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Human small-cell lung cancers show amplification and expression of the N-*myc* gene

(c-*myc* protooncogene/neuroendocrine/gene amplification)

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ABSTRACT **We have found that 6 of 31 independently derived human small-cell lung cancer (SCLC) cell lines have 5- to 170-fold amplified N-*myc* gene sequences. The amplification is seen with probes from two separate exons of N-*myc*, which are homologous to either the second or the third exon of the c-*myc* gene. Amplified N-*myc* sequences were found in a tumor cell line started prior to chemotherapy, in SCLC tumor samples harvested directly from tumor metastases at autopsy, and from a resected primary lung cancer. Several N-*myc*-amplified tumor cell lines also exhibited N-*myc* hybridizing fragments not in the germ-line position. In one patient's tumor, an additional amplified N-myc DNA fragment was observed and this fragment was heterogenously distributed in liver metastases. In contrast to SCLC with neuroendocrine properties, no non-small-cell lung cancer lines examined were found to have N-*myc* amplification. Fragments encoding two N-*myc* exons also detect increased amounts of a 3.1-kilobase N-myc mRNA in N-*myc*-amplified SCLC lines and in one cell line that does not show N-*myc* gene amplification. Both DNA and RNA hybridization experiments show that in any one SCLC cell line, only one *myc*-related gene is amplified and expressed. We conclude that N-*myc* amplification is both common and potentially significant in the tumorigenesis or tumor progression of SCLC.**

Gene amplification represents an important adaptive mechanism allowing selective increased expression of genes whose products are needed by the cell (1, 2). Thus, the finding of amplification and expression of protooncogenes in tumor cells suggests an important role for the protooncogene product in the cellular and clinical biology of these tumors (3–9). We reported previously that eight independent human small-cell lung cancer (SCLC) lines have stable amplification and increased expression or deregulated expression alone of the c-*myc* gene (6, 10). Recent studies have shown increased expression with or without amplification of a c-*myc*-related gene, N-*myc*, in tumors of neural origin, including neuroblastoma and retinoblastoma (7–9). In this paper, we show that six independently derived SCLC lines that express neuroendocrine properties (10–12) also have amplified N-*myc* sequences and increased levels of N-*myc* expression compared to non-N-*myc*-amplified cell lines. In addition, tumor tissues harvested directly from three of these SCLC patients, including one from an untreated primary tumor, also show amplification of the N-*myc* gene. These results demonstrate that either c-*myc* or N-*myc* has been found amplified in 14 of 31 different SCLC lines. This suggests that expression with or without amplification of a *myc*-related gene may play a prominent role in the biology of SCLC.

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MATERIALS AND METHODS

Cloned DNA Fragments. Specific cloned DNA fragments purified from vector sequences were used as probes and are described in the various figure legends. The human c-*myc* plasmids were a gift of Philip Leder and were both obtained from a 12.7-kilobase (kb) *Eco*RI fragment isolated and cloned from human lymphocyte DNA (13). The plasmid pNb-1 containing a human N-*myc* fragment isolated from a human neuroblastoma cell line (7) was provided by Manfred Schwab and J. Michael Bishop. A plasmid containing a human oxytocin (*OT*) gene fragment was derived as described (14).

Additional specific N-*myc* fragments were prepared in our laboratory from a genomic library bacteriophage clone, Ch N-*myc* 4. The clone was isolated from a partial *Mbo* I human placenta library in Ch28 (provided by Philip Leder) (15) using Nb-1 as a probe. Specific fragments in the genomic clone that show homology to the second c-*myc* exon or Nb-1 fragment (1.0-kb *Eco*RI/*Bam*HI placental fragment) and the third c-*myc* exon (2.2-kb *Bgl* II/*Eco*RI placental fragment) were identified by hybridization. Fragments containing these genes were subcloned into pJB327 (14) (see Fig. 3 schematic map).

Cell Lines and Tumor Specimens. The lung cancer cell lines established, grown, and characterized in our laboratory (10, 11) include the following National Cancer Institute (NCI) SCLC lines: H69, H82, H187, H209, H249, H372, H378, N417, H446, H510, H526, N592, and H689; and NCI non-small-cell lung cancer (NSCLC) lines H125 and H157. The human tumor specimens were obtained as part of National Institutes of Health approved clinical research protocols. The neuroblastoma cell line SMS-KCNR (16, 17) was kindly provided by Patrick Reynolds.

DNA and RNA Blot Hybridization. DNA from cell lines was prepared from lysed cells (18) or from guanidine thiocyanate/cesium chloride gradients (19) and were processed as described (18). Solid tissues were first pulverized in liquid nitrogen in a blender before DNA extraction (20). DNA (12 μg) was digested with *Eco*RI, electrophoresed on a 0.8% agarose gel, denatured, and transferred to nitrocellulose essentially as described by Southern (21). Hybridization was performed with 10% dextran sulfate and 40% formamide (22) with ^{32}P-labeled restriction fragment probe, as indicated in various figures, and washed at 52°C in 0.1× SSC (1× SSC = 0.15 M NaCl/0.015 M Na citrate)/0.1% $NaDodSO_4$ as described (23). The degree of N-*myc* amplification was determined by cutting out the appropriate ^{32}P-labeled N-*myc* bands from the Southern hybridization blot seen in Fig. 1*b* and by liquid scintillation counting. Copy number estimates

Abbreviations: SCLC, small-cell lung cancer; NSCLC, non-small-cell lung cancer; kb, kilobase(s); dhfr, dihydrofolate reductase; OT, oxytocin.

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were also determined from serial dilutions of N-*myc*-amplified DNA and these bands were also cut out and counted (Fig. 1*b*). Both approaches yielded similar values.

Total cellular RNA was prepared by guanidine thiocyanate/cesium chloride gradient centrifugation (19) and then poly(A)$^+$ RNA was selected (24). Poly(A)$^+$ RNA (2.5 or 5.0 μg) was denatured and electrophoresed on a 1% or 1.4% agarose formaldehyde gel according to Lehrach *et al.* (25), modified by using 0.22 M formaldehyde in the gel, at 160 V for 4–6 hr. Transfer was as described (26), hybridization was carried out at 42°C in 10% dextran sulfate/50% formamide/4× SSC, and washing was at 65°C in 0.1× SSC/0.1% $NaDodSO_4$.

RESULTS

Detection of a c-myc-Related DNA in SCLC. While searching for c-*myc* amplification in SCLC, we also observed that several SCLC lines contained a faint 2.0-kb *Eco*RI fragment that hybridized to a human second exon c-*myc* probe (Fig. 1*a*). Concurrently, workers in two laboratories reported amplification of a 2.0-kb *Eco*RI c-*myc*-related sequence in human neuroblastomas (7, 27). This sequence, named N-*myc*, was shown to contain limited homology to the second exon of c-*myc* (7). Its human germ-line location is on chromosome 2 (27, 28), a different location from the germ-line c-*myc* gene on chromosome 8 (23). We speculated that this 2.0-kb *Eco*RI fragment cross-hybridizing with human c-*myc* might represent N-*myc* gene amplification in SCLC DNA.

Several SCLC Cell Lines Contain Amplified N-myc DNA Sequences. To confirm this, a human N-*myc* probe (Nb-1) (7) was used in Southern blotting experiments against *Eco*RI-digested genomic DNA from SCLC and NSCLC cell lines. Five SCLC lines (H69, H249, H372, H526, H689) were found to be markedly amplified for the 2.0-kb N-myc DNA when compared to other lung cancer cell line DNAs (Fig. 1*b*). The degree of N-*myc* amplification was determined and we found the following: H69 amplified 85-fold; H249 amplified 150-fold; H372 amplified 35-fold; H526 amplified 115-fold (5.5-kb band) and 135-fold (2.0-kb band); and H689 amplified 170-fold. One DNA from cell line H526 showed equal amplification of another 5.5-kb N-*myc*-hybridizing *Eco*RI fragment. This amplified fragment could be the result of an N-*myc* rearrangement, the loss of an *Eco*RI restriction site during amplification, amplification of an N-*myc* polymorphism, or the presence of a new *myc* gene related to N-*myc*. While most of the cell lines derived from tumor specimens of patients who relapsed after chemotherapy, H526 was started from a tumor sample biopsy prior to chemotherapy (11). None of the SCLC lines with N-*myc* amplification had c-*myc* amplification using the second exon c-*myc* probe (see Fig. 1*a* for examples). Likewise, no SCLC cell lines previously shown to be amplified for the c-*myc* gene (H60, H82, H211, H360, N390, N417, H446, and H524) (6, 10) are amplified for the N-*myc* gene. Of interest, just as the second exon c-*myc* probe containing the region homologous to Nb-1 detects amplified N-*myc* sequences (H249, H526, and H689; Fig. 1*a*), the Nb-1 probe containing this region detects the c-*myc* sequence when it is amplified in genomic DNA (H446; Fig. 1*b*). Occasionally, other faint hybridizing bands of unknown origin were seen with the N-*myc* probe (e.g., the 5.0-kb fragment in H249; Fig. 1*b*).

N-myc DNA Amplification Is Found in Tumor Tissue but Not in Normal Tissue. Tumor biopsies of two patients had generated tumor cell lines H526 and H689 with N-myc DNA amplification. Both tumor and normal tissue obtained at the time of autopsy were examined (Fig. 2 *b* and *c*). Analysis of *Eco*RI-digested DNAs prepared from the cell lines and autopsy tissues of these two patients showed in both cases N-*myc* amplification in the DNA of tumor tissue but not in DNA from normal tissue (Fig. 2 *b* and *c*). The degree of amplification of the 2.0-kb *Eco*RI N-*myc* hybridizing fragments in the autopsy tissue was comparable (by quantitation) to that found in the cell line DNA. These results clearly indicate that the amplification of N-*myc* in SCLC can occur in the patient. In addition, they show that normal tissues in these patients are not amplified for N-myc DNA.

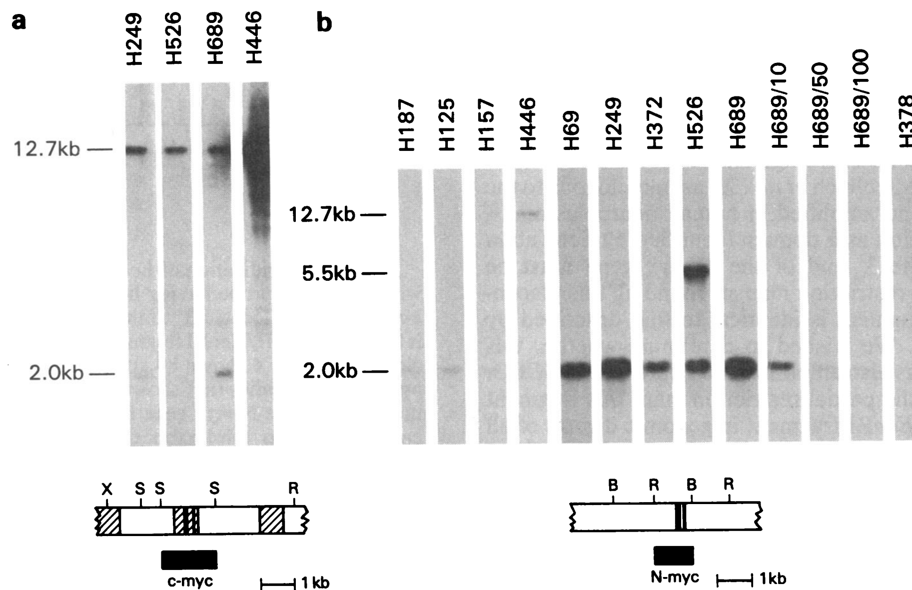

FIG. 1. Hybridization comparison of *Eco*RI digests of representative SCLC and NSCLC cell line DNAs with human c-*myc* (*a*) and N-*myc* (*b*) probes. The human c-*myc* probe is a second-exon 1.6-kb *Sst* I fragment (13) and N-*myc* is a 1.0-kb *Eco*RI/*Bam*HI fragment obtained from clone pNb-1 (7). Hatched blocks indicate exons of the c-*myc* gene; heavy vertical lines indicate regions of homology between c-*myc* and N-*myc* DNA sequences (7); solid blocks show specific probe used for hybridization. B, *Bam*HI site; R, *Eco*RI site; S, *Sst* I site; X, *Xho* I site. Both probes are shown in the 5′ to 3′ orientation. The 12.7-kb position represents the *Eco*RI germ-line c-*myc* gene fragment (23) and the 2.0-kb position is an *Eco*RI germ-line N-*myc* fragment (7). H125 and H157 are NSCLC lines; all other lines are SCLC (10–12). In *b*, 689/10, 689/50, and 689/100 represent 1:10, 1:50, and 1:100 dilutions, respectively, of H689 DNA.

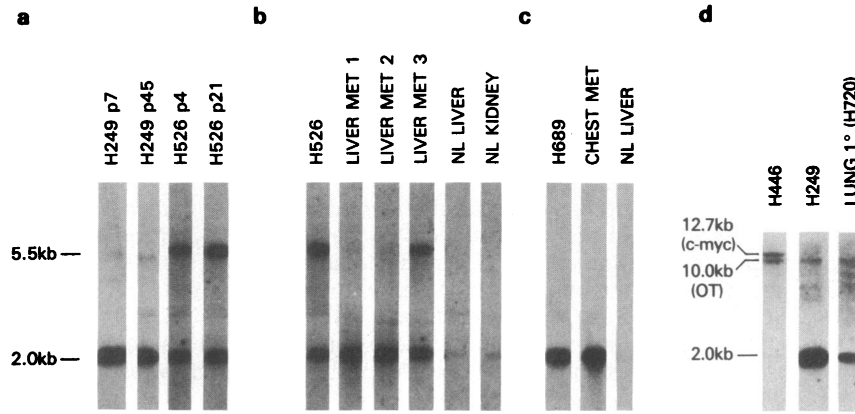

FIG. 2. Hybridization of *Eco*RI-digested SCLC cell line DNAs (H249, passages 7 and 45; H526, passages 4 and 21) (*a*); patient H526 (*b*); and patient H689 (*c*) DNAs from cell line, as well as tumor and normal tissue samples harvested directly from the patients at autopsy. (*d*) SCLC DNAs (c-*myc*-amplified cell line H446; N-*myc*-amplified cell line H249) and primary tumor (H720) to the 1.0-kb *Eco*RI/*Bam*HI N-*myc* probe, Nb-1 (7). p, Passage number; NL, normal tissue; MET, metastatic tissue. In *d*, in addition to the N-*myc* probe, a human *OT* third-exon 0.8-kb *Pst* I/*Pvu* II DNA probe (*OT*) used as a reference single-copy gene is shown (14). The *Eco*RI germ-line position of OT is 10 kb.

In patient H526, it is interesting to note that the 5.5-kb *Eco*RI amplified N-*myc*-related species found in the cell line (established from a bone marrow tumor biopsy sample) was present in only one of the liver metastases, while all tumor tissues from this patient contained the 2.0-kb *Eco*RI-amplified N-*myc* fragment typically seen. On long exposures, no 5.5-kb fragment was seen in normal tissue DNA from this patient, arguing against the idea that this fragment is an inherited restriction fragment length polymorphism of the N-*myc* gene.

We also tested primary lung tumor tissue from an untreated SCLC patient from which cell line H720 has been established (Fig. 2*d*). As a single copy gene control in these experiments, the human oxytocin gene (*OT*) was included in the hybridization reaction (24). Southern blot analyses demonstrate that the 2.0-kb *Eco*RI N-*myc* fragment is amplified (5-fold) in the primary lung tumor tissue. In H720, we also noted additional fragments (Fig. 2*d*). DNA prepared from the cell line H720 also reveals N-myc DNA amplification (data not shown). This finding shows that N-*myc* amplification can take place in the patient prior to metastasis.

An Amplified N-*myc* Domain Related to the Third Exon of the c-*myc* Gene Is Present in SCLC. To extend our N-*myc* amplification studies, we used the Nb-1 N-*myc* fragment as a probe and isolated a human placenta genomic DNA bacteriophage clone (Ch N-*myc* 4). (See *Materials and Methods* and Fig. 3.) A region homologous to the third c-*myc* exon in Ch N-*myc* 4 was identified in a 2.2-kb *Bgl* II/*Eco*RI fragment located ≈2.8 kb 3′ to the fragment equivalent to Nb-1 (Fig. 3, solid bar). This fragment lies in the same region reported earlier by Michitsch *et al.* (30) as homologous to the third c-*myc* exon and amplified in human neuroblastomas, and in the same region as a domain identified by Schwab *et al.* within which the 3′ end of the N-*myc* gene must be contained (31). The restriction map surrounding this placental *Bgl* II/*Eco*RI fragment is identical to that described by Schwab *et al.* (31). We wished to determine whether this region 3′ to Nb-1 was also amplified and expressed in SCLC. As predicted from the partial restriction map, this fragment did detect a 4.2-kb *Eco*RI fragment in genomic digests of all SCLC cell line DNAs (Fig. 3). This fragment is only amplified in those SCLC DNAs that contain the 2.0-kb *Eco*RI-amplified N-*myc* sequence. Note that cell line H187 (which we later show expresses N-*myc*), H446 (amplified for c-*myc*) (6), and H510 (amplified for L-*myc*) (29) do not show N-*myc* amplification.

N-myc DNA Amplification Is Stable Over Time in Cell Culture. Loss of amplified sequences [e.g., the dihydrofolate reductase (*dhfr*) gene] can occur when selection pressure is released (1, 2). We have previously reported that SCLC tumor line H249 lost amplified *dhfr* gene sequences when grown in cell culture in the absence of methotrexate, which had apparently selected for the amplified *dhfr* sequences in the patient (32). However, no apparent change in the amount of N-*myc* gene amplification was noted in cell lines H249 and H526 (Fig. 2*a*) after continuous growth in culture for >30 and >15 weeks (≈70 and ≈50 population doublings), respectively.

We were also able to compare the stability of N-*myc* amplification in the same tumor specimen passaged directly from the patient into tissue culture (H249) or first injected into a nude mouse to form a xenograft (N592). We found that

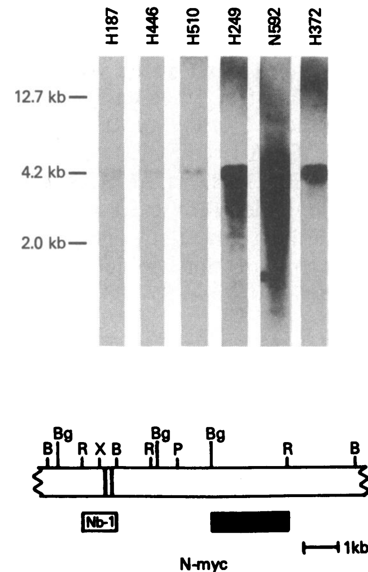

FIG. 3. Hybridization of *Eco*RI digests of SCLC cell line DNAs with an N-*myc* probe having homology with the third exon of the c-*myc* gene and located 3′ to the Nb-1 region of N-*myc*. The probe is the 2.2-kb *Bgl* II/*Eco*RI fragment of human genomic bacteriophage clone Ch N-*myc* 4 shown by solid bar in the schematic diagram. In hybridization studies (not shown), this fragment is known to contain the region of the N-*myc* gene homologous to the third exon of the c-*myc* gene. No third-exon c-*myc* hybridization is detected on fragments 5′ or 3′ of this *Bgl* II/*Eco*RI fragment. The open bar labeled Nb-1 indicates the region corresponding to Nb-1 in phage clone Ch N-*myc* 4. Nb-1 shares homology with the second exon of human c-*myc*. B, *Bam*HI site; Bg, *Bgl* II site; R, *Eco*RI site; X, *Xho* I site; P, *Pvu* II site. The 12.7-kb position represents the *Eco*RI germ-line c-*myc* fragment; the 2.0-kb position represents the *Eco*RI germ-line N-*myc* fragment detected by Nb-1; and the 4.2-kb position represents the observed *Eco*RI fragment identified by the 2.2-kb *Bgl* II/*Eco*RI fragment corresponding to the size predicted by the restriction map of the cloned N-*myc* gene. H446 is known to be amplified for c-*myc* (10), H510 is amplified for L-*myc* (29), and H187 is amplified for neither c-, N-, nor L-*myc* (29). H249 cell culture line and N592 tumor xenograft line were derived from the same tumor sample (11).

N-*myc* was amplified in both H249 and N592 but to an even greater degree in the N592 xenograft line than in the H249 cultured cell line (Fig. 3). We were also able to show that seven independent clones, four derived from H249 and three from H526, all contained the same level of amplified N-*myc* sequences (data not shown). All H249 clones were found to contain approximately equal amounts of the 2.0-kb *Eco*RI fragment, while H526 clones had equal amounts of both 5.5- and 2.0-kb *Eco*RI N-*myc* hybridizing fragments. This suggests that most clonogenic cells in these two lines contain stably amplified N-*myc* sequences and, in H526, that both amplified fragments are present in the same cell.

Detection of N-*myc* Transcripts in N-myc DNA-Amplified SCLC Cell Lines. N-*myc* gene expression was examined in SCLC cell lines and in one neuroblastoma cell line known to express N-*myc* (17) using as probes both Nb-1 (with homology to the second exon of c-*myc*; Fig. 4*a*) and the 3′ *Bgl* II/*Eco*RI placental N-*myc* fragment (having homology to the third exon of c-*myc*; Fig. 4*b*). The neuroblastoma cell line (KCNR) and SCLC cell lines with N-*myc* amplification (H69, H249, H592, and H526) contain a 3.1-kb N-*myc* transcript. The 3.1-kb size of the predominant N-*myc* transcript was determined by using both human (28S and 18S) and *Escherichia coli* (23S and 16S) ribosomal RNAs as standards. This value coincides with that observed for neuroblastoma transcripts (3.2 kb) by Kohl *et al.* (27), but it differs from the 4.0-kb size reported by Schwab *et al.* (33). Several larger transcripts in those lines expressing N-*myc* are seen, including one at 4.7 kb, which probably represent N-*myc* precursor RNAs (33). It is of interest to note that both the Nb-1 probe (Fig. 4*a*) and the 3′ *Bgl* II/*Eco*RI placental N-*myc* fragment probe yield identical results, confirming that this 3′ N-*myc* fragment does contain an exon of the N-*myc* gene. The independently maintained nude mouse heterotransplant cell line N592 started from the same original tumor, as H249 shows more N-*myc* expression than H249 mirroring the DNA amplification results. One SCLC cell line (H187), not amplified for the N-*myc* gene sequence, also expresses a 3.1-kb N-*myc* RNA transcript. This recalls earlier observations that some neuroblastoma (33) and retinoblastoma (9) cell lines, not amplified for N-*myc*, express detectable N-*myc* transcripts. In addition, H526 RNA also contains a 1.8-kb N-*myc* hybridizing transcript (Fig. 4*a*), which could result from transcription of the 5.5-kb *Eco*RI N-*myc* hybridizing DNA fragment (see Fig. 1*b*). In marked contrast, no N-*myc* expression was observed in a NSCLC cell line (H125), two cell lines amplified and expressing the c-*myc* gene (H82 and N417), and three SCLC cell lines with expression of the newly described L-*myc* gene (H209, H378, and H510) (29).

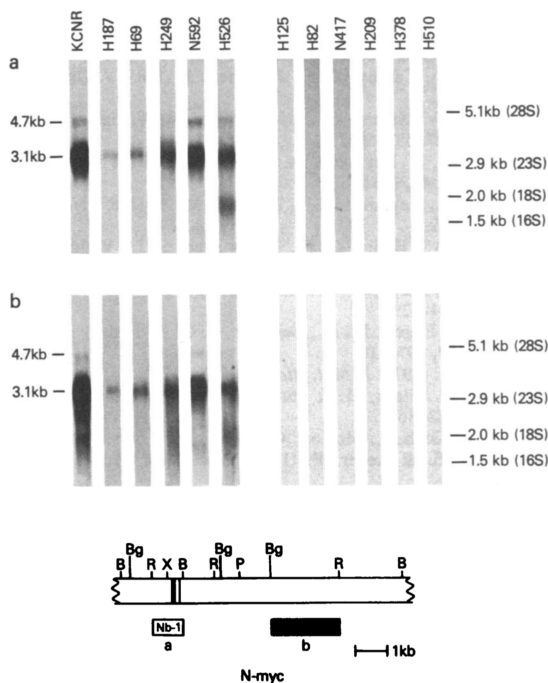

FIG. 4. Hybridization of poly(A)$^+$ cell line RNAs to (*a*) the 1.0-kb *Eco*RI/*Bam*HI N-*myc* probe (Nb-1) (7) and (*b*) the 2.2-kb *Bgl* II/*Eco*RI 3′ placental N-*myc* probe. Open bar labeled Nb-1 indicates probe used in *a*; solid bar indicates probe used in *b*; heavy vertical lines show regions of homology between c-*myc* and N-*myc* DNA sequences (7). B, *Bam*HI site; Bg, *Bgl* II site; R, *Eco*RI site; X, *Xho* I site; P, *Pvu* II site. The 3.1- and 4.7-kb positions indicate N-*myc*-specific transcripts determined using human (28S and 18S) and *E. coli* (23S and 16S) ribosomal RNAs as markers. The six lanes on the right side of both *a* and *b* show no detectable N-*myc* hybridization (lines H125, H82, N417, H209, H378, H510), contained 5 μg of poly(A)$^+$ RNA, and were exposed three times as long (72 hr). The N-*myc*-expressing cell lines (*Left*) (KCNR, H187, H69, H249, N592, H526) contained 2.5 μg of poly(A)$^+$ RNA and were exposed 24 hr.

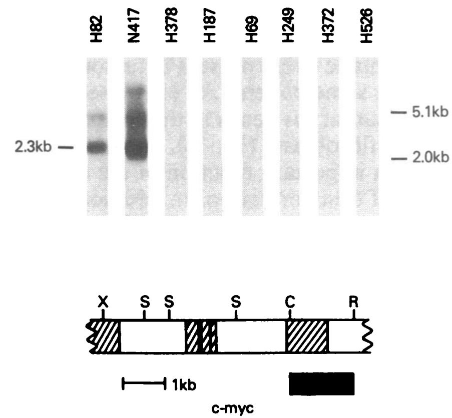

FIG. 5. Hybridization of 2 μg of poly(A)$^+$ cell line RNAs to the 1.5-kb *Cla* I/*Eco*RI c-*myc* probe containing the third c-*myc* exon (13). Hatched blocks indicate exons of the c-*myc* gene; heavy vertical lines indicate regions of homology between c-*myc* and N-*myc* DNA sequences (7); solid block shows specific probe used for hybridization. X, *Xho* I site; S, *Sst* I site; C, *Cla* I site; R, *Eco*RI site. The 2.3-kb position indicates size of mature c-*myc* mRNA while 5.1 and 2.0 kb indicate positions of 28S and 18S human ribosomal RNAs used as size markers.

c-*myc* Genes Are Not Coexpressed in N-*myc*-Expressing SCLC Cell Lines. Fig. 4 shows that c-*myc*-expressing cell lines (H82, N417) do not express N-myc mRNA. We then examined the SCLC cell lines that expressed increased amounts of N-*myc* for c-*myc* expression by using a third exon c-*myc* probe (Fig. 5) No evidence of c-*myc* transcription was seen in the N-*myc*-amplified and -expressing cell lines (H69, H249, H372, and H526), while SCLC cell lines known to be amplified and expressing the c-*myc* gene sequence had abundant c-myc mRNA (H82 and N417). In the SCLC cell lines examined to date, we have found no cell lines to have the c-*myc*, N-*myc*, or L-*myc* genes simultaneously amplified or expressed.

DISCUSSION

N-*myc* amplification and/or expression is observed thus far in three human tumors, all of which have neural or neuroendocrine properties: neuroblastoma (7, 8), retinoblastoma (9), and, as reported here, SCLC. Several observations implicate the N-*myc* gene in the development and/or progression of tumor cells committed to this differentiation pathway. The presence of N-*myc* amplification in human

neuroblastoma is associated with a more advanced clinical stage (4). In neuroblastoma (7, 8, 30) and SCLC, the amplification is a somatic event present in tumor but not in normal tissue from the same patient. An expressed N-*myc* gene can complement mutant *ras* genes in the tumorigenic conversion of rat embryo fibroblasts (31, 34), suggesting that this gene can provide an important function in the establishment and maintenance of transformation. In addition, we have shown here that: (*i*) amplification of the N-*myc* gene can occur in both primary and metastatic lung tumors; (*ii*) amplification is maintained stably over many passages in cell culture, while amplified genes for *DHFR* in the same tumor line were lost (32); and (*iii*) when passaged as a nude mouse xenograft, the amplification and expression of N-*myc* is retained and may increase. All of these facts provide circumstantial evidence for an important role for N-*myc* in the growth of these human tumors.

Previously, we have shown that every example of c-*myc* amplification in SCLC occurred in a morphologic and biochemical variant subclass of SCLC, which is characterized by a distinct large cell morphology, shorter doubling time, and higher cloning efficiency *in vitro*, as well as greatly reduced or absent expression of the neuroendocrine enzyme marker, L-dopa decarboxylase (10, 11). In contrast, N-*myc* amplification is found in both classic (H69, H249) and variant (H372, H526) types of SCLC. The doubling times (36–126 hr) and agarose colony-forming efficiencies (0.6–12%) measured *in vitro* of the N-*myc*-amplified lines overlap the values observed in SCLC with or without c-*myc* amplification (10, 11). Thus, further studies are needed to define the role of the N-*myc* gene in regulating the growth and differentiation of these cells.

We have found a SCLC cell line (H187) that expresses N-myc mRNA without amplification, a feature also seen in some neuroblastomas and retinoblastomas (7, 9). The association of N-*myc* gene expression with neural and neuroendocrine tumors might reflect a specific requirement for the N-*myc* gene product during the normal embryologic development of these cell types. In this regard, *in situ* hybridization studies show increased N-*myc* expression in the undifferentiated neuroblast component of neuroblastomas (31), while induction of neuroblastomas to differentiate using retinoic acid is associated with decreased levels of N-*myc* expression (17).

In addition to expression during normal development, the c-*myc* gene expression is altered by translocation (23), amplification, and probably other as yet unexplained mechanisms. Similarly, while N-*myc* amplification was associated with increased N-*myc* expression, other mechanisms of N-*myc* gene activation must also be considered. One N-*myc*-amplified SCLC (H526) exhibits an amplified N-*myc* restriction fragment heterogeneously distributed in the patient's tumor metastases, while additional N-*myc* hybridizing fragments were seen in other lines (e.g., H249, H720). These hybridizing restriction fragments could represent rearranged N-*myc* genes or amplification of an N-*myc*-related gene.

Recently, we have identified another *myc* family member (which we have called L-*myc*, since it was first detected in a lung cancer) in human SCLC (29). L-*myc* also shares homology with both N-*myc* and the second exon of the c-*myc* gene. Using an L-*myc*-specific probe, five SCLCs with L-*myc* amplification have been found (29). None of these SCLC lines expresses or is amplified for the N-*myc* gene (H378 and H510; Figs. 1, 3, and 4) or the c-*myc* gene (H378; Fig. 5). The results presented here, combined with our prior studies, give a total of 21/31 SCLC lines amplified and/or expressing one of the *myc* family of genes (nine c-*myc*, seven N-*myc*, and five L-*myc*). In SCLC, we have seen expression of only one of these *myc* family members in any individual tumor cell line. These results raise the possibility that deregulation of a *myc*-like gene is a common and perhaps necessary occurrence in some SCLC tumors.

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