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Establishment of a Human Small Cell Lung Carcinoma Cell Line Carrying Amplification of c-*myc* Gene and Chromosomal Translocation of t(3p;6p) and t(12q;17p)

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A transplantable tumor and an *in vitro* culture cell line (GK-T3) were established from metastatic liver tissue of human small cell lung carcinoma (SCLC). Southern blot analysis revealed about 30-fold amplification of c-*myc* gene in the tumor cells in liver, xenografts, and *in vitro* cell line. The degree of c-*myc* amplification was essentially conserved through serial passages in nude mice and cultivation *in vitro*. The level of c-*myc* mRNA was significantly increased in these cells. Cytogenetically, numerical and complex structural abnormalities were observed in GK-T3 cells, including t(3p;6p), t(12q;17p), two homogeneously staining regions (hsrs) and several double minutes (dmins). These results suggest that activation of c-*myc* gene and alteration of gene(s) around these chromosomal breakpoints may play a role in tumorigenesis of GK-T3 SCLC.

Key words: c-*myc* amplification — Small cell lung cancer — Chromosome 3 abnormality — Chromosome 17 abnormality

Small cell lung carcinoma (SCLC) represents approximately 25% of bronchogenic carcinoma.[1)] Clinically, it grows rapidly with a propensity for early widespread metastasis. One of the *myc* gene family, including c-*myc*, N-*myc* and L-*myc*, is frequently amplified in SCLC cells, especially in those obtained from patients with a shorter survival.[2)] In *in vitro* experiments, SCLC cell lines bearing c-*myc* amplification have a tendency to express biologically more aggressive phenotypes,[3)] suggesting that an activation of c-*myc* or related genes contributes to the progression steps of SCLC.[4–7)]

Concerning the tumor suppressor genes, loss of heterozygosity (LOH) of chromosome 3p, 13q, and 17p has been observed in a large proportion of SCLC.[8–11)] Among them, *Rb* gene on chromosome 13q and p53 gene on chromosome 17p seem to be important tumor suppressor genes in SCLC, since these genes are very frequently mutated structurally or suppressed in expression.[12)] With regard to region 3p, recent reports have indicated the presence of tumor suppressor genes for lung cancer at three distinct loci of 3p25, 3p21.3 and 3p14-cen,[13)] as well as homozygous deletion at 3p13-14 in an SCLC cell line.[14)] To elucidate the molecular mechanism of tumorigenesis in SCLC, establishment and characterization of SCLC cell lines bearing abnormalities unique to SCLC would be helpful. Here we report an SCLC cell line (GK-T3) which exhibits amplified c-*myc* gene on two hsrs and complex chromosomal changes including t(3p;6p) and t(12q;17p).

A metastatic liver tissue of SCLC was excised from a 69-year-old man on autopsy and transplanted to nude mice. Xenograft tumor specimens were subsequently excised and a suspension of single cells was transferred to Dulbecco's modified Eagle's medium with 6% fetal calf serum. A cell line, GK-T3, was established two months later, and three independent clones derived from single cells, GK-T3-11, GK-T3-12, and GK-T3-13 were isolated by the limiting dilution method. In the culture medium the cells proliferate in clumps similarly to other SCLC cell lines, and are loosely attached to the bottom of the culture flask. Doubling time was about 24 to 30 h.

To examine the activation of oncogenes, genomic DNAs from the original liver tissue, xenografts, and the established cell line were analyzed by the Southern blot method using nine oncogene probes. As shown in Fig. 1a, DNAs from GK-T3 as well as the original tumor cells were found to carry about 30-fold amplification of c-*myc* gene. On the other hand, a probe for c-*mos*, which is located in the same chromosome 8q as c-*myc*, could not detect amplification of the gene. These results suggest that a limited region on chromosome 8q is amplified. In Southern blotting of DNAs digested with a few restriction enzymes including *Xba*I, *Eco*RI or *Hin*dIII, several

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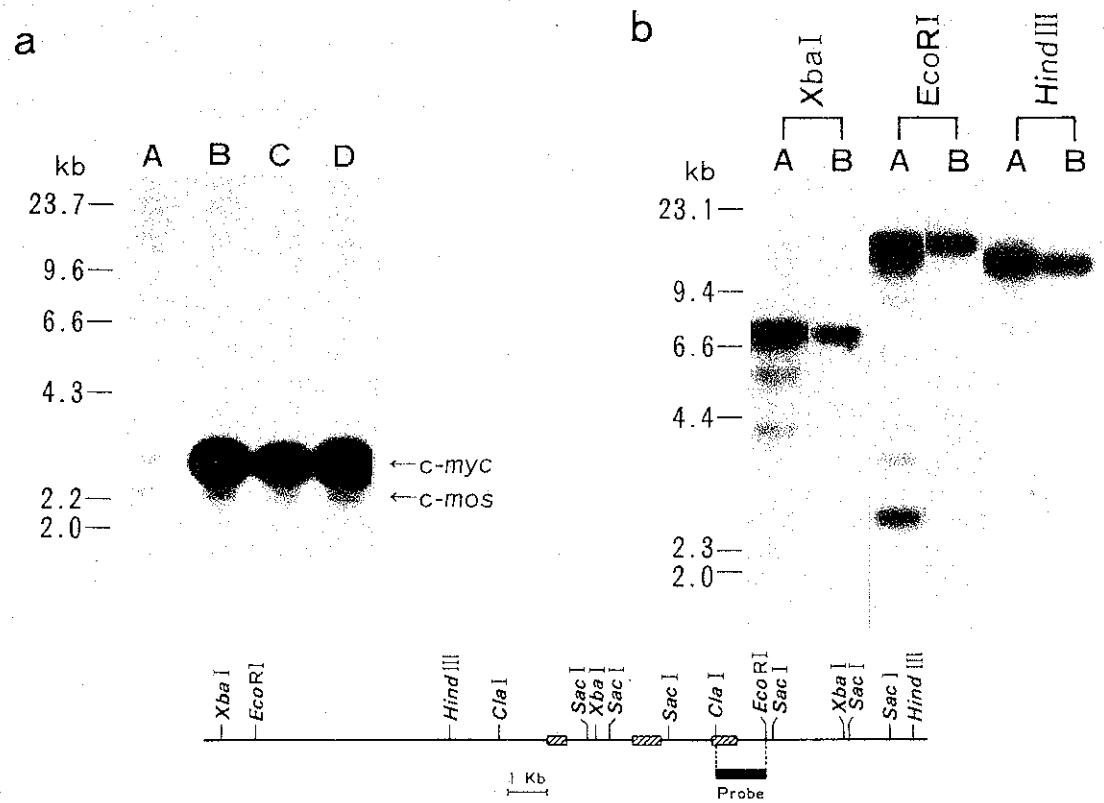

Fig. 1. Amplification of c-*myc* gene in the SCLC tumor, xenograft, and GK-T3 cell line. a) Southern blot analysis of DNA from human placenta (lane A), the original SCLC tumor (lane B), xenograft (lane C), and the established cell line, GK-T3 (lane D). About 10 μg of DNA was digested with restriction enzyme *Sac*I and hybridized with mixed probes of ^{32}P-labeled *Cla*I-*Eco*RI 1.5 kb c-*myc* DNA[18] and *Ava*I-*Bgl*II 0.6 kb c-*mos* DNA.[19] c-*mos* gene was used as an internal control single copy gene to assess the relative amount of c-*myc* gene on the same filter. b) Possible rearrangements of c-*myc* gene in GK-T3 cell line. GK-T3 DNA (0.06 μg) (lane A) and human placenta DNA (1.8 μg) (lane B) were digested with *Xba*I, *Eco*RI and *Hin*dIII followed by hybridization with c-*myc* probe. A schematic representation of the normal human c-*myc* gene is depicted at the bottom. Shaded boxes represent three exons of c-*myc* gene.

Fig. 2. Northern blot analysis of RNA from GK-T3 and various other human cells or cell lines. Poly(A)$^+$ RNA (2 μg) was hybridized with the c-*myc* probe. The arrow indicates a normal 2.3 kb transcript of c-*myc* gene. HL60, acute myelocytic leukemia cell line; U937, histiocytic lymphoma cell line; HEK, primary human embryonic kidney cells; A431, epidermoid carcinoma cell line; Hela, cervical epitheloid carcinoma cell line. Northern blot experiments using constant amounts (10 μg) of total RNA, confirmed by ethidium bromide staining after electrophoresis, gave essentially the same results.

faint bands were detected (Fig. 1b). The biological significance of these possible rearrangements is not clear at this time.

To confirm abnormal expression of c-*myc* gene in GK-T3 cells, Northern blot analysis was carried out using poly(A)-selected cellular RNA prepared by guanidine thiocyanate/cesium chloride centrifugation and oligo-dT column chromatography. As indicated in Fig. 2, expression of c-*myc* in GK-T3 was about 10-fold more abundant than that in Hela, A431 and HEK (human embryo kidney) cells, and at a similar level to that in HL60, which is known to exhibit 20- to 30-fold c-*myc* gene amplification.

Although several *in vitro* cell lines of SCLC were reported to carry amplified c-*myc* gene, it was not confirmed in most cases that the original tumor in patients *per se* exhibited c-*myc* amplification.[15] Since GK-T3 cells were confirmed to have c-*myc* amplification in every stage from the original tumor, through xenografts to the *in vitro* cell line, this cell line should be useful for studies on the molecular mechanism and the structure of c-*myc* gene amplification in SCLC.

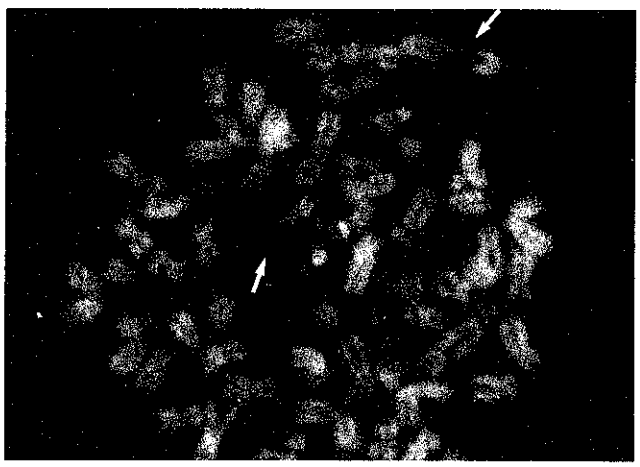

Fig. 4. *In situ* hybridization of GK-T3 cells. Chromosomes from GK-T3 cells were hybridized with ^{3}H-labeled c-*myc* probe. Arrows indicate specific accumulation of silver grains on two hsrs at 14q and 15q of Q-banded metaphase chromosomes.

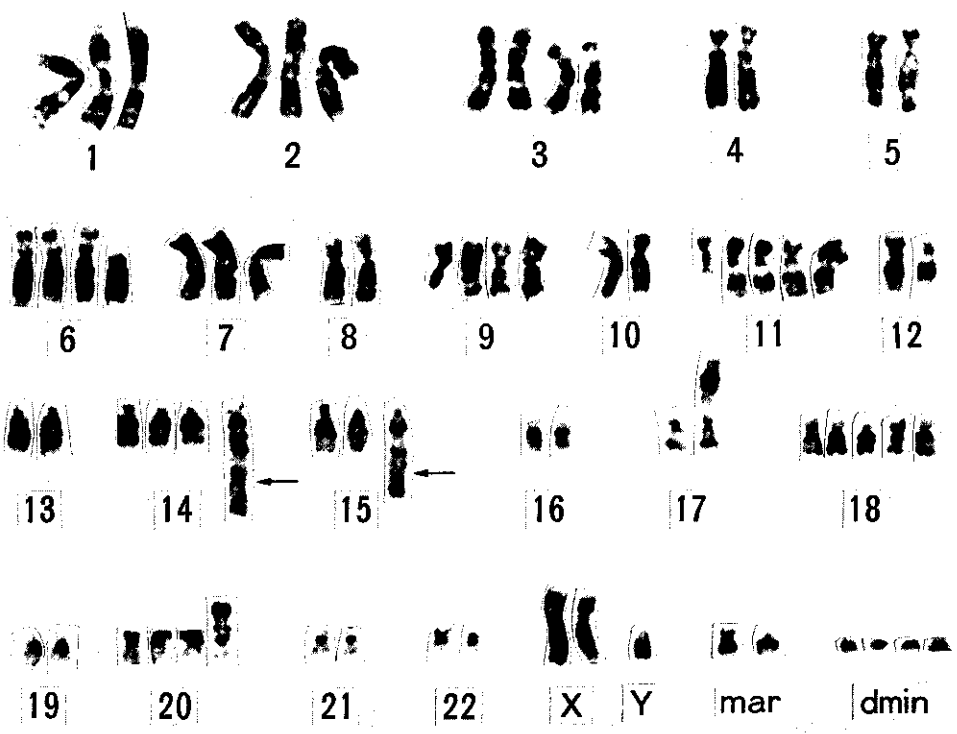

Fig. 3. Representative karyotype of GK-T3 cell line: 69, XXY, −3, +der(3)t(3;6)(p12;p12)x2, −4, −5, +del(6)(p12), −8, +del(9)(q22), −10, +11, +del(11)(q13), −12, del(12)(p12), −13, +14, hsr(14)(q3), hsr(15)(q2), −16, −17, −17, +der(17)t(12;17)(q12;p13), +18, +18, −19, +der(20)t(20;?)(p13;?), −21, −22, +2mar, 4dmin. Arrows indicate two hsrs.

To examine karyotypes of GK-T3, C- or Q-banded chromosomes were prepared by using trypsin-Giemsa or quinacrine mustard stain, respectively. The results revealed that GK-T3 cells had a near-triploid range of chromosomes with complex structural changes (Fig. 3). One of the distinctive cytogenetic features of GK-T3 was the presence of several double minutes (dmins) and homogeneously staining regions (hsrs)[16] on 14q and 15q. Other structural abnormalities consistently observed were unbalanced translocations of t(3;6)(p12;p12) and t(12;17)(q12;p13), del(6)(p12) and del(12)(p12). t(3;6) and t(12;17) may result in alteration of the putative tumor suppressor gene(s) on 3p[10-14] and p53 gene at 17p13,[10,12] respectively. Although 6p12 and 12q12 have not been considered to be particular breakpoints in SCLC, unknown genes at these loci might also be involved in tumorigenesis of GK-T3 cells. Further studies are necessary to confirm genetic changes around these breakpoints.

To examine the chromosome loci for the c-*myc* amplicons, ^{3}H-labeled c-*myc* DNA (*Cla*I-*Eco*RI 1.5 kb) was hybridized *in situ* to chromosomal DNAs.[17] The results indicated that silver grains were specifically accumulated on both hsrs (Fig. 4). Thus, in this GK-T3 cell line, c-*myc* gene was amplified and integrated into two chromosomes, leading to formation of two hsrs. On the other hand, silver grains were not clearly detected on dmins. This may be attributed to the low copy number of the amplified c-*myc* gene in dmins compared to hsrs. Another possibility is that dmins might contain a unit of amplified gene other than c-*myc*.

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