Joining of the c-myc Gene and a Line 1 Family Member on Chromosome 8 in a Human Primary Giant Cell Carcinoma of the Lung

Masayoshi Iizuka, Masahiko Shiraiishi, Michihiro C. Yoshida, Kenshi Hayashi, and Takao Sekiya

Oncogene Division, National Cancer Center Research Institute, 1-1, Tsukiji 5-Chome, Chuo-ku, Tokyo 104 [M. I., M. S., K. H., T. S.], and Chromosome Research Unit, Faculty of Science, Hokkaido University, Kita-ku, Sapporo 060 [M. C. Y.], Japan

ABSTRACT

A rearranged c-myc gene found in a human primary giant cell carcinoma of the lung was analyzed. The rearrangement was found in the region about 6 kilobase pairs upstream of the c-myc gene. The breakpoint was joined to a sequence carrying a Line 1 (LI) family member located on chromosome 8. This in vivo rearrangement of the c-myc gene specific to tumor cells may represent one mechanism of activation of a protooncogene during tumorigenesis or tumor progression in human cancer.

INTRODUCTION

The c-myc oncogene, a human homologue of the transforming gene of avian myelocytomatosis virus, is essential for cell proliferation and cell differentiation (1). However, accumulating evidence suggests that structural alterations of the c-myc gene, such as gene amplification and gene rearrangement, are involved in tumorigenesis in human cancer (2). Rearrangement of the c-myc gene and its implication in tumorigenesis have been studied extensively in hematopoietic cell neoplasms. Juxtaposition of the immunoglobulin loci to the c-myc locus might be involved in tumorigenesis of Burkitt's lymphoma (3, 4). On the other hand, there have been few reports of rearrangement of the c-myc gene in human solid tumors. A rearranged and amplified c-myc gene in double-minute-containing cells derived from a patient with glioblastoma multiforme was reported (5).

In one case of familial renal cell carcinoma, translocation of the c-myc gene associated with a (13;8)(p14.2;q24.13) chromosomal translocation was demonstrated (6). However, the translocation breakpoint in the carcinoma was not found in the region of 1.5 megabases surrounding the c-myc gene (7).

We are interested in structural alterations of the c-myc gene in human solid tumors and previously reported rearrangement of the c-myc gene in a human primary giant cell carcinoma of the lung, in which a breakpoint was found in the region 6 kilobase pairs upstream from the transcription initiation site (8). We cloned the rearranged c-myc gene and analyzed the nucleotide sequence flanking this breakpoint. Here we describe an interesting rearrangement, namely, the joining of a region carrying a Line 1 (LI) family member located on chromosome 8 to the 5' upstream region of the c-myc gene.

MATERIALS AND METHODS

Isolation of DNA and Molecular Cloning of the Rearranged c-myc Gene. High molecular weight DNA from a human primary giant cell carcinoma of the lung that contained both normal and rearranged c-myc alleles was prepared as described previously (8). DNA was completely digested with EcoRI or partially digested with MboI and fragments of appropriate sizes were isolated by sucrose density gradient centrifugation. Fractionated DNA fragments were ligated to λ phage vectors, Charon 4A digested with EcoRI, or EMBL3 digested with BamHI plus EcoRI. The recombinant DNA obtained were packaged into bacteriophage λ particles using an in vitro packaging system (Stratagene). Libraries composed of 1 x 10^6 independent recombinant bacteriophages were amplified and then screened by the method of Benton and Davis (9). Two clones, XNCO301 (Charon 4A vector) and XNCO901 (EMBL3 vector), contained the rearranged DNA fragments, while XNCO906 (EMBL3 vector) carried the upstream region of the breakpoint of the rearranged DNA (Fig. 1A). XNCO302 (Charon 4A vector) and XNCO903 (EMBL3 vector) contained DNA fragments of the normal c-myc allele (Fig. 1B).

Plasmid Clones. A 6.0-kilobase pair HindIII fragment containing the breakpoint and the surrounding regions was isolated from XNCO301 DNA and the clone obtained using a plasmid vector pUC19 was designated as pNCO311. Similarly, a 2.9-kilobase pair EcoRI/KpnI fragment from XNCO301, a 1.5-kilobase pair BamHI/EcoRI fragment, and a 3.2-kilobase pair Sau3A/BamHI fragment from XNCO901 were subcloned using plasmid vectors and designated as pNCO312 (pUC19 vector), pNCO903 (pGEM-2 vector), and pNCO901 (pUC19 vector), respectively. These four plasmid clones carrying the regions indicated in Fig. 2A were used for characterization of the region surrounding the breakpoint. A plasmid clone pNCO907 carrying a 0.5-kilobase pair PsII fragment from pNCO901 was prepared using pUC19 as a vector. The fragment (the D number assignment for this fragment is D85586) carried a unique genomic sequence adjacent to the 3'-end of the L1 family member and was used as a probe for hybridization with DNA from human-mouse somatic cell hybrids. Plasmid clones carrying a 0.2-kilobase pair KpnI/PstI fragment from pNCO311, a 0.6-kilobase pair EcoRI/PstI fragment from XNCO302, and a 1.2-kilobase pair EcoRI/NdeI fragment from XNCO901 were also obtained using pGEM-2 as a vector and subjected to nucleotide sequence analysis. The nucleotide sequences of the DNA fragments were determined by the dideoxy chain termination method of Sanger (10).

DNA Probes. A plasmid clone carrying exon 1 of the c-myc gene was kindly provided by Y. Taya (National Cancer Center Research Institute, Tokyo, Japan) and used as a probe for screening the gene library. A plasmid clone carrying exon 3 of the c-myc gene, used as a probe for hybridization of DNA from human-mouse somatic cell hybrids, was also provided by Y. Taya. Several plasmid clones carrying the L1 family member, T≤B≥G41 DNA (11), and a plasmid clone of c-myb DNA (12) were obtained through the Japanese Cancer Research Resources Bank.

Cell Lines. The human promyelocytic leukemia cell line HL-60 was kindly provided by M. Miwa (National Cancer Center Research Institute). The human colon carcinoma cell line COLO 320 DM was obtained from the American Type Culture Collection.

Southern Blot Analysis. DNA samples (2–6 μg) were digested with appropriate restriction endonucleases under the conditions recommended by the suppliers (Takara Shuzo Co., Ltd., Kyoto, Japan; Toyobo Co., Ltd., Osaka, Japan; and New England Biolabs, Inc., Boston, MA). The DNA fragments obtained were subjected to Southern blot analysis (13) using 0.6% agarose gel and a nylon membrane (Hybond-N; Amersham). DNA on the filter was fixed by heating at 80°C for 2 h and then hybridized with 32P-labeled DNA probes at 42°C (or 50°C) for 16 h in a mixture of 50% formamide, 6 x standard saline-citrate (1 x standard saline-citrate is 0.15 M sodium chloride-0.015 M sodium citrate), 5 x Denhardt's solution (1 x Denhardt's solution is 0.2% bovine serum albumin-0.02% Ficoll-0.02% polyvinylpyrrolid-
RESULTS

Cloning of the Rearranged c-myc Gene and the Nucleotide Sequence of the Region Surrounding the Breakpoint. We screened gene libraries constructed by DNA from a human primary giant cell carcinoma of the lung that had been found to carry a rearranged c-myc gene together with the normal allele (8) and obtained two recombinant λ clones, λNCO301 and λNCO302 (Fig. 1, A and B). These were detected using a DNA probe for exon 1 of the c-myc gene. λNCO901 was detected using a 0.4-kilobase pair Psrl/BglII fragment from λNCO301 DNA as a probe. λNCO906 carrying the region upstream of the breakpoint was detected using a 0.5-kilobase pair Psrl fragment from λNCO901 DNA as a probe. An overlapping λ clone, λNCO903, carrying regions of the normal c-myc allele was also obtained from the library (Fig. 1B). Comparison of the restriction maps of λ clones of the rearranged and normal DNAs revealed that the breakpoint was located in the region flanked by the first HindIII and the second Kpnl recognition sites upstream of the c-myc gene on the rearranged DNA (Fig. 1A). For determination of the exact position and the features of the breakpoint, the nucleotide sequences of a region from the Kpnl site to the Psrl site 200 base pairs further downstream on the rearranged DNA (Fig. 1A) and a region from the first EcoRl site upstream of the c-myc gene to the Psrl site 600 base pairs further downstream on normal DNA (Fig. 1B) were deter-

The nucleotide sequences surrounding the breakpoint of the rearranged DNA (see Fig. 1C). The nucleotides in the sequence identical to those of the 3'-end of the lg-A'KpnI-83 sequence (17) are boxed. The hexanucleotide sequence TGCTTT at the breakpoint is directly repeated on the rearranged lung carcinoma DNA at the 3'-end of the LI sequence (Fig. 2E). The underlined sequence is complementary to the human LI sequence inserted into ir-satellite DNA with 75% homology (16). kb, kilobase pairs.

Fig. 1. (A) Restriction map of the region carrying the rearranged c-myc gene and (B) that of the upstream region of the normal c-myc gene. •, region contiguous to the c-myc gene in the normal DNA; •, region joined to the c-myc locus in the rearranged DNA. Open boxes with numbers, three exons of the c-myc gene. Sp, E, B, H, and K, sites for SphI, EcoRI, BamHI, HindIII, and Kpnl, respectively. The regions cloned into λ vectors are indicated under the DNAs. (C) Nucleotide sequence of the rearranged DNA in the region surrounding the breakpoint and that of the corresponding region of normal DNA. The nucleotide sequence of the strand corresponding to the c-myc sequence is shown. The hexanucleotide sequence boxed is directly repeated at the 3'-end of the LI family member on the rearranged DNA, as shown in Fig. 2E. Arrow, position from which the two nucleotide sequences are different. The underlined sequence is complementary to the human LI sequence inserted into α-satellite DNA with 75% homology (16). kb, kilobase pairs.

Fig. 2. (A) The plasmids clones analyzed are indicated in appropriate regions under the structure of the rearranged DNA. □, DNA at the c-myc locus; ■, DNA joined to the c-myc locus. (B) Structure of an LI family member in the β-globin gene (T/G41). The LI family DNA indicated by an open box was cut into four fragments, a, b, c, and d, which were used as probes for Southern blot analysis in C. Arrow, direction of transcription of the LI family member. (C) Southern blot analysis of plasmid clones carrying fragments surrounding the breakpoint. Plasmid clones were digested with restriction endonucleases as follows: pNCO901 with BamHI and EcoRI (Lane 1); pNCO903 with BamHI and EcoRI (Lane 2); pNCO312 with EcoRI and HindIII (Lane 3); and pNCO311 with HindIII and Kpnl (Lane 4). The probes used are shown in C and indicated under each figure. (D) Alignment of the LI family sequence in the region joined to the c-myc locus. ■, region joined to the c-myc locus. Portions of the LI family sequence hybridized to DNA fragments are indicated under the bar. (E) Nucleotide sequence of the 3'-end region of the LI family member joined to the c-myc locus. The nucleotide sequence corresponding to the LI family member is shown. The nucleotides in the sequence identical to those of the 3'-end of the lg-KpnI-83 sequence (17) are indicated by dots. The da-rich region characteristic of the 3'-end of LI family members is underlined. The hexanucleotide sequence boxed is also observed at the breakpoint of the rearranged DNA (see Fig. 1C). E, B, H, K, and Hp, sites for EcoRI, BamHI, HindIII, Kpnl, and Hpal, respectively, kb, kilobase pairs.

m. The nucleotide sequence surrounding the breakpoint of the rearranged DNA and that of the corresponding region of the normal DNA are shown in Fig. 1C. The arrow in Fig. 1C shows the position from which the two nucleotide sequences differed. As described below, the nucleotide sequence newly joined to the region upstream of the c-myc gene showed 75% homology with that of a human L1 member inserted into α-satellite DNA (16). The hexanucleotide sequence TGCTTT at the breakpoint is directly repeated on the rearranged lung carcinoma DNA at the 3'-end of the L1 sequence (Fig. 2E).

Joining an L1 Family Member to the c-myc Locus. To identify the region joined to the c-myc locus, four plasmid clones, pNCO311, pNCO312, pNCO903, and pNCO901, carrying
DNA fragments in the regions surrounding the breakpoint were constructed (Fig. 2A). Southern blot analysis of human genomic DNA, using a 0.6-kilobase pair KpnI fragment in the pNCO311 clone as a probe, revealed that this fragment contained a human specific repetitive sequence (data not shown). The plasmid clones were first analyzed by Southern blot hybridization using a probe for an Alu sequence (BLUR 8). However, none of the plasmid clones except pNCO901 contained the repeating sequence. We then used TgG41 DNA, a full-length member of the L1 family near the 3' end of the human β-globin gene (11), as a probe and found that all four plasmid clones carried the repeating unit. For determination of the direction of the L1 family member, the TgG41 DNA sequence was divided into four portions designated as “a,” “b,” “c,” and “d” in order in the direction of their transcription, as shown in Fig. 2B, and these fragments were used as probes for Southern blot analysis of the four plasmid clones. The results in Fig. 2C indicate that probe a hybridized to a 1.2-kilobase pair HindIII/KpnI fragment in pNCO311 (Lane 3) and pNCO312 (Lane 4) and a 0.6-kilobase pair KpnI fragment and 4.2-kilobase pair KpnI/HindIII fragment in pNCO311 (Lane 4). Probes b, c, and d hybridized to a 1.7-kilobase pair EcoRI/HindIII fragment in pNCO312 (Lane 3), a 1.5-kilobase pair BamHI/EcoRI fragment in pNCO903 (Lane 2), and a 2.4-kilobase pair EcoRI/BamHI fragment in pNCO901 (Lane 1), respectively. Thus, as summarized in Fig. 2D, the L1 family member was joined to the upstream region of the c-myc gene in a head-to-head mode. We also sequenced a DNA fragment in pNCO901 that hybridized to probe d (Fig. 2E) and found that the region carried a sequence homologous to that of the 3'-end of the Ig-KpnI-83 DNA (17) and a da-rich sequence characteristic of the 3'-end of L1 family members (11, 17, 18). It is noteworthy that the hexanucleotide sequence AAAGCA following the da-rich sequence was directly repeated at the breakpoint, as shown in Fig. 1C. (In Fig. 1C, the nucleotide sequence of the complementary strand is shown.)

Characterization of the Rearrangement. L1 family members are known to be mobile genetic elements, and their insertion into or near the c-myc gene has been demonstrated in canine and human tumors (19, 20). We examined whether the joining of the L1 family sequence to the 5'-region of the c-myc gene in the giant cell carcinoma of the lung was due to insertion of the repeating unit or crossing over between two chromosomal loci. For this, normal and cancer DNAs of the patient were subjected to Southern blot analysis using as a probe a 0.5-kilobase pair PstI fragment in pNCO907 that was located in the region 1.5 kilobase pairs downstream of the 3'-end of the L1 sequence [distal to the c-myc gene (Fig. 3B)]. On digestion with BglII, BamHI, StuI, or PvuII, normal and cancer DNAs gave the same 7-kilobase pairs downstream from the probe binding site (Fig. 3B) and the StuI site 16 kilobase pairs upstream from the probe site. On the other hand, digestion of normal DNA with SphI produced two fragments of 22 and 7 kilobase pairs that hybridized to the probe, indicating the presence of restriction fragment length polymorphism at the locus (Fig. 3A, Lane 5N). Although the signal intensity of the 22-kilobase pair fragment was not the same as that of the 7-kilobase pair fragment in this particular experiment, this restriction fragment length polymorphism was evident by analysis of DNAs from 17 unrelated individuals. Seven of the 17 individuals were heterozygous and gave both 22-kilobase pair and 7-kilobase pair SphI fragments, while the remaining 10 individuals were homozygous. When tumor DNA was digested with SphI, a 19-kilobase pair fragment instead of the normal 22-kilobase pair fragment was produced besides the 7-kilobase pair fragment (Fig. 3A, Lane 5C). The reduced signal intensity observed at the position of the 22-kilobase pair fragment in Lane 5C might be due to the presence of normal cells in the tumor specimen. Because no SphI site is present in the region between the site corresponding to the probe and the position of the breakpoint, production of the 19-kilobase pair fragment must be due to rearrangement of the allele producing the 22-kilobase pair fragment. Simple insertion of the L1 family member cannot explain the decrease in the chain length of the fragment from 22 to 19 kilobase pairs. These results indicate that the joining of the L1 family sequence to the 5'-region of the c-myc gene was not due to insertion of the sequence as a mobile element but to recombination of the chromosomal region carrying the L1 family member to that carrying the c-myc gene.

Joining of the L1 Family Member on Chromosome 8 to the c-myc Gene. We next examined the origin of the region joined to the c-myc gene. The 0.5-kilobase pair PstI fragment in pNCO907 carrying a unique genomic sequence was hybridized to cellular DNAs from a series of human-mouse somatic cell hybrids. The DNA probe hybridized to a 4.7-kilobase pair BamHI fragment of DNA from hybrid cell lines that carried human chromosome 8 (Fig. 4A, Lanes 3–8). For confirmation of this specific hybridization to chromosome 8 DNA, DNAs from hybrid cell lines were hybridized with a DNA probe specific for the c-myc gene which is on chromosome 8 (21–23). Besides the 6-kilobase pair fragment corresponding to the mouse c-myc gene, the c-myc specific probe hybridized to a 23-kilobase pair BamHI fragment of DNAs from the same cell lines (Fig. 4B). The results, summarized in Table 1, indicate that DNA sequence joined to the c-myc locus on chromosome 8 is located on the same chromosome. This type of DNA rearrangement could be produced by deletion of a portion of the chromosome or by inversion or translocation of DNA within the chromosome. For characterization of the region joined to the c-myc locus, the 0.5-kilobase pair PstI fragment in pNCO907 (the D number assignment for this fragment is D8S586) was hybridized to DNAs from two human tumor cell

3 Unpublished results.
B

Fig. 4. Southern blot hybridization of a unique genomic sequence adjacent to the 3’-end of the L1 family member in the rearranged DNA to BamHI-digested DNA from human-mouse somatic cell hybrids. (A) The 0.5-kilobase pair PstI fragment in pNCO907 was 32P labeled and hybridized to a blot containing 6 μg of digested DNA from hybrids 7-1D2 (Lane 1), 8-2 (Lane 2), SC3 (Lane 3), 6-1 (Lane 4), 11-6 (Lane 5), 1a (Lane 6), 4B1 (Lane 7), 3B5 (Lane 8), and C1-17 (Lane 9) and from the mouse cell line B62 (Lane 10) and the human cell line HE2144 (Lane 11). The human chromosomal complement in each hybrid is shown in Table 1. (B) The blot in A was subjected to hybridization using a 32P-labeled DNA fragment carrying exon 3 of the c-myc gene as a probe. The BamHI fragment of 23.0-kilobase pair contains the human c-myc gene, while the 6.0-kilobase pair BamHI fragment corresponds to the mouse counterpart.

lines carrying the amplified c-myc gene, the human promyelocytic leukemia cell line HL-60 (24, 25), and the human colon carcinoma cell line COLO 320 DM (26). As shown in Fig. SC, the 0.5-kilobase pair PstI fragment could hybridize with polymorphic EcoRI fragments of 4.8 and 1.3 kilobase pairs, when DNAs from five unrelated individuals were analyzed. Upon digestion of DNAs from the lung carcinoma and the HL-60 cells with EcoRI, signal intensities of the DNA probe hybridized to the fragments were similar between the two DNAs (Fig. 5A, Lanes 1 and 2). This result indicated that the region hybridized to the DNA probe was not amplified in HL-60 DNA. On the other hand, COLO 320 DM DNA gave a strong signal at the position of a 1.3-kilobase pair EcoRI fragment, suggesting coamplification of the probed region with the c-myc gene (Fig. 5A, Lane 3). To confirm that the strong signal for the 1.3-kilobase pair EcoRI fragment of COLO 320 DM DNA was due to amplification and not to a larger amount of DNA on the blot, the same blot was subjected to hybridization using a probe for the c-myb gene (Fig. 5B). The results indicated that the amount of COLO 320 DM DNA on the blot was less than the amounts in blots of the other two DNAs. Thus we concluded that the region joined to the c-myc gene in the giant cell carcinoma of the lung was included in an amplification unit of the c-myc gene in COLO 320 DM cells, but not in a unit in HL-60 cells.

DISCUSSION

In this work, we analyzed the structure of the rearranged DNA near the c-myc oncogene in a human primary giant cell carcinoma of the lung. Results indicated that a region carrying an L1 family member located on chromosome 8 was joined to the c-myc gene through interstitial deletion, inversion, or translocation within the chromosome. The region joined to the c-myc gene was included in an amplification unit of the c-myc gene in a human colon carcinoma cell line COLO 320 DM. If there is no chromosomal rearrangement in the c-myc amplification unit in the COLO 320 DM cells, this observation may indicate that the region joined to the c-myc gene is located near the c-myc locus on chromosome 8. However, it should be noted that the amplification units in human tumors containing amplified c-myc genes were reported to be variable. One of the several possibilities is that the different amplification units are the result of chromosomal rearrangements prior to amplification (27).

Rearrangement of protooncogenes could be present in cancer cells due to (a) a primary event essential for tumorigenesis in an earlier stage of cancer, (b) a secondary event required for tumor progression or clonal selection in later stages of cancer, or (c) a random DNA aberration due to fragility of chromosomes in neoplastic cells. It is hard to determine which of these mechanisms was responsible for the rearrangement of the c-myc locus in the giant cell carcinoma of the lung described here. Because the breakpoint of the rearrangement was located about 6 kilobase pairs upstream of the c-myc gene as in a Burkitt lymphoma (28, 29), the break was probably not a fortuitous event. By analogy with cases in Burkitt lymphoma (3, 4), the DNA joined to the c-myc locus might play a key role in activation of the c-myc gene. In the region joined to the c-myc locus, we found an almost complete sequence of an L1 family member. Joining of an L1 family member to the c-myc locus has been observed in a canine transmissible venereal tumor (19). In this case, an 1.8-kilobase pair DNA showing 62% homology with an L1 family member and containing a dA-rich tail was inserted into the region upstream to the first exon of the c-myc gene. Insertion of an L1 family member into the c-myc locus was also demonstrated in a human breast carcinoma. This L1 family member was present within the second intron of the rearranged c-myc gene (20). However, the effect of these joined L1 family members on expression of the c-myc gene are unknown.

A rearrangement of the c-myc gene similar to that in the primary giant cell carcinoma of the lung analyzed in this study has been found in a region about 7.5 kilobase pairs upstream of the c-myc gene in a human cell line of giant cell carcinoma of the lung, C-Lu65 (8). Our unpublished data revealed that the region joined to the c-myc locus in C-Lu65 cells was located on chromosome 8, suggesting the joining of the two regions on the same chromosome through interstitial deletion, inversion, or translocation. However, the features of the region joined to the c-myc locus in the C-Lu65 cell line remain to be clarified. Because both the primary giant cell carcinoma of the lung and the cell line of the carcinoma carry rearrangement of the c-myc gene brought about through DNA aberration on the same chromosome, the common features might be significant in
REARRANGEMENT OF THE c-myc GENE IN LUNG CANCER

Table 1 Chromosomal localization of unique genomic sequence adjacent to the 3'-end of the L1 family member joined to the c-myc gene in a human giant cell carcinoma of the lung

<table>
<thead>
<tr>
<th>Cell hybrid*</th>
<th>Human chromosome present</th>
<th>Hybridization to probe*</th>
</tr>
</thead>
<tbody>
<tr>
<td>H/B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7-1D2</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8-23</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5C3</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6-1</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>H/F</td>
<td></td>
<td></td>
</tr>
<tr>
<td>III-6</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>1a</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4B1</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3B5</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>C/B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C1-17</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

% of discordance 78 56 56 33 56 44 44 0 78 33 33 56 44 56 44 56 67 33 33 33 44 56 44 78

* H/B, H/F, and C/B represent hybrid clones between human HE2301 and mouse B82 cells, human HE2144 and mouse FM3A cells, and human chronic myeloid leukemia and mouse B82 cells, respectively.

The 0.5-kilobase pair PstI fragment shown in Fig. 3B was used as a probe.

Fig. 5. Southern blot hybridization of a unique genomic sequence adjacent to the 3'-end of the L1 family member in the rearranged DNA to EcoRI-digested DNAs from human tumor cell lines with an amplified c-myc gene and from placentas of unrelated individuals. (A) The 0.5-kilobase pair PstI fragment in pNCo907 was 32P labeled and hybridized to a blot containing DNA from a human primary giant cell carcinoma of the lung (Lane 1), the human promyelocytic leukemia cell line HL-60 (Lane 2), and the human colon carcinoma cell line COLO 320 DM (Lane 3). (B) The same blot as for A was subjected to hybridization using a 32P-labeled DNA fragment from the c-myc gene as a probe. (C) The 32P-labeled 0.5-kilobase pair PstI fragment was hybridized to placenta DNAs from five unrelated individuals.

ACKNOWLEDGMENTS

We thank Y. Shimosato and H. Iwahana for providing surgical specimens and placenta DNAs, respectively. We also thank Y. Sakaki for providing the T/G41 DNA clone and for detailed information on the clone.

REFERENCES


REARRANGEMENT OF THE c-myc GENE IN LUNG CANCER

30. Yoshimoto, K., Hirohashi, S., and Sekiya, T. Increased expression of the c
Joining of the c-myc Gene and a Line 1 Family Member on Chromosome 8 in a Human Primary Giant Cell Carcinoma of the Lung

Masayoshi Iizuka, Masahiko Shiraishi, Michihiro C. Yoshida, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/50/11/3345

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, use this link http://cancerres.aacrjournals.org/content/50/11/3345.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.