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REARRANGEMENT OF THE c-*myc* GENE IN TWO GIANT CELL CARCINOMAS OF THE LUNG

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The c-*myc* oncogene was found to be rearranged in a human cell line of giant cell carcinoma of the lung (C-Lu65) and in a human primary giant cell carcinoma of the lung (LuC38C). The rearrangements in C-Lu65 and LuC38C were in regions about 7.5 kb and 6 kb, respectively, upstream from the transcription initiation site. No rearrangement of the c-*myc* gene was observed in a non-cancerous portion (LuC38N) of the lung of the patient who carried LuC38C. These results suggest that rearrangement of the c-*myc* gene may play some role in tumorigenesis of giant cell carcinoma of the lung.

Key words: c-*myc* — Rearrangement of gene — Giant cell carcinoma of the lung

The c-*myc* gene is the cellular gene homologous to a transforming gene (v-*myc*) identified in avian myelocytomatosis virus.[1] Accumulating evidence indicates that genetic changes involving c-*myc* structure and the deregulation of its expression are correlated with tumorigenesis of various animal tumors.[2–4] Amplification and overexpression of the c-*myc* gene have been described in a variety of human tumors.[5–11] There are many reports of rearrangement of the c-*myc* gene associated with chromosomal translocation in hematopoietic malignancies. In Burkitt lymphoma cells, a translocation joins the c-*myc* gene to a region encoding one of the immunoglobulin genes.[12] In addition to findings in B-cells, translocation of the gene for the constant region of the T-cell receptor to a region 3′ to the c-*myc* gene in T-cell leukemias has also been reported.[13] A translocation involving the c-*myc* gene has also been found in cells other than hematopoietic cells, that is, in a hereditary renal cell carcinoma.[14] Thus, chromosomal translocation involving the c-*myc* gene is thought to be closely related to these diseases.

These observations prompted us to examine other human cancers for rearrangement of the c-*myc* gene, as a possible mechanism of its activation. This paper reports rearrangement of the c-*myc* gene found in two giant cell carcinomas of the lung; one a cell line and the other a specimen obtained at surgery. High-molecular-weight DNAs were prepared by the method of Blin and Stafford[15] from cultured cell lines and lung cancer tissues resected in the National Cancer Center Hospital. The DNAs were digested with restriction endonucleases and then subjected to Southern blot analysis[16] using a cloned DNA fragment corresponding to the 5′ flanking region and exon 1 of the c-*myc* gene as a probe.

In Fig. 1, the DNAs from two cell lines (C-Lu65 and C-Lu99), which were established from human giant cell carcinomas of the lung transplanted into athymic nude mice,[17] are compared to those from a human promyelocytic leukemia cell line (HL60) and human placenta. The levels of c-*myc* mRNA were as high in the two giant cell carcinoma cell lines as in HL60.[8] Corresponding to the high levels of the transcripts, amplification of the c-*myc* gene was observed in C-Lu65[18] and HL60,[5] but not in C-Lu99.[8] Upon digestion of the DNAs with *Eco*RI, all samples tested gave a DNA fragment with a chain length of 12.5 kb that was expected from the germ line c-*myc* DNA (Fig. 3a), although its copy number was high in HL60 and C-Lu65, reflecting the c-*myc* gene amplification. On the other hand,

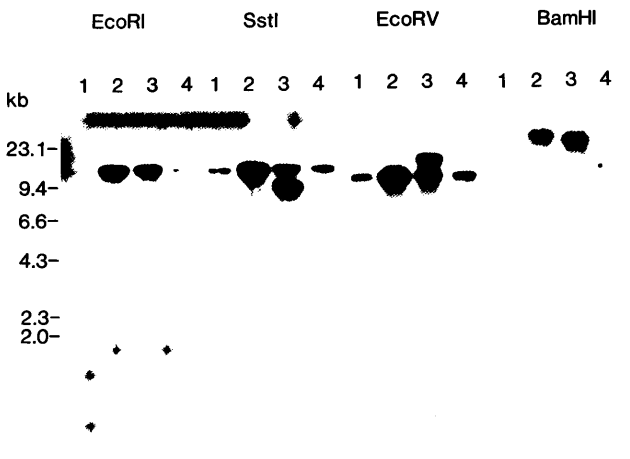

Fig. 1. Southern blot analysis of the c-*myc* gene in human tumor cell lines. Cell lines were maintained in RPMI1640 medium supplemented with 10% fetal calf serum. Samples of 5 μg of each DNA digested with *Eco*RI, *Sst*I, *Eco*RV or *Bam*HI were separated by electrophoresis on a 0.7% agarose gel and transferred to nitrocellulose filters as described by Southern.[16] The filters were baked at 80° and then hybridized to the nick-translated c-*myc* (*Hin*dIII-*Xba*I fragment, Fig. 3) probe. The hybridization and washing conditions were as described previously.[8] Lanes 1, 2, 3 and 4 contain DNA isolated from human placenta, HL60, C-Lu65 and C-Lu99, respectively.

with the use of *Sst*I, *Eco*RV and *Bam*HI, abnormal fragments of 9, 16 and 21 kb were observed in C-Lu65 in addition to the normal-sized fragments of 12.5, 10 and 26 kb, respectively. In the particular experiments shown in Fig. 1, the non-amplified 26 kb *Bam*HI fragment in the placenta or C-Lu99 was scarcely detectable, but it could be detected on longer exposure during autoradiography. From the results in Fig. 1 and other experiments (data not shown), the new sites for restriction endonucleases in C-Lu65 were concluded to be as summarized in Fig. 3b. These results clearly indicate that a significant portion of the amplified c-*myc* gene in C-Lu65 is rearranged in the region between the sites for *Eco*RV and *Eco*RI about 7.5 kb upstream from the transcription initiation site. A representative karyotype of C-Lu65 revealed that one-third of the cells had double minute chromosomes, but no aberration was found on chromosome 8 in which the c-*myc* gene was located.[17] This observation suggests that the rearrangement of the c-*myc* gene in C-Lu65 is not due to a typical translocation event like those found in Burkitt lymphomas.

We then analyzed DNAs from 50 primary lung tumors, including two giant cell carcinomas of the lung (LuC38C and LuC52C). Of these surgical specimens, one (LuC38C) of the two giant cell carcinomas was found to carry a rearranged c-*myc* gene. Figure 2 compares the DNA from LuC38C with that from a non-cancerous portion (LuC38N) of the lung of the same patient, who was a 72-year-old man with no family history of neoplastic disease and with no history of chemotherapy or radiation. Digestions of the DNAs with the restriction endonucleases *Kpn*I, *Pvu*II, *Bam*HI, *Sst*I, *Eco*RV and *Eco*RI, all resulted in a second DNA band (5, 6.3, 24.5, 9.3, 10.6 and 15 kb, respectively) only in the case of LuC38C, in addition to the

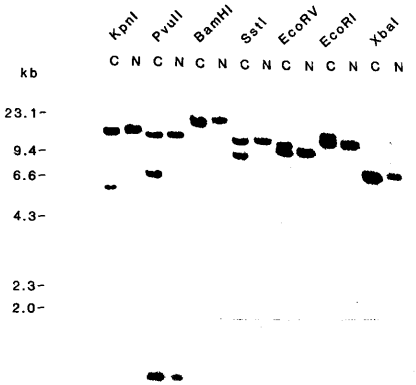

Fig. 2. Southern blot analysis of the c-*myc* gene in LuC38C and LuC38N. Samples of 5 μg of each DNA digested with *Kpn*I, *Pvu*II, *Bam*HI, *Sst*I, *Eco*RV, *Eco*RI or *Xba*I were separated on a 0.7% agarose gel and transferred to nitrocellulose filters. Hybridization to the c-*myc* probe was done under the same conditions as for Fig. 1. C and N indicate lanes containing DNA isolated from Lu C38C and LuC38N, respectively.

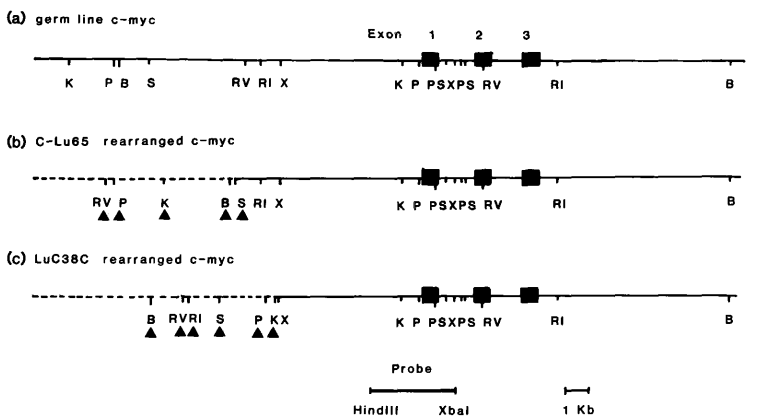

Fig. 3. Restriction map of the c-*myc* gene rearranged in C-Lu65 and LuC38C DNA. The dotted lines in C-Lu65 and LuC38C indicate the DNA joined to the c-*myc* gene by rearrangement. The closed triangles indicate the restriction sites found in the rearranged DNAs. B, *Bam*HI; K, *Kpn*I; P, *Pvu*II; RI, *Eco*RI; RV, *Eco*RV; S, *Sst*I; X, *Xba*I.

DNA fragments expected from the normal c-*myc* allele (14, 13, 26, 12.5, 10 and 12 kb, respectively). On the other hand, digestions of the DNAs with *Xba*I gave only one fragment of 7.4 kb that was expected from the germ line DNA (Fig. 3a). In the case of LuC38N, the bands of larger than 7.4 kb were due to incomplete digestion. The restriction sites that appeared newly in LuC 38C DNA are summarized in Fig. 3c. Since the same amounts of DNAs from LuC38C and LuC38N were compared, the results

in Fig. 2 also indicate that the c-*myc* gene was not amplified in the cancerous portion. In a separate experiment, the copy numbers of the c-*myc* gene in LuC38C, LuC38N and a normal human placenta were confirmed to be the same (data not shown). These results clearly demonstrate that one of the c-*myc* alleles in LuC38C, but not in LuC38N, is rearranged in the region between the *Eco*RI and *Xba*I sites about 6 kb upstream from the transcription initiation site (Fig. 3c). Since no cytogenetical data were available in the case of LuC38C, it is unknown whether translocation involving chromosome 8 had occurred. However, the apparent rearrangement of the c-*myc* gene in at least one allele specifically in the cancerous portion of the lung strongly indicates that this rearrangement was involved in tumorigenesis of the giant cell carcinoma.

Giant cell carcinoma is a unique and special type of lung cancer. The most striking clinical feature of patients with this type of carcinoma is their short survival time because of the rapid growth of the tumor and its resistance to various therapies.[19] Aberrant expression of the c-*myc* gene might be related with tumorigenesis of this special type of cancer. We detected rearrangement of the c-*myc* gene in two of four giant cell carcinomas of the lung: a cell line and a surgical specimen. Of the other two giant cell carcinomas, the cell line C-Lu99 has been shown to have as high a level of c-*myc* mRNA as that in C-Lu65.[8] However, in C-Lu99 and in the other surgical specimen (LuC52C), we could not detect any rearrangement of the c-*myc* gene in a region as far as 15 kb upstream from the 1st exon of the c-*myc* gene. Analysis of further upstream regions of these specimens seems necessary.

Except in Burkitt lymphomas and in T-cell leukemias, c-*myc* gene rearrangement has rarely if ever been reported, especially in primary cancers. In the two giant cell carcinomas of the lung, the breakpoints were located in a short region 6 to 7.5 kb upstream from the transcription initiation site. Some Burkitt lymphomas were found to carry a breakpoint in a similar region of c-*myc* gene.[20] In Burkitt lymphomas, the c-*myc* gene is joined to a region of one of the immunoglobulin genes that is expressed extensively in B-cells and, in consequence, the expression of the c-*myc* gene is thought to be deregulated.[12] In this respect, identification of the region joined to the c-*myc* gene in giant cell carcinomas seems important and interesting. Studies on this problem are in progress.

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