ki-ras); (B) plasmid pYd1-1.

addition, knowledge of the chromosomal location of the mouse c-Ki-ras gene would be useful for exploring possible chromosomal changes involved in the activation of this oncogene in some tumors, and could provide a stimulus to investigate whether genetic markers linked to the c-Ki-ras locus in the mouse are conserved in the human genome. In order to determine the mouse chromosome(s) from which the amplified DNA sequences of the Y1 cells originated, we utilized a series of Chinese hamster x mouse somatic cell hybrids that segregate mouse chromosomes. The presence in the hybrids of mouse-specific DNA fragments homologous to sequences amplified in the Y1 cells was determined by Southern blot analysis.

For these studies, three amplification-related probes were used: (1) v-Ki-ras clone pHiHi3; (2) cDNA clone k1; (3) plasmid pYd1-1. In Southern blot analysis, ^{32}P-labeled DNA from recombinant pYd1-1 detects a single 3.8 kb EcoRI fragment in control mouse DNA (Fig. 4, lane 1), and a fragment approximately 9 kb in size in Chinese hamster DNA (Fig. 4, lane 2). This probe hybridizes to two fragments, 4.8 and 3.8 kb in size, amplified in DNA from the Y1 cells (Fig. 4, lane 8). Detection of the 3.8 kb band in hybrid cell DNA indicated the presence of sequences homologous to the pYd1-1 probe. In the fifteen hybrid cell lines examined, the 3.8 kb fragment segregated concordantly only with mouse

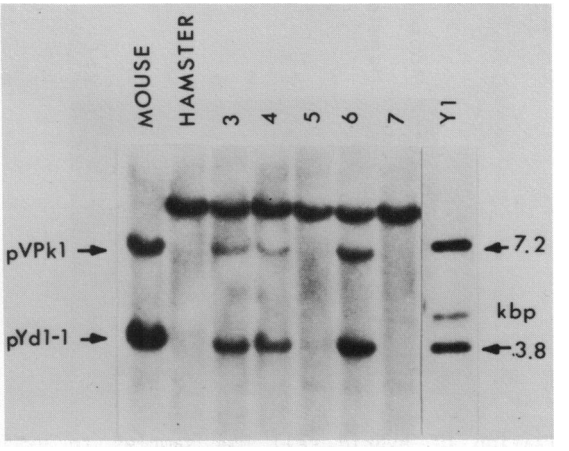

Fig. 4. Southern blot analysis of hybrid DNAs. DNA samples from control 3T3 mouse cells (6 µg, lane 1), parental Chinese hamster cells (10 µg, lane 2), hamster-mouse hybrids (10 µg/lanes 3-7), and Y1-HSR cells (0.5 µg, lane 8), digested to completion with EcoRI, were hybridized simultaneously to probes k1 and pYd1-1. Hybrid cell DNAs in lanes 3, 4 and 6 were scored as positive for the presence of both pYd1-1- and k1-related sequences; those in lanes 5 and 7 were scored as negative for both.

chromosome 6. All other chromosomes were excluded by three or more discordant clones (Fig. 6).

Sequences homologous to cDNA clone k1 are present in a 7.2 kb EcoRI fragment in mouse cells, but no signal was detected with the Chinese hamster DNA under the hybridization conditions used (Fig. 4). In hybrid cell DNAs, the presence of the k1-related 7.2 kb fragment segregated concordantly only with mouse chromosome 6 (Fig. 6).

EcoRI-digested mouse cell DNA hybridized to v-Ki-ras sequences reveals fragments approximately 11 (previously reported as 9.5), 8.5, 5, 1.5 and 0.5 kb in size (6,26). Chinese hamster DNA exhibits a large number of fragments after digestion with EcoRI, as well as with other restriction enzymes (35,36). Some of these fragments can be seen in Fig. 5. However, despite the large number of Chinese hamster bands, the mouse-specific 11 kb EcoRI fragment could still be distinguished (Fig. 5), allowing us to score for its presence in hybrid cell DNAs. In the twelve hybrids analyzed with this probe, homologous sequences segregated concordantly with the sequences detected by hybridization to probes pYd1-1 and k1 and, thus, with mouse chromosome 6 (Fig. 6).

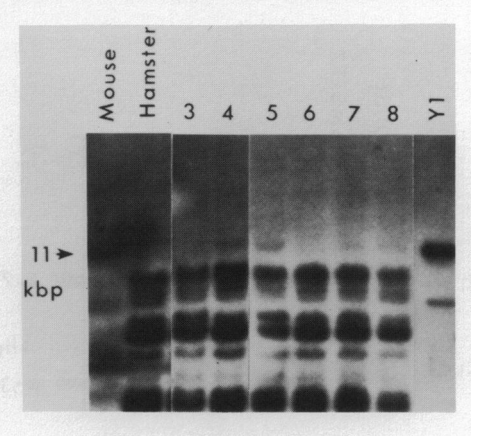

Fig. 5. Hybridization of hybrid cell DNA samples to v-Ki-ras probe pHiHi3. Samples of 5 μg of each DNA from control 3T3 mouse cells (lane 1), parental Chinese hamster cells (lane 2), hamster-mouse hybrid cells (lanes 3-8), or 0.5 μg of DNA from Y1-HSR cells (lane 9) were digested with EcoRI. Only that portion of the autoradiogram relevant for scoring of the 11 kb mouse-specific fragment is shown. Hybrid cell DNAs in lanes 4, 5, 7, and 8 were scored as positive for the presence of the 11 kb fragment; those in lanes 3 and 6 were scored as negative.

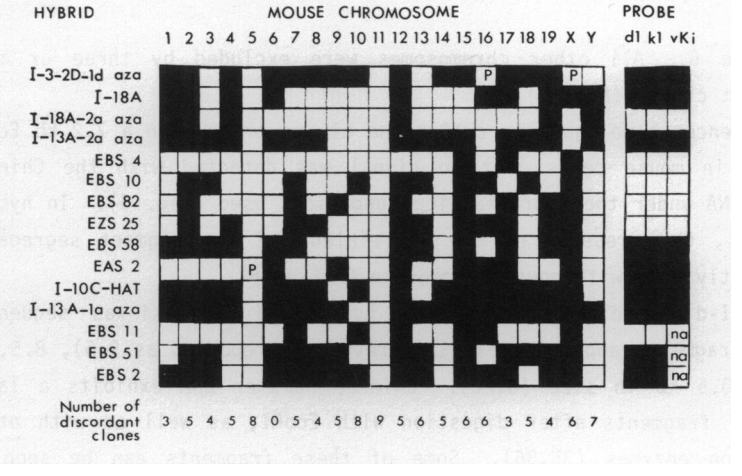

Fig. 6. Chromosomal assignment of amplification-related sequences using a panel of Chinese hamster x mouse somatic cell hybrids. A dark square indicates the presence of an intact chromosome at a frequency >0.1 copies/cell; an open square indicates that the chromosome is absent or present at very low frequency (≤0.1 copies/cell); (P) Only part of chromosome present, data not used; (na) Hybrid not analyzed; (d1) plasmid pYd1-1; (k1) cDNA pVPk1; (vKi) v-Ki-ras clone pHiHi3.

The multiple bands seen in Southern blots of Chinese hamster DNA probed with v-Ki-ras sequences are consistent with an increased number (amplification) of related DNA segments in these cells (35). We found a similar pattern of multiple bands in Chinese hamster DNA probed with cDNA clone cYKi2 (data not shown). This observation prompted us to examine the relative abundance of Ki-ras-related RNAs in the Chinese hamster cells. We found that the level of Ki-ras-related transcripts in the Chinese hamster cell line was similar to that of 3T3 mouse cells, and much less than that present in Y1 cells (Fig. 3A). Therefore, whatever the organization of the extra Ki-ras-related sequences in the Chinese hamster cells, these data indicate that they are not ordinarily expressed.

DISCUSSION

We have constructed a cDNA library from Y1 mouse adrenal tumor cell RNAs and have isolated cDNA clones complementary to an amplified c-Ki-ras mouse gene that is homologous to the human c-Ki-ras2 gene. One of these cDNAs (cYKi2) is derived from the coding region of this gene, while other cDNAs isolated represent segments of the 3' non-translated regions of the 5.2 and 2.0 kb c-Ki-ras transcripts.

The amplification and resulting enhanced expression of the c-Ki-ras gene in the Y1 cells facilitated the isolation of cDNAs and genomic clones derived from this cellular oncogene. With most other cell lines, such efforts have been hampered by the relatively low abundance of c-Ki-ras transcripts, which usually comprise approximately 0.05% or less of the polyadenylated RNAs (34). Screening of the Y1 cDNA library using a differential hybridization protocol indicated that the c-Ki-ras transcripts comprise at least 0.35% of the cDNAs present. This is probably an underestimate, since clones with small inserts (like cYKi2) may have been overlooked in our initial screening efforts.

Not enough data are yet available to establish how the amplification and enhanced expression of the c-Ki-ras gene in the Y1 mouse adrenal tumor cells might contribute to the initiation and/or maintenance of the tumorigenic properties of these cells. It will be important to determine if the c-Ki-ras gene amplified in the Y1 cells has an altered structure that might affect its regulation or the interaction of its product with other molecules. The data we have obtained thus far indicate that no structural changes have occurred. Based on the level of c-Ki-ras gene

amplification and the relative size of the HSR in the Y1 cells, we estimate that the amplified unit is roughly 1000-2000 kb. Thus, it could well include other genes that act independently or in concert with c-Ki-ras to promote the transformed state. In this report, we provide evidence that at least one other transcription unit has also been amplified. Further, our mapping studies show that the DM- and HSR-associated amplified DNA sequences analyzed, including a c-Ki-ras gene, are present on chromosome 6 in normal mouse cells.

The human c-Ki-ras2 gene has been mapped to chromosome 12 (36,37). Also located on human chromosome 12 (in short arm region p12--pter) are the genes for the enzymes lactate dehydrogenase B (LDHB), triose phosphate isomerase (TPI), and glyceraldehyde phosphate dehydrogenase (GAPD)(38). In the mouse, the homologous loci, Ldh-2, Tpi, and Gapd are on chromosome 6 (39). Therefore, we hypothesize that c-Ki-ras2 is part of a linkage group conserved in evolution that is located on the short arm of human chromosome 12 and on mouse chromosome 6.

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