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# *myc* Family Gene Abnormality in Lung Cancers and Its Relation to Xenotransplantability[1]

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## ABSTRACT

**In order to study the relationship between tumor transplantability to the nude mouse and abnormality of the *myc* family genes (c-*myc*, N-*myc*, L-*myc*) in human primary lung cancers, 32 various lung cancers were analyzed for abnormality of the *myc* family genes by Southern blot hybridization, and were transplanted s.c. into nude mice. Southern blot analysis showed that four non-small cell carcinomas and three small cell carcinomas had amplified c-*myc* and L-*myc* genes, respectively. Allelic deletion of the L-*myc* gene was observed in seven cancers, of which two also had an additional band of the c-*myc* gene or amplification of the L-*myc* gene. No abnormality of the N-*myc* gene was observed in this series. Of 13 cancers with abnormality of the *myc* family genes, 11, including all tumors with *myc* gene amplification, were transplantable to nude mice. Of 19 tumors without any abnormalities of the *myc* family genes, however, only five were transplantable to nude mice ($P < 0.005$). These results indicate that abnormality of the *myc* family genes, especially gene amplification, might promote tumorigenic ability in xenotransplantation of lung cancers and this phenomenon might be closely related to the function of the *myc* gene.**

## INTRODUCTION

Recent advances in molecular biology have revealed that some oncogenes are closely related to the development, progression and differentiation of human cancers (1–3). In human cancers, some oncogenes show various abnormalities such as point mutation (4, 5), amplification (6–9), rearrangement (10–12), and overexpression (9, 13). Among them, abnormalities of the *myc* family genes, especially c-*myc* gene amplification, have frequently been detected in various cancer tissues and cell lines such as leukemia (6, 8), and cancers of the colon (7), stomach (14–16), breast (17), and lung (9). The c-*myc* gene in the cell seems to have various biological functions such as growth control (18, 19), cellular transformation (20), and differentiation (21). In the *myc* family genes, N-*myc* gene amplification has been shown to correlate closely with the tumor stage and patient prognosis in neuroblastoma cases (22, 23). Furthermore, L-*myc*, a gene sharing nucleotide sequence homology with both c-*myc* and N-*myc*, has been found to be amplified in cell lines and tumors of small cell carcinoma of the lung (24), but the biological significance of L-*myc* gene amplification has not been elucidated yet.

Abnormality of the *myc* family genes, especially of c-*myc*, has often been observed in various lung cancers such as giant cell carcinomas, small cell carcinomas and adenocarcinomas (9, 25–28). In order to determine the biological significance of *myc* family gene abnormality in human lung cancers, we transplanted human lung cancers into nude mice and studied the relationship between tumor transplantability and the presence of *myc* family gene abnormalities. We demonstrated that lung cancers with *myc* family gene abnormalities, especially gene amplification, were more tumorigenic in nude mice than lung cancers without the abnormalities.

## MATERIALS AND METHODS

**Tumors.** The tumors used in this study were primary lung cancers surgically resected in the National Cancer Center Hospital. During the period from September 1986 to August 1987, 32 primary lung cancers were used for nude mouse transplantation and for DNA analysis. They consisted of five small cell carcinomas, seven large cell carcinomas, nine adenocarcinomas, seven squamous cell carcinomas, two adenosquamous carcinomas, and one each of undifferentiated carcinoma of the small cell type and adenoid cystic carcinoma.

**Transplantation of Lung Cancers into Athymic Nude Mice.** BALB/c athymic nude male mice, 6–10 weeks old, were purchased from CLEA Japan Inc., Tokyo, Japan, and maintained in a specific pathogen-free condition. Freshly excised tumor tissues were placed aseptically in RPMI 1640 medium (GIBCO Laboratories, Grand Island, NY) and minced to small fragments of about 2 $mm^3$. Three or four tumor fragments were implanted s.c. into the right flank of two or three athymic nude mice. The tumor tissue adjacent to the transplanted tumor was stored at −80°C until use for DNA analysis and the remaining tumor was used for histological analysis. Animals with transplanted lung cancers were checked for tumor growth twice a week for 3 months. The result was judged to be a tumor take if the transplanted tumor increased to more than 5 mm in diameter, and the presence of cancer growth was confirmed histologically.

**Light Microscopic Study.** Primary tumors and xenografts in nude mice were fixed in 20% formalin and embedded in paraffin. 3-μm-thick sections were stained with hematoxylin and eosin.

**DNA Isolation and Southern Blot Hybridization.** DNA was extracted from frozen stored primary lung cancer tissues and xenografts in nude mice according to the method of Blin and Stafford (29). Samples (5 μg) of isolated DNA were digested with a restriction enzyme (*Eco*RI, *Pvu*II, or *Bam*HI) as recommended by the supplier (Takara Shuzo Co., Ltd., Kyoto, Japan). The DNA was separated by electrophoresis in 0.7% agarose gel and the fractions were transferred to nitrocellulose filters by the method of Southern (30). Probes [a 3.5-kilobase pair *Hin*dIII-*Xba*I fragment from the first exon of c-*myc* (26), a 2.0-kilobase pair *Eco*RI-*Eco*RI fragment from the second exon of N-*myc* (31), and a 1.8-kilobase pair *Sma*I-*Eco*RI fragment of L-*myc* (24)] were labeled with [$\alpha$-$^{32}$P]dCTP (Amersham, Buckinghamshire, England) by nick translation. Hybridization was performed at 42°C for 16 h in a mixture of 6× standard saline citrate, (SSC; 1× SSC is 0.15 M NaCl/0.015 M sodium citrate), 10 mM EDTA, 5× Denhardt's solution, 0.5% sodium dodecyl sulfate, 100 μg of denatured salmon sperm DNA per ml, 10% dextran sulfate and 50% formamide. The membrane was washed with 0.2× SSC at 50°C. The blots were autoradiographed at −80°C using Kodak XRP-5 film with an intensifying screen. Autoradiographs were quantitated by a Bio-Rad Model VD620 (Richmond, CA) recording densitometer. As a control, DNA from normal lung tissue of each patient was used. A 2.2-kilobase pair *Eco*RI-*Hin*dIII fragment of c-*mos* (32) and a 0.6-kilobase pair *Eco*RI-*Sal*I fragment of N-*ras* (33) were also used as control probes, because c-*mos* and c-*myc* are located on chromosome 8 and N-*ras* and L-*myc* are located on chromosome 1 (28). More than three copies per haploid genome more than the control probe were considered to be due to gene amplification. Allelic deletion of the L-

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*myc* gene was looked for by using a restriction fragment length polymorphism after *Eco*RI digestion of the L-*myc* gene (24). This deletion was detectable only in tumors showing heterozygosity for the L-*myc* gene.

**Statistical Analysis.** Fisher's exact test was used for analysis of the relationship between *myc* family gene abnormality and the xenotransplanted tumor.

## RESULTS

**Histological Type and Tumor Transplantability.** As shown in Table 1, 16 (50%) of the 32 transplanted tumors were successfully transplanted into nude mice. Among the major histological types of lung cancers, large cell and squamous cell carcinoma showed the highest transplantability, followed by small cell carcinoma. One of the five transplantable large cell carcinomas contained areas of giant cell carcinoma. Transplantable squamous cell carcinomas consisted of three moderately differentiated and two poorly differentiated tumors. The three transplantable small cell carcinomas were of the intermediate cell type. Transplantability of adenocarcinomas was the lowest.

No significant relationship between the clinical stage of the tumor and its transplantability was observed (data not shown).

***myc* Family Gene Abnormalities and Transplantability.** Southern blot analysis showed various *myc* gene abnormalities in DNAs extracted from primary lung cancers (Table 2). Amplification of the c-*myc* gene was found in two large cell carcinomas including the tumor with a giant cell component, and in one each of squamous cell carcinoma and adenocarcinoma. The large cell carcinoma with a giant cell component revealed the greatest amplification of the c-*myc* gene (about 10-fold) (Fig. 1). Three small cell carcinomas showed L-*myc* gene amplification (Fig. 2). Allelic deletion of the L-*myc* gene was found in five cancers (Fig. 3). Furthermore, one each of squamous cell carcinoma and small cell carcinoma with allelic deletion of L-*myc* were also associated with an additional band of c-*myc* and L-*myc* gene amplification, respectively. The additional band was located at the 8-kilobase pair position when the DNA digested by *Pvu*II was hybridized with the c-*myc* probe including exon 1. Further study concerning this additional band is in progress. There was no detectable N-*myc* gene abnormality in the 32 primary lung cancers.

Table 1 *Transplantability of various lung cancers to nude mice*

A total of 32 various lung cancers were transplanted s.c. into nude mice. The histology of transplantable lung cancers were variable.

| Histological type | No. of tumors with take/ No. of tumors transplanted |
|---|---|
| Large cell carcinoma | 5/7 |
| Small cell carcinoma | 3/5 |
| Adenocarcinoma | 2/9 |
| Squamous cell carcinoma | 5/7 |
| Adenosquamous carcinoma | 1/2 |
| Undifferentiated carcinoma small cell type | 0/1 |
| Adenoid cystic carcinoma | 0/1 |
| Total | 16/32 |

Table 2 *Histological type and abnormality in myc family genes*

The abnormalities of the *myc* family genes were observed in various histological types of the lung cancer.

| *myc* gene abnormality | c-*myc* amp[a] | L-*myc* amp[a] | L-*myc* del[b] | Total |
|---|---|---|---|---|
| Large cell carcinoma | 2/7 | 0/7 | 2/4 | 4/7 |
| Small cell carcinoma | 0/5 | 3/5 | 1[c]/5 | 3/5 |
| Adenocarcinoma | 1/9 | 0/9 | 2/7 | 3/9 |
| Squamous cell carcinoma | 1/7 | 0/7 | 2[d]/4 | 3/7 |
| Adenosquamous carcinoma | 0/2 | 0/2 | 0/1 | 0/2 |
| Undifferentiated carcinoma small cell type | 0/1 | 0/1 | 0/0 | 0/1 |
| Adenoid cystic carcinoma | 0/1 | 0/1 | 0/0 | 0/1 |

[a] amp, amplification.
[b] del, deletion. L-*myc* deletion was detectable only in tumors heterozygous for the L-*myc* gene.
[c] Also with amplification of L-*myc*.
[d] Including a tumor with an additional band of c-*myc*.

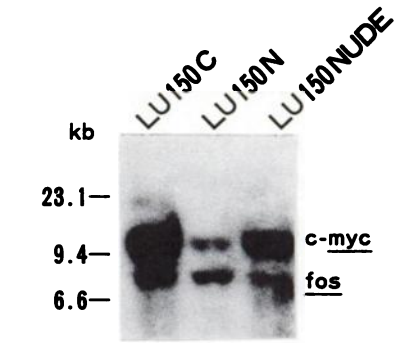


Fig. 1. c-*myc* gene amplification in a large cell carcinoma with a giant cell component. DNA from the primary tumor (*LU150C*) and normal lung tissue (*LU150N*) of the patient and from the xenograft (*LU150NUDE*) was digested with *Eco*RI and hybridized with $^{32}$P-labeled c-*myc* and *fos* probes. The c-*myc* band (*top band*) is more intense in the primary tumor and xenograft than in the normal lung tissue. Intensity of the *fos* band is almost equal.

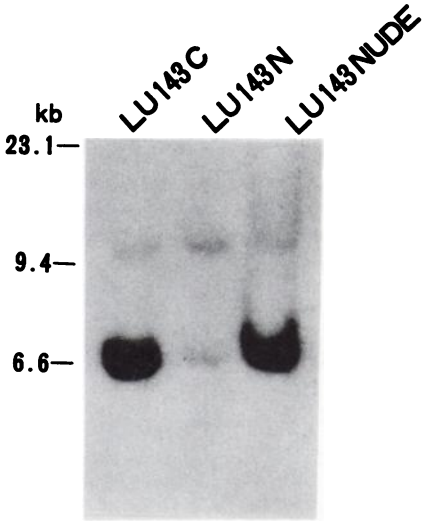


Fig. 2. L-*myc* gene amplification in a small cell carcinoma. DNA from the primary tumor (*LU143C*) and normal lung tissue (*LU143N*) of the patient and from the xenograft (*LU143NUDE*) was digested with *Eco*RI and hybridized with a $^{32}$P-labeled L-*myc* probe. This patient has L-*myc* gene heterozygosity, showing two bands of 6.6 and 10 kilobase pair. The 6.6 kilobase pair band is amplified in the primary and xenograft.

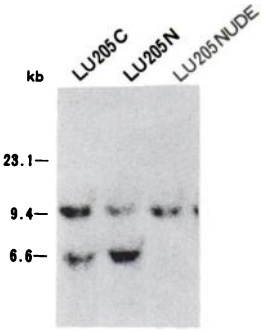


Fig. 3. L-*myc* deletion in a large cell carcinoma. Southern blot analysis was performed on *Eco*RI-digested DNA extracted from the primary tumor (*LU205C*), normal lung tissue (*LU205N*) of the patient and the tumor of the xenograft (*LU205NUDE*) using a $^{32}$P-labeled L-*myc* probe. This patient shows L-*myc* gene heterozygosity, of two bands of 6.6 and 10 kilobase pair. However, the intensity of the 6.6-kilobase pair band of the primary tumor is less strong than that of normal lung tissue and the band is completely deleted in the xenograft.



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Table 3 *Relationship between abnormality in myc family genes and tumor transplantability to nude mice*

Lung cancers with abnormalities of the *myc* family genes showed higher tumor-take rate in nude mice than those without them.

| *myc* gene abnormality | c-*myc* amp[a] | L-*myc* amp[a] | L-*myc* del[b] | Not detected | Total |
|---|---|---|---|---|---|
| No. of tumors with take | 4 | 3[c] | 5[c,d] | 5 | 16 |
| No. of tumors transplanted | 4 | 3 | 7 | 19 | 32 |

[a] amp, amplification.
[b] del, deletion. L-*myc* deletion was detected only in tumors heterozygous for L-*myc*.
[c] Including a tumor with amplification and deletion of L-*myc*.
[d] Including a tumor with an additional band of c-*myc* with deletion of L-*myc*.

The relationship between the *myc* family gene abnormalities and tumor transplantability is shown in Table 3. Of 13 lung cancers with abnormalities of *myc* family genes, 11 including all those with L- and c-*myc* gene amplification were successfully transplanted into nude mice. Of five tumors with only L-*myc* gene deletion, three were transplanted into nude mice. On the other hand, only five of 19 tumors without any detectable abnormalities of *myc* family genes were transplantable. The lung cancers with *myc* family gene abnormalities showed higher tumor-take rate in nude mice than those without any abnormalities of the *myc* family genes ($P < 0.005$).

Eight xenotransplanted tumors were examined for abnormalities of the *myc* family genes. The remaining tumors did not grow large enough for DNA extraction within the period of 3 months. No significant difference in *myc* family gene abnormalities was observed in xenotransplanted tumors compared with those in the original tumors (Fig. 1–3). Of the eight patients, six had *myc* gene abnormalities in the primary lung cancer.

## DISCUSSION

In this study, 50% of the human primary lung cancers were successfully transplanted into nude mice. Previous reports showed a tumor-take rate of primary lung cancers of 42 to 71%, depending on the histological type of the cancer (34–38). In general, large cell carcinoma was easily transplantable to nude mice. However, the xenotransplantation of adenocarcinoma, especially of the well-differentiated type, was quite difficult (35, 36). In Japan, the transplantability of primary lung carcinoma was reported to be less than 50% (35, 36, 38), because adenocarcinomas were the most frequent histological type of lung cancer and a large number of adenocarcinomas were included in those experiments. Our results on tumor transplantability obtained in this study seem to be reasonable compared to those in previous reports.

Amplification of the c-*myc* gene has been reported for various primary lung cancers, both non-small and small cell carcinomas, and cell lines derived from lung cancers (9, 25–28). In small cell carcinomas, c-*myc* gene amplification was reported to be often present in the variant type, but rare in the classic type (39). However, a recent study showed that c-*myc* gene amplification and overexpression is frequent in cultured small cell carcinomas, both variant and classic types (40). In our study no c-*myc* gene amplification was detected in small cell carcinomas. In contrast, L-*myc* gene amplification was found in three of five small cell carcinomas. Nau *et al.* found L-*myc* gene amplification in a small cell carcinoma and four small cell carcinoma cell lines (24). Probably L-*myc* gene amplification in small cell carcinomas is more frequent than reported previously.

This study demonstrates that lung cancers with abnormalities of the *myc* family genes, especially c- and L-*myc* gene amplification, are more easily transplantable to nude mice than those without any detectable abnormalities of the *myc* family genes. In a transplantation experiment using a human breast carcinoma cell line, Modjitahedi *et al.* showed an increased level of c-*myc* gene amplification in transplanted tumors in nude mice, and concluded that increased levels of both amplification and expression of the c-*myc* gene might have an important function in the progression of the malignant phenotype and/or in the proliferation of the cells (41). Shibuya *et al.* detected c-*myc* gene amplification in three of 16 transplanted human gastric cancers in nude mice and also found that a rapidly growing and poorly differentiated tumor had an increased level of c-*myc* gene expression, but that a slowly growing and better differentiated tumor did not (15). In small cell carcinoma cell lines, Gazdar *et al.* found that the cells with c-*myc* gene amplification had higher colony-forming efficiency and shorter generation time than those without c-*myc* gene amplification (39). Summarizing these observations, c-*myc* gene amplification as well as its expression may have a close relation to the maintenance or increase of malignant properties of carcinoma cells. Since there was the possibility of elevated expression of the c-*myc* gene in five transplantable lung cancers without any *myc* family gene abnormality in the present study, further study by Northern blot analysis is necessary.

Xenotransplanted tumors are important materials for cancer research. However, Nakazato *et al.* found c-*myc* gene amplification in two of 11 human gastric cancers transplanted into nude mice, although no such amplification was detected in 30 surgically resected tumors. They stated that the cells with an amplified c-*myc* gene might grow preferentially or the amplification of c-*myc* might occur during passage in nude mice (14). Our present study shows that cancers with amplified *myc* family genes are more apt to grow in nude mice than cancers without any *myc* family gene amplification. Therefore, we must keep it in mind that the rate of *myc* gene amplification in the xenotransplanted tumors and also in cultured tumor cells may be higher than in primary tumors.

The role of N- and L-*myc* genes is not yet known. N-*myc* gene amplification in neuroblastoma was reported to be closely related to the clinical stage of the tumor (18, 19). A significant relation between L-*myc* gene amplification and clinical stage has not been reported as far as we know. Our study showed no relationship between *myc* family gene abnormality and clinical stage of the lung cancers (data not shown). However this study suggested that the lung cancers with abnormalities of the L-*myc* gene as well as cancers with c-*myc* gene amplification are easily transplantable in nude mice.

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