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Analysis of Cellular Oncogene Amplification in Human Lung Cancers

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SUMMARY

Twenty-six lung cancer tissues from operation or autopsy and eight lung cancer cell lines were examined for an amplification of the ten different cellular oncogenes, c-*myc*, N-*myc*, L-*myc*, c-*erb*B-1, c-*erb*B-2, c-*fos*, v-*sis*, c-H-*ras*, v-K-*ras*, and N-*ras*, by the Southern blot hybridization. In small cell lung cancer(SCLC), one SCLC tissue(13-1) out of four SCLC tissues and one SCLC cell line contained three fold amplifications of the L-*myc* oncogene. In non-SCLC, one adenocarcinoma cell line(SLC-51) and one large cell carcinoma cell line(SLC-30) showed an amplification of the c-*myc* oncogene at four fold normal amount and twenty fold amplifications of the c-*erb*B-1 oncogene respectively, out of twenty-two non-SCLC tissues and seven non-SCLC cell lines. Other lung cancer tissues and cell lines did not show any detectable amplifications or rearrangements of oncogenes. These results suggest that an amplification of the studied oncogenes may not always be associated with carcinogenesis even in lung cancer cell lines. Compared with lung cancer cell lines, the frequency of an amplification of oncogenes in lung cancer tissues was low, especially in non-SCLC tissues.

Key words: Oncogene, Human lung cancer, Gene amplification

INTRODUCTION

More than fifty oncogenes have been isolated and shown to serve critical functions in cellular proliferations and reactions to external signals(1-3). An amplification and rearrangement of oncogenes, *myc* oncogene family, *erb*B oncogene, and *ras* oncogene family, in lung cancer have been reported during past several years.

An amplification of a family of *myc* oncogenes (c-*myc*, N-*myc*, and L-*myc*) in lung cancers has been mainly reported in small cell lung cancer(SCLC) (4-8). An

amplification of the c-*myc* and N-*myc* oncogenes was also observed in adenocarcinoma of the lung(9, 10). Restriction fragment length polymorphism(RFLP) of the L-*myc* oncogene in lung cancer has been studied in correlation to clinical stage and metastasis(11). The c-*myc* oncogene product is a well-studied nuclear protein, and appear to be involved in regulations of cell division and differentiation(12, 13, 14).

An amplification of the c-*erb*B-1 and c-*erb*B-2 oncogenes has been also reported in human lung cancer(15, 16). However, an amplification of the c-*erb*B-2 oncogene does not occur in lung adenocarcinoma tissues at a high frequency(10). It was demonstrated that an amplification of the c-*erb*B-2 oncogene was correlated with the clinical stage and lymphnode involvement of breast carcinomas(17, 18).

The mutated c-H-*ras* oncogene which carries a point mutation at the 61st amino acid of the normal counterpart was reported as an activated oncogene in various human lung carcinomas(19). It was also reported that an amplification of the two oncogenes, c-K-*ras*-2 and c-*myc*, occurred in a human lung giant cell carcinoma cell line, accompanied with a point mutational activation of the c-K-*ras*-2(20). Allelic deletion of the c-H-*ras* was detected frequently in lung cancer tissues (16), while its amplification was rare(10, 21, 22).

An amplification of the c-*sis* oncogene has been reported in an osteogenic sarcoma, but not in other human malignant diseases including lung cancers(10). An amplification of the c-*fos* oncogene was not detected in ninety-five samples of various human malignant diseases(10).

Recently, cytogenetic studies have revealed deletions at specific chromosomal sites in chromosomal analysis of lung cancers(23-26), implying that in addition to dominant oncogenes recessive genetic changes of chromosomes may play roles in the genesis of lung cancer(26).

In this report, we have analyzed the genetic structure of ten different oncogenes (c-*myc*, N-*myc*, L-*myc*, c-*erb*B-1, c-*erb*B-2, c-*fos*, v-*sis*, c-H-*ras*, v-K-*ras*, and N-*ras*) in twenty-six lung cancer tissues and eight lung cancer cell lines using the Southern blot hybridization technique. We found amplifications of the c-*myc*, L-*myc* or c-*erb*B-1 oncogenes in one tissue and two cell lines. A relationship between an amplification of oncogenes and prognosis of lung cancers was discussed.

MATERIALS AND METHODS

Tumors and cell lines

Human lung cancer tissues were obtained from surgery or autopsy in Sapporo Medical College Hospital: eleven adenocarcinomas, six squamous cell carcinomas, four small cell carcinomas, four large cell carcinomas and one adenosquamous carcinoma. Pathologically normal tissues adjacent to carcinoma tissues were also

examined when available. Tissues were frozen at −70℃ immediately after operation or autopsy. Eight cell lines of human lung cancer were established in our laboratory (unpublished); three adenocarcinomas(SLC-18, SLC-37, and SLC-51), one small cell carcinoma(SLC-28), three large cell carcinomas(SLC-27, SLC-30, and L4-5D) and one adenosquamous carcinoma(SLC-8). All of the cell lines were grown in RPMI1640 medium containing 10% fetal calf serum.

Isolation of DNA

High-molecular-weight DNA was isolated from frozen tissues as follows. Frozen tissues were minced on ice by a pair of scissors, and suspended in ten fold volumes of a digestion buffer containing 10 mM Tris HCl pH 7.5, 10 mM EDTA, 150 mM NaCl, 0.5% sodium dodecyl sulfate (SDS) and 500μg/m*l* of pronase (Calbiochem Co.). For an extraction of high-moleular-weight DNA from established cell lines, 1 m*l* of the digestion buffer was added to 0.1m*l* packed volume of cells. Samples were incubated overnight at 37℃ and extracted twice with an equal volume of phenol-chloroform(2:1). DNA-containing aqueous phase was overlayed with two volumes of 95% ethanol(27). High-molecular-weight DNA was collected by winding gently with a glass rod, and dissolved in TE buffer (10 mM Tris HCl pH 7.5, 1 mM EDTA).

Probe DNAs

Specific DNA fragments were prepared by digestions of oncogene-carrying plasmids with restriction enzymes and used as probes. The human c-*myc* probe is the 1.4 kilobase(kb) *Eco*RI/*Cla*I fragment of the plasmid pMC41-3RC(28). N-*myc* probe is the 1.0 kb *Eco*RI/*Bam*HI fragment of the plasmid pNb-1(29). L-*myc* probe is the 1.8 kb *Sma*I/*Eco*RI fragment of the plasmid pLmyc10, which was provided by Dr. Minna(7). A probe for the c-*erb*B-1 oncogene was the 2.4 kb *Cla*I fragment of the plasmid pE7 which carries a complementary DNA to the EGF receptor-specific mRNA(30). A probe for the c-*erb*B-2 oncogene is the 0.44 kb *Bam*HI fragment of the plasmid pKX044 which was provided by Drs. Yamamoto and Toyoshima(31). C-H-*ras* probe is the 6.6 kb *Bam*HI fragment of the plasmid pEJ 6.6(32). V-K-*ras* probe is the 1.0 kb *Eco*RI fragment of the plasmid HiHi-3 which was provided by Dr. Kakinuma(33). N-*ras* probe is the 0.5 kb *Sal*I/*Nco*I fragment of the plasmid p6a-1 which was provided by Dr. Kuzumaki(34). C-*fos* probe is the 3.0 kb *Xho*I/*Nco*I fragment of the plasmid ph-c-fos(35). V-*sis* is the 1.28 kb *Pst*I fragment of the plasmid pC60#1(36). The 0.4 kb *Pst*I fragment of the plasmid pHRL-83-BR(37) that spans the fourth intron of the human cardiac actin gene was used as an internal marker. Restriction enzymes (*Bam*HI, *Eco*RI, *Hin*dIII, *Cla*I, *Nco*I, *Pst*I, *Sac*I, *Sal*I, *Sma*I, and *Xho*I) were purchased from

TAKARA SHUZO Co.. The probe DNA was labeled by the method of random prime oligolabeling using (α-^{32}P)dATP and (α-^{32}P)dCTP (Amersham Co.)(38). The specific activity of probe DNA was $1\text{-}2\times10^8$ cpm/μg DNA.

Southern blot hybridization

High-molecular-weight DNA(20 μg) was digested with *Eco*RI or *Sst*I, electrophoresed on a 0.9% agarose gel, denatured, and transferred to a nitrocellulose filter essentially as described by Southern(39). Lambda DNA fragments digested with *Hin*dIII were electrophoresed as molecular weight markers. Hybridization was performed by an incubation of the filter with ^{32}P-labeled DNA probe at 68℃ in 6× SSC (1×SSC=0.15 M NaCl/0.015 M sodium citrate) /0.1% SDS/ 5×Denhardt's solution (1×Denhardt's solution=0.02% Ficoll/0.02% polyvinylpyrrolidone/0.02% BSA) /denatured sheared salmon sperm DNA(100 μg/m*l*) for 14 hrs or more. After hybridization, the filter was washed twice with 250 m*l* of 2×SSC/0.1% SDS at room temperature and then washed twice with 250 m*l* of 0.1×SSC/0.1% SDS at 55℃. The filter was exposed for autoradiography at −70℃ for one week. In some experiments, after autoradiography, the nitrocellulose filter was boiled in distilled water for 15 minutes, and prehybridized with the hybridization buffer described above. The second cycle hybridization was carried out with the cardiac actin gene as a probe. Quantitation of oncogene amplification level was perfomed with a densitometer (MODEL620 VIDEO DENSITOMETER, BIO-RAD Co.) of the developed X-ray films (Fuji RX). Greater than 3 fold of amplification of density compared with the control was defined as positive amplification of oncogene(10).

RESULTS

Amplifications of myc family oncogenes in human lung cancer

High-molecular-weight DNAs were extracted from eight lung cancer cell lines and twenty-six lung cancer tissues, in which sixteen tumors were derived from surgery and ten tumors were from autopsy. DNAs were digested with *Eco*RI, transferred onto a nitrocellulose filter, and hybridized with a *myc*-specific probe (Materials and Methods). The probe detected a single band in a normal placenta and human primary fibroblasts, representing the *Eco*RI fragment of the c-*myc* oncogene with 12.5 kb in a size (Fig. 1A, lanes 1 and 2). In lung cancer cell lines, one(SLC-51) of three adenocarcinoma cell lines showed four fold amplifications of the c-*myc* oncogene, compared with normal placenta and human primary fibroblasts (Fig. 1A, lane 5). Other cell lines including one SCLC cell line(SLC-28) and lung cancer tissues investigated did not show an amplified c-*myc* oncogene (Fig. 1A, lanes 3 and 4, see Table 1).

The L-*myc* probe detected a single band with 6.6 kb or 10.0 kb in sizes or two

bands with 6.6 kb and 10.0 kb in *Eco*RI digested cellular DNA, because of the *Eco*RI RFLP of the L-*myc* oncogene(7). One SCLC tissue had three fold amplifications of the L-*myc* oncogene (Fig. 1B, lane 3), compared with normal placenta and human primary fibroblasts (Fig. 1B, lanes 1 and 2) and with an adjacent non-cancerous portion (Fig. 1B, lane 4). Three other SCLC tissues and twenty-two non-SCLC tissues did not show any amplifications(see Table 1). An amplification of the N-*myc* oncogene was undetectable in any lung cancer tissues and all cell lines examined(see Table 1). The results were summarized in Table 1. No rearrangements of the c-*myc*, L-*myc* and N-*myc* oncogenes were observed in the *Eco*RI cleavage profile in any lung cancer tissues and cell lines examined (data not shown).

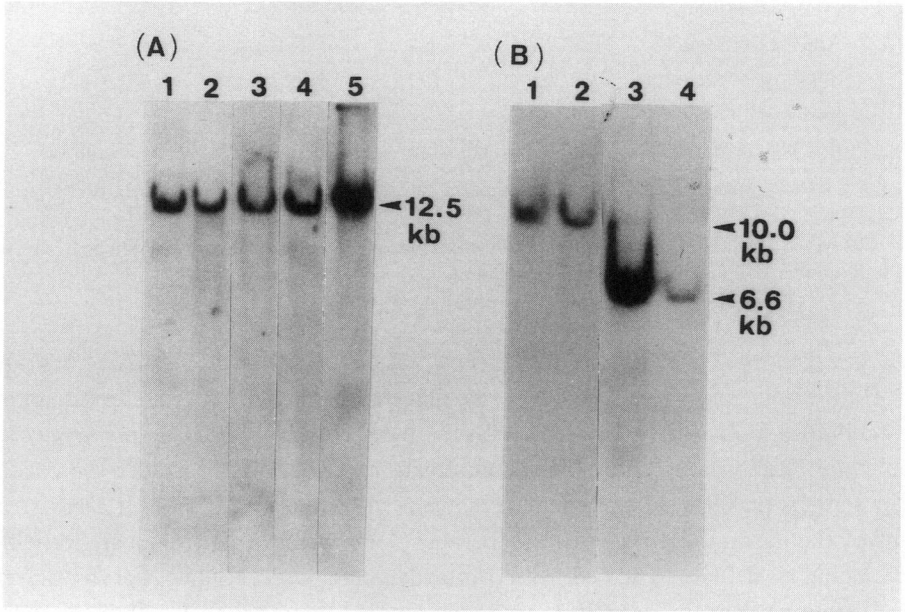

Fig. 1 Southern blot hybridization analysis of the c-*myc* and L-*myc* oncogenes in human lung cancer DNA. High-molecular-weight DNAs were prepared from human lung cancer tissues and lung cancer cell lines, as described in materials and methods. DNAs(20 μg) were digested with *Eco*RI, separated by electrophoresis in 0.9% agarose gels and analyzed by the Southern blot hybridization using ^{32}P-labeled probes specific for the c-*myc*(panel A) and L-*myc* oncogenes(panel B). DNAs of normal human placenta and human primary fibroblasts were used as internal controls. Molecular sizes were estimated by relative mobilities to the *Hin*dIII digests of lambda DNA. (A) c-*myc*: lane 1, human placenta ; lane 2, human primary fibroblasts ; lane 3, adenocarcinoma cell line SLC-18 ; lane 4, adenocarcinoma cell line SLC-37 ; lane 5, adenocarcinoma cell line SLC-51. (B) L-*myc*: lane 1, human placenta ; lane 2, human primary fibroblasts ; lane 3, small cell carcinoma tissue 13-1 ; lane 4, non-cancerous portion adjacent to small cell carcinoma tissue 13-1.

Table 1 *Amplification of the myc family oncogenes in human lung cancer.*

Histology	c-*myc*	N-*myc*	L-*myc*
Lung cancer tissues			
Adenocarcinoma	0/11(3)	0/11(3)	0/11(3)
Squamous cell carcinoma	0/6 (2)	0/6 (2)	0/6 (2)
Small cell carcinoma	0/4 (2)	0/4 (2)	#1/4 (2)
Large cell carcinoma	0/4 (2)	0/4 (2)	0/4 (2)
Adenosquamous carcinoma	0/1 (1)	0/1 (1)	0/1 (1)
Lung cancer cell lines			
Adenocarcinoma	1/3	0/3	0/3
Small cell carcinoma	0/1	0/1	0/1
Large cell carcinoma	0/3	0/3	0/3
Adenosquamous carcinoma	0/1	0/1	0/1

Lung cancer tissues were derived from surgery or autopsy, and samples obtained from autopsies were shown in parentheses. One large cell carcinoma cell line(L4-5D) was established from a sample obtained from autopsy, and the others were established from lung cancer cells in pleural effusion (unpublished).

#: Small cell carcinoma tissue obtained from surgery.

Amplifications of the c-erbB-1 and c-erbB-2 oncogenes in human lung cancer

High-molecular-weight DNAs were digested with *Eco*RI, transferred onto a nitrocellulose filter, and hybridized with the c-*erb*B-1 or c-*erb*B-2 specific probes. The same filter was used for the second cycle hybridization to detect the cardiac actin gene as an internal marker. This probe yielded a single 13.0 kb *Eco*RI fragment of the cardiac actin gene(Fig. 2B, lanes 1-6) which was previously identified by Gunning *et al.*(36). The c-*erb*B-1 probe detected several bands representing the *Eco*RI fragments of the c-*erb*B-1 oncogene ranging from 1.5 to 9.0 kb in sizes(Fig. 2A, lanes 1-6). Compared with normal placenta and human primary fibroblasts (Fig. 2A, lanes 1 and 6), one large cell carcinoma cell line(SLC-30) showed twenty fold amplifications (normalized to an internal marker) of the c-*erb*B-1 oncogene (Fig. 2A, lane 2), but two adenocarcinoma cell lines and one large cell carcinoma cell line did not contain an amplification of the c-*erb*B-1 oncogene(Fig. 2A, lanes 3, 4, and 5).

The c-*erb*B-2 probe detected two bands representing the *Eco*RI fragments of the c-*erb*B-2 oncogene with 14.0 kb and 9.6 kb in sizes(Fig. 2C). Compared with normal placenta(Fig. 2C, lane 1), none of six lung carcinoma cell lines revealed an amplification of the c-*erb*B-2 oncogene(Fig. 2C, lanes 2-7). Eleven lung adenocar-

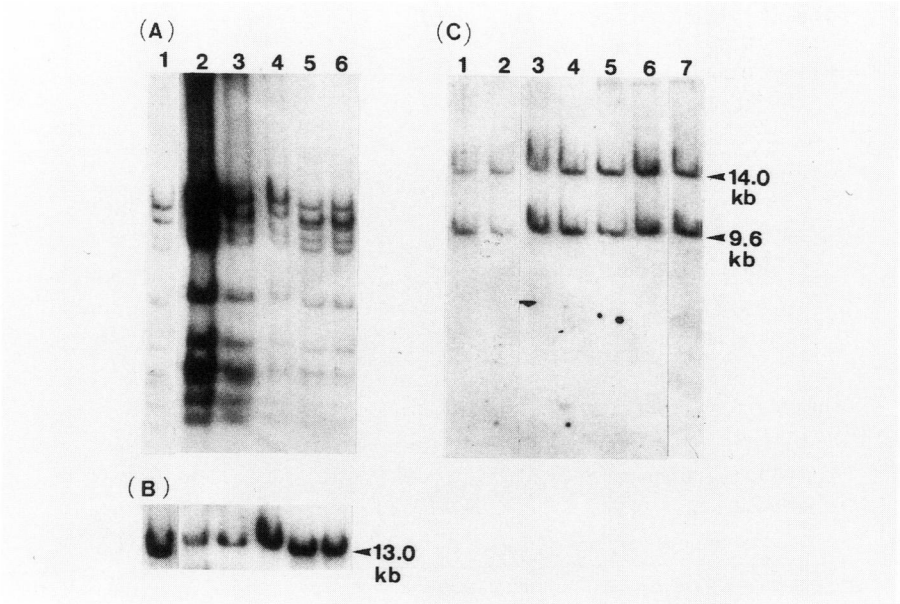

Fig. 2 Southern blot hybridization analysis of the c-*erb*B-1 and c-*erb*B-2 oncogenes in human lung cancer. Southern blot hybridization was performed as described in Fig. 1, using probes specific for the c-*erb*B-1(panel A) and c-*erb*B-2(panel C) oncogenes. The second cycle hybridization was carried out with the cardiac actin gene as a probe(panel B), as described in materials and methods. (A) c-*erb*B-1 : lane 1, human placenta ; lane 2, large cell carcinoma cell line SLC-30 ; lane 3, adenocarcinoma cell line SLC-37 ; lane 4, adenocarcinoma cell line SLC-51 ; lane 5, large cell carcinoma cell line L4-5D ; lane 6, human primary fibroblasts. (B) cardiac actin : Lanes are the same as panel A. (C) c-*erb*B-2 : lane 1, human placenta ; lane 2, adenosquamous carcinoma cell line SLC-8 ; lane 3, large cell carcinoma cell line SLC-27 ; lane 4, small cell carcinoma cell line SLC-28 ; lane 5, adenocarcinoma cell line SLC-37 ; lane 6, adenocarcinoma cell line SLC-51 ; lane 7, large cell carcinoma cell line L4-5D.

cinoma tissues and one other lung adenocarcinoma cell line did not show an amplification of the c-*erb*B-2 oncogene (data not shown). The result was summarized in Table 2. No rearrangements of the c-*erb*B-1 and c-*erb*B-2 oncogenes were observed in the *Eco*RI cleavage profile in any lung cancer tissues and cell lines examined (data not shown).

Amplifications of the c-fos, v-sis, c-H-ras, v-K-ras, and N-ras oncogenes in human lung cancer tissues

High-molecular-weight DNAs were digested with *Eco*RI for analyses of the c-*fos* and v-*sis* oncogenes and with *Sst*I for c-H-*ras*, v-K-*ras*, and N-*ras* oncogenes, transferred onto a nitrocellulose filter, and hybridized with the corresponding oncogene-specific probe. The c-*fos* probe detected a single band in normal placenta,

Table 2 *Amplification of the c-erbB-1 and c-erbB-2 oncogenes in human lung cancer.*

Histology	c-*erb*B-1	c-*erb*B-2
Lung cancer tissues		
Adenocarcinoma	0/11(3)	0/11(3)
Squamous cell carcinoma	0/6 (2)	0/6 (2)
Small cell carcinoma	0/4 (2)	0/4 (2)
Large cell carcinoma	0/4 (2)	0/4 (2)
Adenosquamous carcinoma	0/1 (1)	0/1 (1)
Lung cancer cell lines		
Adenocarcinoma	0/3	0/3
Small cell carcinoma	0/1	0/1
Large cell carcinoma	1/3	0/3
Adenosquamous carcinoma	0/1	0/1

Origins of lung cancer tissues and lung cancer cell lines are described in legend of Table 1. Parentheses show numbers of samples obtained from autopsies.

Table 3 *Amplification of oncogenes in human lung cancer tissues.*

Histology	c-H-*ras*	v-K-*ras*	N-*ras*	c-*fos*	v-*sis*
Lung cancer tissues					
Adenocarcinoma	0/7(2)	0/9(3)	0/4(1)	0/9(3)	0/8(3)
Squamous cell carcinoma	0/6	0/5(1)	0/1	0/8(2)	0/8(2)
Small cell carcinoma	0/2	0/2	0/2	0/2	0/2
Large cell carcinoma	0/1(1)	0/2(2)	N. D.	0/3(2)	0/4(2)
Adenosquamous carcinoma	N. D.	0/1(1)	N. D.	0/1(1)	0/1(1)

Origins of lung cancer tissues are described in legend of Table 1. Parentheses show numbers of samples obtained from autopsies.
N. D.: not done

representing the *Eco*RI fragment of the c-*fos* oncogene with 9.0 kb in a size(Fig. 3, lanes 1 and 8). Ten non-SCLC tissues and one SCLC tissue did not contain an amplification of the c-*fos* oncogene, compared with a normal placenta(Fig. 3, lanes 2-7 and 9-13). In other lung cancer tissues, the c-*fos* oncogene was not amplified (see Table 3). An amplification of the v-*sis* and *ras* family oncogenes was also undetectable in lung cancer tissues examined(see Table 3). The results were summarized in Table 3. No rearrangements of these five oncogenes were observed in the *Eco*RI or *Sst*I cleavage profiles in any lung cancer tissues examined (data not

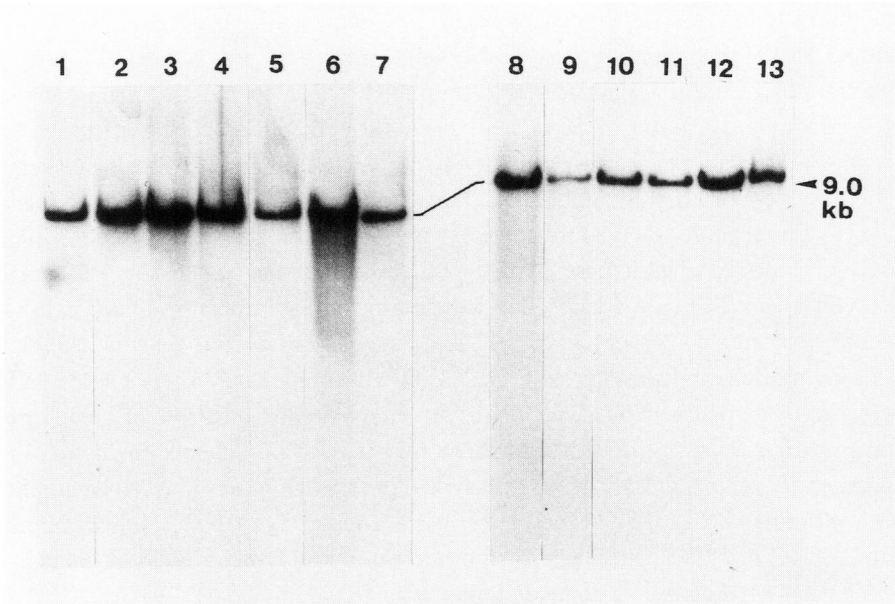

Fig. 3 Southern blot hybridization analysis of the c-*fos* oncogene in human lung cancer. Southern blot hybridization technique using the probe specific for the c-*fos* oncogene was carried out as described in Fig. 1. lane 1, human placenta ; lane 2, squamous cell carcinoma 05-1 ; lane 3, adenocarcinoma 07-1 ; lane 4, squamous cell carcinoma 08-1 ; lane 5, adenocarcinoma 11-1 ; lane 6, adenocarcinoma 14-1 ; lane 7, small cell carcinoma 15-1 ; lane 8, human placenta ; lane 9, adenocarcinoma 06-1 ; lane 10, adenosquamous carcinoma 19-1 ; lane 11, squamous cell carcinoma 20-1 ; lane 12, adenocarcinoma 34-1 ; lane 13, adenocarcinoma 37-1.

shown).

DISCUSSION

Oncogenes are classified into five groups(40), depending upon the structure, function and cellular localization of the oncogene products: protein kinase group (e. g. c-*src*), GTP binding protein group (e. g. c-H-*ras*), cellular growth factor group (e. g. c-*sis*) (42), nuclear protein group (e. g. c-*myc*), and cellular membrane hormone receptor group (e. g. c-*erb*B) (41). In numerous human tumors and tumor cell lines, some oncogenes have been reported to exhibit amplifications (43), rearrangements(43), increased expressions(15, 44), and point mutations(19). We analyzed an amplification of several oncogenes in four oncogene groups with the exception of protein kinase group in human lung cancers.

SCLC cell lines have been studied intensively to understand the biochemical, morphological, biological and clinical behavior of human lung cancers(45). SCLC

is a distinct, aggressive neoplasm with neuroendocrine properties(45). SCLC accounts for approximately 25% of all lung cancers in the U.S.A.(45), however approximately 12% in Japan(46). An amplification of oncogenes in lung cancers was predominantly found in SCLC cell lines(43). In our experiment, the L-*myc* oncogene was amplified in one case out of four SCLC tissues(Table 1). Gazdar *et al.*(45) have reported that SCLC cell lines are classified into three subclasses by analysis of morphology, growth characteristics and biochemical profiles: classic SCLC cell line, biochemical variant SCLC cell line, and morphological variant SCLC cell line. The c-*myc* oncogene was found to be amplified in SCLC cell lines, especially the morphological variant SCLC, suggesting a role for the c-*myc* oncogene in malignant behavior of SCLC(4, 45). Wong *et al.*(5) extracted DNAs from paraffin-embedded SCLC specimens and found an amplification of the c-*myc* or N-*myc* oncogenes with greater than three fold in 12% of the specimens analyzed. Johnson *et al.* reported that the *myc* oncogene family has been shown to be amplified in approximately 30% of SCLC cell lines(47). They divided their cell lines into two groups, SCLC cell lines established from untreated and from treated patients. An amplification of the *myc* oncogene family was found in 11%(2/19) of SCLC cell lines established from untreated patients, whereas it was found in 44%(11/25) of SCLC cell lines established from treated patients.

In our study on non-SCLC, investigated twenty-two tissues did not show an amplification of *myc* oncogene family. Saksela *et al.*(9) reported an amplification of the N-*myc* oncogene in one adenocarcinoma of twenty-five non-SCLC tissues. In the established lung cancer cell lines in this study, the c-*myc* oncogene was amplified in one adenocarcinoma cell line(SLC-51), out of seven non-SCLC cell lines(Table 1). SLC-51 cell line was established from pleural effusion of adenocarcinoma in a patient treated with chemotherapeutic agents, and the primary tumor was unusually aggressive; this patient died one month after the occurrence of lung caner. The frequency of an amplification of *myc* oncogene family in non-SCLC cell lines has not been well documented. Little *et al.* reported that one cell line of five non-SCLC cell lines showed an amplification of the c-*myc* oncogene(4). To delineate an amplification of *myc* oncogene family with lung cancer patient's prognosis, further analysis of the *myc* oncogene amplification will be required.

An amplification of the c-*erb*B-1 oncogene has been detected in non-SCLC, adenocarcinoma(1/15), squamous cell carcinoma(2/7), and large cell carcinoma (2/2) (16). Hunts *et al.* reported that an amplification of the c-*erb*B-1 oncogene was detected in one out of five squamous cell carcinoma tissues of the lung(15). In this study, an amplification of the c-*erb*B-1 oncogene was not detected in all of twenty-two non-SCLC tissues(Table 2). However, an amplification was detected in one large cell carcinoma cell line(SLC-30) (Fig. 2A, lane 2). None of eight cell

lines and twenty-six lung cancer tissues showed an amplification of the c-*erb*B-2 oncogene, including lung adenocarcinoma tissues and cell lines(Table 2). Cline *et al.*(16) reported an amplification of the c-*erb*B-2 oncogene in one adenocarcinoma out of twenty-one non-SCLC tissues. Our results suggest that an amplification of the c-*erb*B-2 oncogene may not play a main role in the evolution of non-SCLC.

An amplification of the c-*sis* oncogene in osteogenic sarcoma has been reported, however it was not detectable in various primary malignant diseases or squamous cell carcinoma of the lung transplanted into nude mice(10, 22). In our study, an amplification of the c-*fos* and v-*sis* oncogenes was not detected in lung cancers. An amplification of the v-*sis* oncogene and c-*fos* oncogene may not be associated with carcinogenesis of lung cancer or may not be a main event. An amplification of *ras* family oncogenes was not detected in all of the lung cancers examined. The *ras* family oncogenes are rarely amplified in lung cancer(10, 21, 22), while allelic deletion of the c-H-*ras* was detected frequently in lung cancer tissues(16). It is likely that an amplification of *ras* family oncogene may not mainly be associated with lung cancer.

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