myc Family DNA Amplification in Small Cell Lung Cancer Patients’ Tumors and Corresponding Cell Lines

Bruce E. Johnson,1 Robert W. Makuch,2 Alfreda D. Simmons, Adi F. Gazdar, David Burch, and Alan W. Cashell

NCI-Navy Medical Oncology Branch, National Cancer Institute and Naval Hospital [B. E. J., A. D. S., A. F. G.], Pathology Department, Naval Hospital [D. B. A. W. C.], Bethesda, Maryland 20814, and the Division of Biostatistics, Yale University School of Medicine [R. W. M.], New Haven, Connecticut 06510

ABSTRACT

Tumor specimens procured from 38 different small cell lung cancer patients were studied for DNA amplification of the myc family of protooncogenes (c-myc, N-myc, and L-myc). Six of the 38 specimens (16%) had 4-fold or greater myc family DNA amplification (N-myc in 4 and L-myc in 2). All 6 tumors with amplification came from patients who had received combination chemotherapy. The myc family gene copy number of the DNA prepared from 9 tumor cell lines established from these 38 patients was similar to the myc family gene copy number in the DNA prepared from fresh tumor specimens from these same patients. myc family DNA amplification is present in 16% of small cell lung cancer patients’ tumors and the amplification pattern in the tumor cell lines is representative of the fresh tumors obtained from the same patients.

INTRODUCTION

DNA amplification and mRNA expression of the myc family of protooncogenes (c-myc, N-myc, and L-myc) has been identified in tumor cell lines and in tissues from small cell lung cancer patients by numerous investigators (1–9). DNA amplification of the myc family of protooncogenes has been associated with an altered clinical outcome in small cell lung cancer and other human cancers. c-myc DNA amplification in tumor cell lines established from chemotherapy treated small cell lung cancer patients has been associated with a shortened survival (6). In addition, N-myc DNA amplification in tumors from untreated childhood neuroblastoma patients has been linked with advanced stage disease and rapid tumor progression (10, 11). Yakota et al. (12) examined a wide variety of epithelial cancers and associated c-myc DNA amplification with advanced aggressive disease. In the present study, molecular genetic characterization of myc family DNA amplification in small cell lung cancer tumor specimens was undertaken in patients who have had systematic initial evaluations and complete clinical follow-up. Thus we are able to examine the relationship between the clinical outcomes and myc family DNA amplification in this adult malignancy.

Previous observations on the role of myc family DNA amplification in small cell lung cancer have been restricted primarily to analysis of tumor cell lines (1–9) and paraffin fixed small cell lung cancer tissue (13). There is a paucity of data about myc family protooncogene amplification in a series of fresh tumor specimens from small cell lung cancer patients or any other single type of adult malignancy. In addition, the degree of correlation between small cell lung cancer cell line amplification and fresh tumors has not been defined. We studied fresh tumors from small cell lung cancer patients who also had tumor cell lines established and their myc family amplification status studied (6). Therefore, we can define the relationship between protooncogene amplification in tumor cell lines and tumors from the same small cell lung cancer patients.

MATERIALS AND METHODS

The tumor specimens adequate for molecular genetic analysis were obtained from 38 different small cell lung cancer patients treated at the NCI-Navy Medical Oncology Branch from 1982 to 1987, 34 during necropsy examination and 4 during surgical biopsy. Normal tissue from the same patient was available for analysis from 34 of the 38 patients. The tumor and normal specimens were frozen at −70°C until analysis.

The normal and tumor tissues were finely minced and disrupted in isotonic buffer plus 0.4% sodium dodecyl sulfate detergent, and DNA was prepared by the method of Heiter et al. (14). Tumor cell lines established from 9 of the 38 patients were harvested during log phase growth, and DNA was prepared as previously described (6). The DNA was digested with the restriction endonucleases EcoRl, BamHI, or SstI, electrophoresed in 0.8% agarose gels, and transferred to nitrocellulose by the method of Southern (15). The filters were hybridized to a 32P radiolabeled Clal-EcoRl third exon c-myc fragment (c-myc probe) (16), an EcoRl-BamHI second exon N-myc fragment (N-myc probe) (17), or a Small-EcoRl third exon L-myc fragment (L-myc probe) (2). Small cell lung cancer cell lines previously shown to be amplified for c-myc, N-myc, and L-myc (N417, H720, and H378, respectively) were used as controls for amplified tumor cell lines (1, 2, 5). The variations in the amount of DNA loaded per lane were adjusted by standardizing the myc family gene signal to the signal made by either a 32P radiolabeled single copy PvuII-PvuII gastrin releasing peptide fragment (gastrin releasing peptide probe) (18) or a PstI-PvuII oxytocin fragment (oxytocin probe) (19).

DNA amplification was quantitated by serial dilution of DNA to obtain a hybridization signal equivalent to a single-copy signal from DNA prepared from normal tissue or a nonamplified small cell lung cancer cell line (e.g., a 20-fold amplification is indicated if a 1:20 dilution is necessary to achieve single-copy intensity). The presence of an amplified myc family signal was also confirmed by densitometric comparison of the signal from the tumor DNA versus the signal from the normal DNA standardized by the signal from the single copy gene control (gastrin releasing peptide or oxytocin probe). The myc family gene was considered to be amplified if the signal from the tumor DNA was at least 4-fold greater than the normal tissue DNA signal.

The clinical characteristics and course of the small cell lung cancer patients who had their tumor studied for myc family DNA amplification were reviewed. The initial stage, date of initial chemotherapy, response to therapy, date tumor was obtained for study, and the date of death were noted. If a tumor cell line was established at any time during the life of the 38 small cell lung cancer patients whose fresh tumor specimen had been studied, the date the tumor tissue was obtained for cell culture studies was identified.

Statistical methods for the analysis of the data included Fisher’s exact test for the analysis of the 2 × 2 contingency tables and use of the nonparametric Wilcoxon rank sum test for the analysis of continuous variables (20). For the analysis of survival data, the Kaplan-Meier method was used to examine the data graphically (21). The generalized Wilcoxon test of Gehan (22) was used to compare the survival times between patients with and without myc family DNA amplification. Survival time was measured from the data on study to the date of death. All P values are of the two-sided type. Finally, to calculate the proba-
probability that by chance alone myc family DNA amplification of a tumor cell line and a tumor from the same small cell lung cancer were the same, we assumed: (a) the observations were independent; and (b) the a priori probability of a myc match (c-myc, N-myc, L-myc, or no myc) between a tumor and the corresponding cell line was 0.25. The binomial distribution was then used to calculate the probability that all the pairs are matched by chance alone.

RESULTS

The tumors from small cell lung cancer patients analyzed for myc family DNA amplification were obtained at different times during the patients' treatment. Of the 38 tumor specimens, 32 were harvested during necropsy examinations of patients who died after administration of combination chemotherapy. Six other tumor samples were harvested prior to the patient's receiving combination chemotherapy (4 from surgical biopsies and 2 from postmortem examinations).

The DNA from 4 patients' tumors (patients 1, 2, 3, and 4) was amplified for the N-myc protooncogene while the normal tissue obtained from the same patients had a normal diploid copy number (Fig. 1). Dilutional studies of the N-myc signal from the tumor DNA prepared from patients 1–4 showed approximately 4-, 20-, 20-, and 10-fold greater intensity, respectively, compared to the signal from DNA prepared from normal tissue from the same patient. The tumor DNA of patients 5 and 6 had L-myc amplification while a diploid copy was present in the normal tissue from these patients (Fig. 2). The L-myc signal from the tumor DNA from patients 5 and 6 showed approximately 10-fold greater intensity compared to the signal from the DNA prepared from normal tissues from the same patients. The DNA from all 38 tumors had a signal intensity equivalent to that of normal tissue when hybridized to a radiolabeled c-myc fragment. All 6 tumor specimens with myc family DNA amplification came from patients who had been treated with combination chemotherapy.

There was no marked difference in clinical characteristics and course between patients whose tumors had myc family DNA amplification and those whose tumors did not. Compared to patients whose tumors did not have myc family DNA amplification, patients whose tumors had myc family DNA amplification presented with a similar proportion of women (2 of 6
were amplified for one of the myc family of genes (4 N-myc expressing abundant amounts of one of the myc family of protoon- and 2 L-myc). The incidence of myc family DNA amplification from 38 small cell lung cancer patients' tumors showed 6 (16%) patients. The probability of this event occurring by chance alone was markedly different from that seen in the other 32 patients whose tumors were not amplified (median, 11 months; range, 0–92; \( P = 0.63 \)) or from that observed in large series of chemotherapy treated small cell lung cancer patients (23–25).

Nine of these 38 patients (27%) had tumor cell lines established and have had their myc gene copy number previously reported (6). Six of the cell lines were established at the same time in the patient's treatment course as the tumor tissue was obtained; 3 at diagnosis prior to initiation of chemotherapy and 3 at the time of tumor progression following chemotherapy administration. The remaining 3 tumor cell lines were established prior to initiation of chemotherapy and the tumor specimens adequate for DNA analysis were obtained at relapse following chemotherapy administration. myc family DNA amplification was present in both the tumor cell lines and tumors from two patients. The tumor cell line H526 was established from the bone marrow of patient 2 prior to the initiation of combination chemotherapy while the tumor specimens were harvested at postmortem examination 4 months later after combination chemotherapy and chest irradiation. The cell line H689 was established from the pleural effusion of patient 3 after the patient had relapsed following administration of combination chemotherapy and the tumor was obtained 4 weeks later at his postmortem examination. N-myc DNA amplification was present in both the tumor cell lines and tumor specimens from these patients and to a similar degree (H526, 40-fold; patient 2, 20-fold; and H689, 40-fold, patient 3, 20-fold; Fig. 1). The other 7 cell lines established from patients whose tumors were studied in this series had diploid copy numbers for the myc family genes, in agreement with the findings in their respective tumors (Table 1). Therefore, the copy number for all three myc family genes was similar in all nine matched tumor cell lines and tumors from the same small cell lung cancer patients. The probability of this event occurring by chance alone is extraordinarily small \( (P < 0.001) \).

**DISCUSSION**

Small cell lung cancer cell lines are commonly amplified and express abundant amounts of one of the myc family of protooncogenes, c-myc, N-myc, and L-myc (1–9). This study of DNA from 38 small cell lung cancer patients' tumors showed 6 (16%) were amplified for one of the myc family of genes (4 N-myc and 2 L-myc). The incidence of myc family DNA amplification identified in small cell lung cancer patients' tumors in this study is similar to the incidence of myc family DNA amplification identified in other recent investigations of adult solid tumors. Wong et al. (13) studied DNA from paraffin embedded small cell lung cancer tissue and showed that 5 of 45 (11%) had greater than 3-fold amplification of c-myc (2 examples) or N-myc (3 examples). Yakota (12) demonstrated c-myc DNA amplification in 8 of 71 (11%) tumors from patients with a wide variety of epithelial neoplasms which did not include any small cell lung cancer tumors. In this study we have examined DNA prepared directly from small cell lung cancer tumors and have extended the analysis of myc family genes to include L-myc. DNA amplification of the myc family is still detected in a small minority (16%) of these patients' tumors. Although we observed an incidence (4 of 38) of N-myc DNA amplification in small cell lung cancer tumors similar to that of Wong et al. (3 of 45), we did not find any examples of c-myc amplification in the fresh small cell lung cancer tissue. It is possible that we could not detect the lower levels of c-myc amplification (5- and 7-fold) Wong et al. (13) detected using the slot blot technique. Alternatively, our patient cohort may not have had any c-myc DNA amplification in their tumors, or it may not have been large enough to rule out this low probability of occurrence with high statistical power \( (i.e., \text{in excess of } 80\%) \).

The copy number of the myc family genes in the amplified tumors is similar to that reported by other investigators studying N-myc and L-myc in small cell lung cancer cell lines and tumors. The 4-, 10-, 20-, and 20-fold DNA amplification of N-myc described in the 4 small cell lung cancer tumors in this study is similar to the 14-, 22-, and 33-fold amplification of N-myc in the small cell lung cancer tissue studied using slot blot techniques of DNA extracted from paraffin blocks. Although the N-myc copy number of the tumor cell lines H526 and H689 (40- and 40-fold, respectively) reported here is less than that previously reported by our group (115-fold for H526 and 170-fold for H689), it is consistent with the N-myc copy number for H689 depicted in the dilutional studies (5). The higher estimates previously reported are potentially explained by the alternative method of quantitation used by cutting out the appropriate \( ^{32}P \)-labeled N-myc bands from the Southern hybridization blot and counting the bands in a liquid scintillation counter (5). The degree of L-myc DNA amplification reported here (patient 5, 10-fold; and patient 6, 10-fold) is similar to the single case (patient 5) previously reported by our group (2). Although we can compare only 4 N-myc DNA amplified tumors from these small cell lung cancer patients, the N-myc DNA amplification pattern was not obviously similar to the bimodal pattern described in childhood neuroblastoma where tumor DNA appears to be amplified either 3–10-fold or 100–300-fold (10, 11).

myc family DNA amplification had been shown previously to be associated with a poor clinical outcome. Studies of c-myc DNA amplification in tumor cell lines established from treated small cell lung cancer patients and N-myc DNA amplification in tumors obtained from untreated patients with childhood neuroblastoma have associated shortened survival with myc family DNA amplification (6, 11). In addition Yakota et al. (12) associated c-myc DNA amplification with aggressive disseminated primary tumors. This study of myc family DNA amplification in 38 tumor specimens obtained from small cell lung cancer patients showed that the 6 patients whose tumors had N-myc or L-myc DNA amplification did not have a remarkably different clinical course than patients whose tumors did not. One must be careful in interpreting the significance of

| myc family oncogene amplification of tumor cell lines and tumors from the same small cell lung cancer patients |
|-------------------------------------------------|---------------------------------|
| myc family gene | Cell line | Tumor |
| c-myc | 0 | 0 |
| N-myc | 2 | 2 |
| L-myc | 0 | 0 |
| No myc family | 7 | 7 |
| DNA amplification | Total 9 | 9 |

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DNA amplification of the myc family genes in small cell lung cancer because the survival of these patients is short (less than 12 months). This shortened survival makes it difficult to identify a biological variable which may actually be important in the genesis and progression of small cell lung cancer because it does not obviously change the observed short survival. In agreement with the previous observation that myc family DNA amplification is more prevalent in tumor cell lines established from chemotherapy treated small cell lung cancer patients' tumors (6), the present study showed myc family DNA amplification was observed solely in tumor specimens obtained from chemotherapy treated small cell lung cancer patients although we studied only six tumors from untreated patients.

In addition, we have demonstrated the myc family DNA copy number was similar in 9 tumor cell lines and tumor specimens from the same patients. Because of the potential growth advantage demonstrated by higher cloning efficiencies and shorter doubling times of c-myc amplified small lung cancer cell lines in cell culture (4, 26), some degree of selection for amplification in vitro may occur. However, we have not encountered this in the tumor cell lines and tumors studied thus far.

Thus, our ability to study DNA samples obtained from small cell lung cancer patients' cell lines and tumor specimens for myc family amplification allows certain observations to be made. myc family DNA amplification is present in 16% of the patients studied and was observed only in patients who had received combination chemotherapy. The myc family DNA amplification pattern is identical in 9 tumor specimens and 9 tumor cell lines obtained from the same small cell lung cancer patients suggesting that the DNA amplification of the myc family of protooncogenes is not exclusively an artifact of cell culture.

REFERENCES

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