myc Family DNA Amplification in 107 Tumors and Tumor Cell Lines from Patients with Small Cell Lung Cancer Treated with Different Combination Chemotherapy Regimens

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ABSTRACT

We studied 107 specimens (38 tumors and 69 tumor cell lines) from 90 patients with small cell lung cancer to determine the characteristics and clinical situations of patients from whom tumor cell lines could be established and the myc family DNA copy number. The proportion of extensive stage small cell lung cancer patients from whom a tumor cell line could be established prior to the initiation of therapy increased during the 10 years of the study (P < 0.001). Amplification of one of the myc family genes occurred in 3 of 40 (8%) of the untreated patient specimens compared to 19 of 67 (28%) of the treated patient specimens (P = 0.01). The myc family DNA amplification occurred in 17 of 54 (31%) of the specimens from patients treated with cyclophosphamide-based combinations and 2 of 13 (15%) of the specimens from patients treated with etoposide/cisplatin (P = 0.25). Both tumors and tumor cell lines were obtained from 17 patients with small cell lung cancer and the myc family DNA copy number was similar in 16 of the 17 patients. We conclude that: (a) myc family DNA amplification occurs more commonly in specimens from treated than untreated patients; (b) there are no prominent differences in the frequency of amplification following treatment with different chemotherapy regimens; and (c) myc family DNA amplification is similar in tumors and tumor cell lines from the same patients.

INTRODUCTION

Three of the members of the myc family, c-myc, N-myc, and L-myc, have been shown to be amplified in tumors and tumor cell lines from patients with small cell lung cancer (1-10). c-myc amplification has been the most frequently observed and is associated with a variant form of small cell lung cancer cell lines that have a more rapid growth rate than the classic type (1, 11-13). We have previously shown that myc family DNA amplification is more common in tumors and tumor cell lines derived from small cell lung cancer patients previously treated with combination chemotherapy (8, 14). Furthermore, DNA amplification of c-myc in tumor cell lines established from chemotherapy-treated patients is associated with a shortened survival time (14).

Since these initial observations were made, the combination of etoposide plus cisplatin has been introduced and has become one of the common initial treatments of patients with small cell lung cancer (15-17). This regimen was adopted as the induction chemotherapy regimen for our extensive stage small cell lung cancer protocol beginning in 1983 (18). This study also incorporated systemic pretreatment biopsies of patients' cancer for in vitro drug sensitivity testing. On the basis of the in vitro drug sensitivity results, patients were treated during the second 12 weeks of therapy with a chemotherapy regimen to which their tumor cells were most sensitive. If no in vitro results were available, patients were treated with an empiric regimen. Therefore, we decided to determine if the proportion of tumor cell lines established from our untreated patients with small cell lung cancer increased after 1983 with the introduction of systematic pretreatment tumor biopsies of extensive stage patients. We also wished to extend our initial observations of myc family DNA amplification to include 107 tumor specimens and tumor cell lines from patients with small cell lung cancer treated over the last 10 years with etoposide plus cisplatin as well as cyclophosphamide-based combinations.

MATERIALS AND METHODS

We obtained tumor and/or tumor cell line specimens for myc family DNA analyses from 90 of the 306 patients with small cell lung cancer treated at the National Cancer Institute-Navy Medical Oncology Branch from 1977 through 1986. The patients underwent staging evaluations as described previously (19-21). The patients were classified as having limited disease (defined as tumor limited to one hemithorax and bilateral hilar, mediastinal, and supraclavicular nodes) or extensive disease (defined as tumor spread beyond these areas). The limited stage patients were treated with cyclophosphamide-based combination chemotherapy with or without irradiation (19). The induction chemotherapy for the patients with extensive stage small cell lung cancer consisted of either etoposide plus cisplatin (18) or a cyclophosphamide-based combination regimen. The cyclophosphamide-based combinations included CMC/VAP3 (20), CAPO (21), and CAV (22). An attempt to establish cell lines from the patients' tumor specimens was made as described previously (11, 12, 18). Specimens were identified as having been obtained prior to the initiation of chemotherapy or after one or more courses of combination chemotherapy.

c-myc, N-myc, and L-myc DNA copy number of the tumor and tumor cell line DNA was determined as previously described (8). The presence of high molecular weight DNA from the tumors and tumor cell lines was confirmed by agarose gel electrophoresis of the undigested DNA. Quantification of the DNA amplification was determined by densitometric comparison of the signal from the sample DNA to the signal of a single copy gene control (i.e., gastrin-releasing peptide or oxytocin probe) (8). The copy number of the myc genes was considered to be amplified if the signals were 4-fold greater than the single copy gene controls.

The survival time of the patients was calculated from the date of initial chemotherapy. Follow-up was available on all patients. The differences in frequency of amplification between the totals of the treated and untreated groups and between the totals of the two treated groups were statistically compared using the $\chi^2$ test for comparing

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"The abbreviations used are: CMC/VAP, cyclophosphamide, methotrexate, thomustine, vincristine, doxorubicin, and procarbazine; CAPO, cyclophosphamide, doxorubicin, etoposide, and vincristine; CAV, cyclophosphamide, doxorubicin, and vincristine."
RESULTS

A total of 107 small cell lung tumors and tumor cell lines from 90 patients were evaluated for myc family DNA amplification. There were 38 tumor specimens and 69 tumor cell lines. These were obtained from 66 patients with extensive stage and 24 patients with limited stage disease. Seventeen patients had both tumor and tumor cell line specimens available for myc family DNA analyses.

Fig. 1A depicts the stages of the 306 small cell lung cancer patients entered onto our protocols from 1977 to 1986. Fig. 1B displays the number of previously treated versus untreated patients as the sources of the 69 tumor cell lines. There was an increase in the proportion of tumor cell lines established from untreated patients with extensive stage small cell lung cancer from 1977 through 1986 (P < 0.001). In 1983 the extensive stage small cell lung cancer study was initiated which included systematic biopsy of tumors prior to the initiation of chemotherapy treatment. The percentage of untreated patients with extensive stage small cell lung cancer who had tumor cell lines established increased from 10 of 119 (8%) during 1977–1982 to 24 of 78 (31%) after the start of the new study in 1983 (P < 0.001).

Forty-five, 5, and 4 specimens were obtained from patients initially treated with the cyclophosphamide-based combinations, CMC/VAP, CAV, and CAPO, respectively. Thirteen specimens were derived from patients treated with etoposide plus cisplatin induction therapy. Forty specimens were obtained from patients who had not received chemotherapy.

Fig. 2 contains autoradiograms of DNA from small cell lung cancer cell lines hybridized to c-myc and gastrin-releasing peptide DNA fragments. DNAs from H847 and H1304 show c-myc amplification. Dilutional studies revealed c-myc DNA copy numbers of 20 and 40 for NCI-H847 and NCI-H1304, respectively. Autoradiograms of other c-myc-amplified tumor cell lines have been published previously (14). The other tumors and tumor cell lines studied did not have c-myc DNA amplification.

Table 1 gives the distribution of myc family DNA amplification of the small cell lung cancer tumors and tumor cell lines.

![Fig. 2. Autoradiograms of DNA from small cell lung cancer cell lines hybridized to c-myc and gastrin-releasing peptide fragments. The tumor cell line DNA was hybridized to a 32P-labeled Clal-EcoRI third exon c-myc fragment (23) and a Prul-PvuII gastrin-releasing peptide fragment (24). NCI-N417 is a cell line previously shown to be amplified for c-myc (1). It demonstrates an intense 12.5-kilobase (kb) EcoRI c-myc fragment signal. NCI-H372 is a cell line previously examined and known to be non-c-myc amplified (1). NCI-H847 and NCI-H1304 have multiple copies of the c-myc fragment. The single copy 6.1-kilobase EcoRI gastrin-releasing peptide fragment signal appears below the c-myc fragment signal.](https://cancerres.aacrjournals.org/content/51/9/1709/F2.large.jpg)
which have N-myc DNA amplification and have been described specimens from patients 2 (NCI-H526) and 3 (NCI-H689) the results of each are reported separately. The myc family both tumor and tumor cell line DNA available for analyses and matched specimens. This included tumor and tumor cell line among the different treatment groups. Seventeen patients had fragment signal. (kb) BamHl gastrin-releasing peptide fragment signal appears below the L-myc (8). NCI-H209 and BL-I are cell lines that a tumor cell line previously shown to be amplified for L-myc (3). It demonstrates DNA amplification pattern was identical for 16 of the 17 digested with BamHl and hybridized to a "P-labeled Smal-EcoRI L-myc fragment (3) and a PvuII-PvuII gastrin-releasing peptide fragment (24). NCI-H378 is a tumor cell line previously shown to be amplified for L-myc (3). It demonstrates C. (Fig. 3) Autoradiograms of DNA from small cell lung cancer cell lines hybridized to L-myc and gastrin-releasing peptide fragments. The tumor cell line DNA digested with BamHl and hybridized to a 32P-labeled Smal-EcoRI L-myc fragment (3) and a PvuII-PvuII gastrin-releasing peptide fragment (24). NCI-H378 is a tumor cell line previously shown to be amplified for L-myc (3). It demonstrates an intense L-myc fragment signal (8). NCI-H209 and BL-I are cell lines that have been previously examined and known to be non-L-myc amplified (3). NCI-H889 has multiple copies of the L-myc fragment. The single copy 3.1-kilobase (kb) BamHl gastrin-releasing peptide fragment signal appears below the L-myc fragment signal.

Table 1 Distribution of myc family DNA amplification of small cell lung cancer tumors and tumor cell lines among the different treatment groups. Seventeen patients had both tumor and tumor cell line DNA available for analyses and the results of each are reported separately. The myc family DNA amplification pattern was identical for 16 of the 17 matched specimens. This included tumor and tumor cell line specimens from patients 2 (NCI-H526) and 3 (NCI-H689) which have N-myc DNA amplification and have been described previously (8). The other 14 did not have evidence of myc family DNA amplification. In the other matched specimens, the tumor cell line (NCI-H847) demonstrated c-myc DNA amplification while the tumor (from patient 7) did not (Fig. 4A). The DNA from patient 7's tumor was examined and found to be degraded (Fig. 4B). Review of his clinical course showed that his small cell lung cancer had been treated with chemotherapy for 3 months before his death from infectious complications. The tumor tissue from his postmortem examination was reviewed and found to be mostly necrotic cells with little evidence of cancer (Fig. 5A). We suspect that there were insufficient viable tumor cells for accurate evaluation of myc family DNA amplification in the tumor specimen.

Table 2 summarizes the myc family DNA amplification results for the tumor and tumor cell line specimens. The data are divided into cyclophosphamide-based combinations versus etoposide plus cisplatin versus untreated groups. The individual myc family member DNA amplifications are given. Amplification of one of the myc family genes occurred in 19 of 67 (28%) of the treated patient specimens compared to 3 of 40 (8%) of the untreated patient specimens (P = 0.01). The amplification in the treated patient specimens was composed of 17 of 54 (31%) of the cyclophosphamide-based combination specimens and 2 of 13 (15%) of the etoposide plus cisplatin specimens (P = 0.25).

Fig. 6 depicts survival from the initiation of chemotherapy of small cell lung cancer patients whose tumor cell lines established at relapse had c-myc family DNA amplification compared to patients whose cell lines established at relapse did not. Patients whose cell lines had DNA amplification of c-myc lived a shorter time than patients whose cell lines did not (P = 0.002). Patients whose tumor cell lines had DNA amplification of N-myc or L-myc lived a similar period of time as those whose did not (P = 0.78 and P = 0.99, respectively; data not shown).

Fig. 7 shows decreasing numbers of myc family DNA-amplified tumor cell lines from previously treated patients from 1977.
myc FAMILY DNA AMPLIFICATION IN SMALL CELL LUNG CANCER

1. To 1986. There were fewer myc family DNA amplified tumor cell lines established after the introduction of etoposide/cisplatin induction chemotherapy in 1983. Ten of 18 (56%) of tumor cell lines established from previously treated small cell lung cancer patients from 1977 to 1982 had myc family DNA amplification compared to 3 of 17 (18%) previously treated patients whose tumor cell lines were established after 1983 ($P = 0.02$). The linear trend from 1977 through 1986 did not achieve standard statistical significance ($P = 0.14$).

2. Table 2 myc family DNA amplification of small cell lung cancer tumors and tumor cell lines

<table>
<thead>
<tr>
<th></th>
<th>c-myc</th>
<th>N-myc</th>
<th>L-myc</th>
<th>Total</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBS</td>
<td>6/54 (11)</td>
<td>6/54 (11)</td>
<td>5/54 (9)</td>
<td>17/54 (31)</td>
<td>0.32</td>
</tr>
<tr>
<td>VP/PL</td>
<td>1/13 (8)</td>
<td>1/13 (8)</td>
<td>0/13 (0)</td>
<td>2/13 (15)</td>
<td>0.01</td>
</tr>
<tr>
<td>Untreated</td>
<td>0/40 (0)</td>
<td>1/40 (2)</td>
<td>2/40 (5)</td>
<td>3/40 (8)</td>
<td>0.02</td>
</tr>
</tbody>
</table>

$a$ $P_1$ refers to the comparison of the total CBS versus VP/PL numbers, while $P_2$ refers to the comparison of the total CBS and VP/PL versus untreated numbers.

$BC$: cyclophosphamide-based combination; VP/PL, etoposide-cisplatin.

$\frac{\%}{100}$ Numbers in parentheses, percentage.

Fig. 5. A, photomicrograph of a tumor specimen from patient 7. A necrotic autopsy specimen from patient 7 reveals no viable tumor cells. The poor condition of this specimen is consistent with the degraded DNA present in figure 4. H & E, $\times 230$. B, photomicrograph of a tumor specimen from patient 2. A well-preserved autopsy specimen contains a metastatic focus of small cell lung cancer in the liver of patient 2. The tumor is composed of groups of poorly differentiated small cells with hyperchromatic nuclei with nuclear molding and scanty cytoplasm characteristic of small cell lung cancer. There is no necrosis. A rim of normal liver tissue is in the lower left hand corner. H & E, $\times 230$.

DISCUSSION

In this paper we have extended our previous observations of myc family DNA amplification in tumors and tumor cell lines from patients with small cell lung cancer. In addition to the 44 small cell lung cancer cell lines (14) and 38 tumors (8) that have been reported previously, we have studied the myc family DNA copy number from an additional 25 tumor cell lines and have collectively analyzed all 107 specimens from the 90 different patients with small cell lung cancer. We have continued to observe that myc family DNA amplification is more frequently present after treatment with different combination chemotherapy regimens and that DNA amplification of c-myc in tumor cell lines established from chemotherapy-treated patients is associated with a shortened survival time similar to our previous reports (8, 14).

Other investigators who have studied 9–18 tumor cell lines established from different patients with small cell lung cancer have found myc family DNA amplification in 50–83% of the cell lines (6, 7, 10). Thirteen of 44 (30%) small cell lung cancer cell lines had myc family DNA amplification in our previous study (14) and 3 of 25 (12%) tumor cell lines reported in this current study had myc family DNA amplification. We believe
this decrease in myc family DNA amplification observed in our tumor cell lines occurs in part because an increased proportion of the tumor cell lines analyzed are established from previously untreated patients, 19 of 44 (43%) in the previous report compared to 15 of 25 (60%) of the new tumor cell lines reported in this study (Fig. 1B). In addition, because of the decreased proportion of myc family DNA amplified tumor cell lines established from previously treated patients (P = 0.25), this is approximately one-half the rate of myc family DNA amplification observed in tumor cell lines established from patients treated with cyclophosphamide-based combinations (31%). Additional studies of more small cell lung cancer cell lines and tumors will be needed before one can resolve this issue.

In contrast to the relatively high incidence of myc family DNA amplification in tumor cell lines from patients with small cell lung cancer, studies of 17-45 tumors obtained from different patients with small cell lung cancer have found myc family DNA amplification in only 11-24% (5, 8-10). Therefore, it is unlikely that myc family DNA amplification is required for the initial steps of malignant transformation.

There has been a linear trend from 1977 through 1986 that an increasing proportion of our extensive stage patients have had a cell line established from tumors biopsied prior to the initiation of therapy (P < 0.001). This has been associated with the introduction in 1983 of the current extensive stage small cell lung cancer study which links the selection of the chemotherapy to in vitro chemosensitivity testing of the patients' tumor cells (18). We believe this report is the first to show a link between using the patients' tumors for individualized chemotherapy determinations and increasing the proportion of cell lines established from a defined cohort of patients entering a study.

We have continued to observe myc family DNA amplification more commonly in patients with small cell lung cancer who have been treated previously with combination chemotherapy. However, the proportion of myc family DNA-amplified cell lines which are established from chemotherapy-treated patients with small cell lung cancer is smaller since 1983. In addition, the small cell lung cancer cell lines (10) which have been obtained from patients before and after chemotherapy treatment have not developed myc family DNA amplification in the specimens obtained after chemotherapy administration. Therefore, additional studies will need to be done to determine if the chemotherapy treatment can actually induce myc family DNA amplification in tumor and/or tumor cell line specimens obtained before and after cytotoxic therapy.

REFERENCES
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