Changes in the Phenotype of Human Small Cell Lung Cancer Cell Lines after Transfection and Expression of the c-*myc* Proto-oncogene

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Abstract

Small cell lung cancer growing in cell culture possesses biologic properties that allow classification into two categories: classic and variant. Compared with classic small cell lung cancer cell lines, variant lines have altered large cell morphology, shorter doubling times, higher cloning efficiencies in soft agarose, and very low levels of L dopa decarboxylase production and bombesin-like immunoreactivity. C-*myc* is amplified and expressed in some small cell lung cancer cell lines and all c-*myc* amplified lines studied to date display the variant phenotype. To investigate if c-*myc* amplification and expression is responsible for the variant phenotype, a normal human c-*myc* gene was transfected into a cloned classic small cell lung cancer cell line not amplified for or expressing detectable c-*myc* messenger RNA (mRNA). Clones were isolated with one to six copies of c-*myc* stably integrated into DNA that expressed c-*myc* mRNA. In addition, one clone with an integrated neo gene but a deleted c-*myc* gene was isolated and in this case c-*myc* was not expressed. C-*myc* expression in transfected clones was associated with altered large cell morphology, a shorter doubling time, and increased cloning efficiency, but no difference in L dopa decarboxylase levels and bombesin-like immunoreactivity. We conclude increased c-*myc* expression observed here in transfected clones correlates with some of the phenotypic properties distinguishing c-*myc* amplified variants from unamplified classic small cell lung cancer lines.

Introduction

Some previously identified biologic activities of the c-*myc* gene in mammalian cells include the ability to immortalize rat fibroblast cells (1) and to cooperate with *ras*-like oncogenes to induce tumorigenic conversion of primary rat embryo fibroblast cells (2). In addition, c-*myc* gene expression appears to stimulate transfected rat fibroblasts to form tumors in nude mice and syngeneic rats (3) and is associated with breast cancer in transgenic mice (4). Further, c-*myc* expression is induced by a wide variety of mitogens including serum (5), platelet derived growth factor, fibroblast growth factor (FGF), epidermal growth factor (EGF) (6–8), and partial hepatectomy (9, 10). Increased c-*myc* expression in transfected cell lines is also associated with a higher cloning efficiency in soft agar and a more rapid growth rate of rat fibroblast cells in low serum conditions (1, 3).

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Small cell lung cancer (SCLC)[1] cells offer another opportunity for studying the biologic role of c-*myc*. The variant subset of SCLC cell lines has a four- to 60-fold DNA amplification of c-*myc* and shows high expression of this gene (11, 12). In addition, a large amount of immunoreactive c-*myc* protein has been demonstrated in a c-*myc* amplified variant SCLC cell line, N417 (13). Compared with the more common classic SCLC lines, which do not express c-*myc*, variant cell lines have altered morphology in cell culture (growth in loose chains), altered athymic nude mouse xenotransplant histology (large cells with abundant cytoplasm and prominent nucleoli), and different biochemical properties from classic SCLC. The variants produce low or undetectable levels of L dopa decarboxylase, a marker of neuroendocrine differentiation. They also produce low or undetectable levels of bombesin-like immunoreactivity (gastrin releasing peptide), a putative autocrine growth factor in small cell lung cancer (14). The SCLC variants also have a more rapid doubling time, a 10-fold higher soft agarose cloning efficiency, and are less radiosensitive (15–17). Thus, the two types of cell lines derived from different patients with the same malignancy have easily evaluable phenotypic differences and the more aggressive variant phenotype is closely associated with c-*myc* amplification and expression.

Although a close association with the more aggressive variant phenotype of SCLC cell lines has been demonstrated, the causal role for c-*myc* in generating the variant phenotypic has not been proven. The recent development of electroporation (18–20) for introducing DNA into cells growing in suspension has allowed us to introduce a normal human c-*myc* gene covalently linked to the neomycin resistance gene, aminoglycoside 3′ phosphotransferase (*neo*), into a classic SCLC cell line. Transformants expressing the transfected DNA can then be selected using the aminoglycoside antibiotic G-418. Thus, we are able to ask if c-*myc* could be expressed and affect the phenotype of a classic SCLC cell line and compare the transformants to amplified variant SCLC cell lines.

Methods

Cell lines and cell culture. Three SCLC cell lines, H209, H146, and N417, representing cell lines with different levels of c-*myc* mRNA expression, were used as controls and have been characterized as previously described (11, 15, 16). H209 is a classic SCLC cell line that does not express detectable c-*myc* mRNA. H146 has properties intermediate between a classic and a variant and expresses c-*myc* mRNA at levels intermediate between H209 (classic) and N417 (variant). N417 is variant SCLC cell line amplified 50-fold for c-*myc* and expressing abundant

1. *Abbreviations used in this paper:* FCS, fetal calf serum; kb, kilobase; SCLC, small cell lung cancer.

amounts of c-*myc* mRNA. The cell line H209 was cloned in soft agarose and one of the clones, DD1, was used for the electroporation. The cells were grown under standard conditions (15, 16).

Electroporation. A c-*myc-neo* plasmid was constructed by inserting a 12.7 Eco RI human c-*myc* gene cloned from the leukocytes of a normal volunteer into the Eco RI site of pBR 327. In addition, a 3.4-kilobase (kb) Bam HI *neo* gene was constructed by ligating Bam HI linkers to the Pvu II site of the Pvu II-Bam HI fragment containing the SV 40 promoter and neomycin resistance gene from pSV2 *neo* plasmid. This Bam HI fragment was inserted into the Bam HI site of the pBR 327-*myc* plasmid. This plasmid was linearized with Pvu I and electroporated with 5×10^6 cells from cloned cell line H209 DD1 at 2 KV at 0°C (18). After electroporation, the cells were resuspended at 1×10^6 cells/ml in 10% fetal calf serum (FCS)–RPMI 1640 for 1 wk, and were then selected at 800 μg/ml of G-418 (real concentration, 370 μg/ml). 5×10^4 cells were placed in each well of a 96-well microtiter dish and 35 clones were isolated.

Preparation of DNA and RNA. Cells were grown to a density of 2–5×10^6 cells/ml in log phase culture. Total cellular RNA and DNA was prepared from the same cells as described by Chirgwin (21) and Hieter (22).

Southern, Northern, and S_1 nuclease analysis. Southern blots were prepared, hybridized, washed, and exposed as described by Southern (23) and Little (11). Northern blots were prepared, hybridized, washed, and exposed on radiographs as described by Little (11). The DNA restriction fragments labeled by nick translation were an Rsa 1-Xba 1 fragment containing the first exon (first exon probe), an Eco RI-Cla 1 fragment containing the third exon (third exon probe), and a Bam HI-Bam HI fragment containing the neomycin gene (neo probe). The same Northern blot was washed at 70°C and rehybridized to a human beta actin probe to control for the amount of mRNA (24). S_1 nuclease studies were carried out as described by Battey (25). A Pvu II-Pvu II fragment containing the 5′ promoter region of the first exon (promoter region probe) was used for the S_1 studies.

Soft agarose cloning and doubling times. Cells growing in log phase were made into a single cell suspension plated in 0.3% agarose to yield 30–1,000 colonies per 40-mm dish. The colonies of >50 cells were counted at 2–3 wk. Each doubling time was determined by plating 2.5 $\times 10^4$ cells/ml in 2 ml of RPMI plus 10% FCS in 24-well macrotiter dishes (Gibco, Long Island, NY) and counting the cells in duplicate from two wells every 2 d. One-fourth to one-half of the medium was replaced every 2 d. The doubling times for each replicate were then determined independently by a biostatistician (Robert W. Makuch) using a standard approach (26, 27).

Cell culture morphology and nude mouse histology. The cell culture photographs were taken of cell lines and transfected clones growing in log phase growth in 10% FCS-RPMI 1640 2 d after feeding. 2×10^7 cells were injected subcutaneously into nude mice. The tumors were harvested simultaneously at 6–12 wk, fixed in formalin, embedded in paraffin, and typical sections were stained with hematoxylin and eosin. The histologic sections were examined and photographed by a pathologist unaware of the origin of the tissue.

Dopa decarboxylase and bombesin-like immunoreactivity. L dopa decarboxylase production was determined as previously described (28) and bombesin-like immunoreactivity was determined as previously described (29).

Results

Neo gene integration and expression. The cloned cell line, NCI H209 DD1, possesses all previously described properties of a classic SCLC cell line and does not express detectable c-*myc* mRNA. The c-*myc-neo* plasmid (Fig. 1) was linearized by cleavage in vector sequences with Pvu I and transfected into H209 DD1 using electroporation. 35 independent clones were selected using the aminoglycoside antibiotic G-418. DNA from all selected clones studied contained at least one copy of the neomycin resistance gene. The clones had a spectrum of in vitro morphologies and five (identified as clones A, B, C, D, and E) were selected for further study. The clones were compared to three nontransfected small cell lung cancer cell lines expressing different amounts of c-*myc* mRNA (H209, H146, and N417).

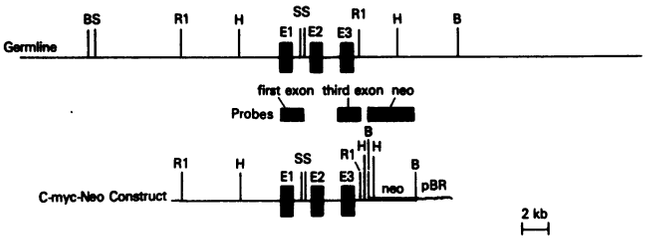

Figure 1. Restriction endonuclease maps of the c-*myc* and the c-*myc-neo* construct. This is a schematic representation of the germ-line human c-*myc* locus and the c-*myc-neo* construct. The solid boxes numbered E1–E3 denote the exons of c-*myc*. The solid bar in the c-*myc-neo* construct labeled *neo* denotes the 3.4-kb *neo* gene described in Methods. The wavy line labeled pBR represents pBR 327 sequences. The solid bars between the two schematic representations labeled the first exon, third exon, and *neo* probes represent DNA fragments subsequently used for hybridizations and are described in Methods. The partial restriction map is shown in schematic representation of the c-*myc* germline locus and the c-*myc-neo* construct, with the following cleavage sites: B, Bam HI; S, Sst I; RI, Eco RI; and H, Hind III.

We first tested for integration of the c-*myc-neo* construct into H209 genomic DNA (Fig. 2). As expected, the three untransfected control SCLC cell lines (H209, H146, and N417) have no evidence of the *neo* gene. In contrast, the five transfected clones each contain a 3.4-kb Bam HI fragment with an intact *neo* gene fragment. In addition, clone C and E have additional bands after Bam HI digestion which may represent a deletion or rearrangement of a portion of the 3.4-kb Bam HI *neo* fragment. All five transfected clones have the 0.7-kb Hind III fragment containing the SV 40 promoter and enhancer region necessary for *neo* expression in mammalian cells (Fig. 2). Another Hind III DNA fragment hybridizing to the *neo* probe is generated when Hind III cuts once in the *neo* gene and the second site is donated by the next Hind III site available in the flanking host genome. These larger Hind III fragments are different sizes in all five transfected clones, which indicates the integration occurred at different sites. They also allow estimation of the number of copies of the *neo* gene integrated into the host genome. Clone A, B, and D each have one copy, clone C has two copies, and clone E has six copies and the different sizes of *neo* fragments generated by Hind III digestion suggest the transfected gene integration into the host genome is unlinked and is probably random. Finally, as expected, Northern blot analysis shows no evidence of *neo* expression in the parent host cell line, H209, while 1.4-kb *neo* mRNA is present in all five transfected clonal derivatives of H209 growing in selective medium containing the aminoglycoside antibiotic, G-418.

C-myc gene integration. To determine the number of c-*myc* genes introduced by transfection, three nontransfected control cell lines and five transfected clones were hybridized to the third and first exon probes after various restriction enzyme digestions (Fig. 3). The first two lanes show the parental untransfected cell line, H209, and H146 genomic DNA digested with Eco RI. Neither cell line has c-*myc* gene amplification. The third lane shows N417 which is highly amplified for c-*myc* genes as has been

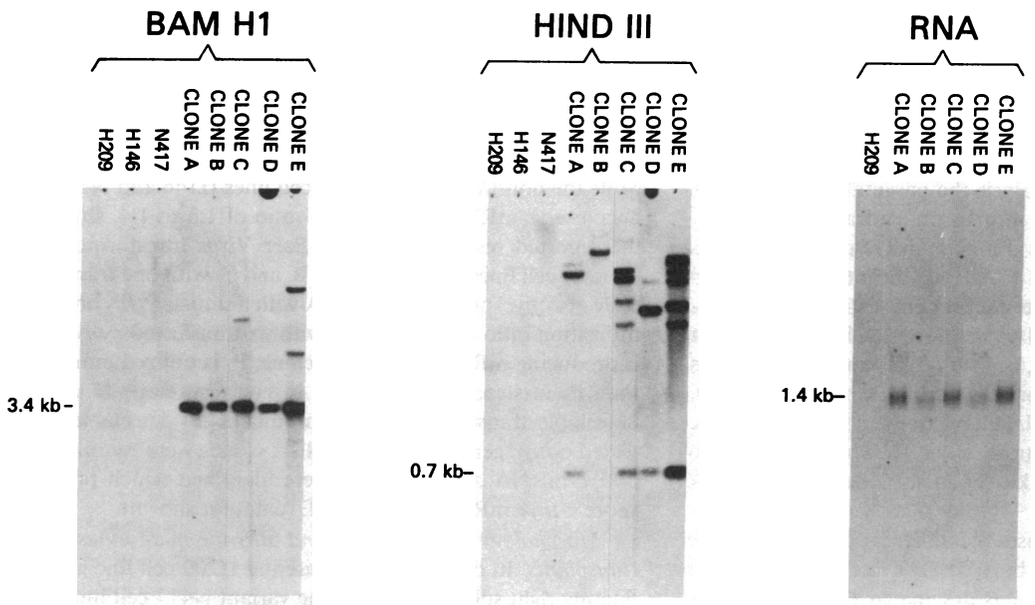

Figure 2. Neo gene integrations and expression. In the first two panels, 10 μg of DNA from three control SCLC cell lines and five transfected clones was digested with the designated restriction endonucleases, electrophoresed in 0.8% agarose gels, transferred to nitrocellulose paper, and hybridized with ^{32}P-labeled *neo* fragment. The third panel represents 10 μg of total cellular RNA isolated from the host H209 and five transfected clones growing in log phase, electrophoresed in a 0.8% agarose formaldehyde gel, blotted to nitrocellulose, and hybridized with ^{32}P-labeled DNA (*neo* probe in Fig. 1).

previously described (11). Genomic DNA from clones A, C, and E digested with Eco RI give fragments other than the 12.7 kb c-*myc* insert in the c-*myc-neo* construct. It appears the transfected 12.7 kb c-*myc* sequence from the plasmid is rearranged or deleted during transfection and subsequent integration in clones A, C, and multiple times in clone E. The rearrangement or deletion appears to include sequences 5′ to the c-*myc* gene because the c-*myc-neo* Hind III site 27 bp from the Eco RI site immediately flanking the third c-*myc* exon is retained in all five clones (Fig. 2, Hind III digest). The DNA from the five transfected clonal derivatives of H209 was hybridized to the most 5′ c-*myc* fragment in the construct, a 1.1-kb Eco RI-Bgl II fragment. Three of the additional bands from clone E were not seen, showing that in at least some cases the 5′ c-*myc* flanking sequences furthest from the gene are deleted from the construct integrated into the host DNA (data not shown).

The parental cell line, H209, and all five transfected clones retain the 11.4-kb Hind III germ line c-*myc* fragment (Fig. 3, panel 2). In addition, clones A, B, C, and E have new bands at 8.8 kb, the predicted size of a Hind III c-*myc* fragment from the c-*myc-neo* construct (Fig. 1). Clone D is missing this novel sized Hind III band despite the presence of a *neo* gene (Fig. 2). Parental H209 and all five clones show the germ line 23-kb Bam HI fragment containing the third exon of c-*myc* (Fig. 3, panel 3). In addition, clones A, B, C, and E show differently sized bands. The Bam HI cleaves once in the c-*myc-neo* construct at the 5′ origin of the neo gene and once at the next flanking genomic Bam HI site 5′ to the integrated portion of c-*myc-neo* construct. Clone D again shows no evidence of a novel third exon hybridizing fragment from the c-*myc-neo* construct. The germ line 14.7 kb Sst I fragment containing the first exon is seen in the H209 parental cell line and all five clones (Fig. 3, panel 4). In

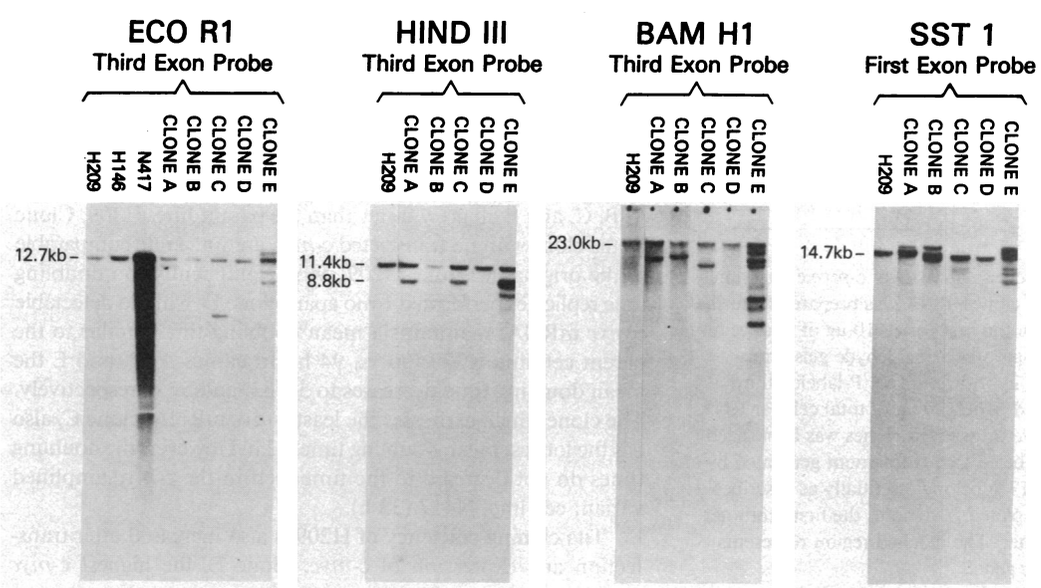

Figure 3. Southern analysis of DNA from three control SCLC cell lines and five clones transfected with c-*myc*. DNA was prepared from cell lines and clones growing in log phase. 10 μg was digested with restriction endonucleases, electrophoresed in 0.8% agarose, transferred to nitrocellulose paper, and hybridized to two different ^{32}P-labeled fragments. The third exon and first exon probes of c-*myc* are described in Methods. A partial restriction map is shown in schematic representation of the c-*myc* germline locus and the c-*myc-neo* construct in Fig. 1.

addition, multiple sized fragments from the c-*myc-neo* construct are seen hybridizing to a c-*myc* exon 1 probe in clones A, B, C, and E. No exogenous c-*myc* first exon is seen in clone D.

Expression of transfected c-myc genes. C-*myc* expression in the three nontransfected control cell lines, H209, H146, and N417, and five H209 derived transfected clones differ markedly (Fig. 4). By Northern blot analysis the parental cell line H209 expresses no detectable c-*myc* mRNA. In contrast, N417 is amplified for c-*myc* and produces abundant mRNA. Cell line H146 is not amplified for c-*myc* but makes significant levels of c-*myc* mRNA (Fig. 4). Dilution experiments demonstrate H209 is expressing <1/20 of the mRNA produced by H146 (data not shown). Transfected clones A, B, C, and E all contain exogenous c-*myc* DNA and all make normal size c-*myc* mRNA (2.3 kb). Clone E produces more mRNA than clone A and B while clone C produces the least. In contrast, clone D, which contains no exogenous c-*myc* DNA, makes no detectable c-*myc* mRNA (Fig. 4).

The human c-*myc* gene uses two different promoters in the first exon separated by 170 bp (25). The more 5′ of these is designated P_1 and the more 3′ is designated P_2. We therefore performed S_1 nuclease protection studies of c-*myc* mRNA using a promoter region probe (0.84 kb Pvu II-Pvu II fragment, Fig. 4). Using this probe, P_1-initiated transcripts yield a 520 base S_1 protected transcript, while P_2-initiated transcripts generate a 350-base protected species (Fig. 4, second panel). The parental H209 cell line was again seen not to produce detectable c-*myc* mRNA while the nontransfected control cell lines H146 and N417 produce c-*myc* mRNA with a P_1/P_2 ratio of 1:3 to 1:4, similar to the reported results for Epstein-Barr Virus transformed lymphocyte cell lines (12). Clones A, B, and C, with one transfected copy of c-*myc*, produce an mRNA with a similar P_1/P_2 promotor utilization ratio of 1:3. Clone E with six transfected c-*myc* genes is producing mRNA, but in this clone, P_1 is utilized more often than P_2 in steady state mRNA. In contrast, clone D with no detectable transfected c-*myc* gene makes no detectable transfected c-*myc* gene. An additional six clones were examined for c-*myc* mRNA and no clones were identified which produced more c-*myc* mRNA than clone E (data not shown).

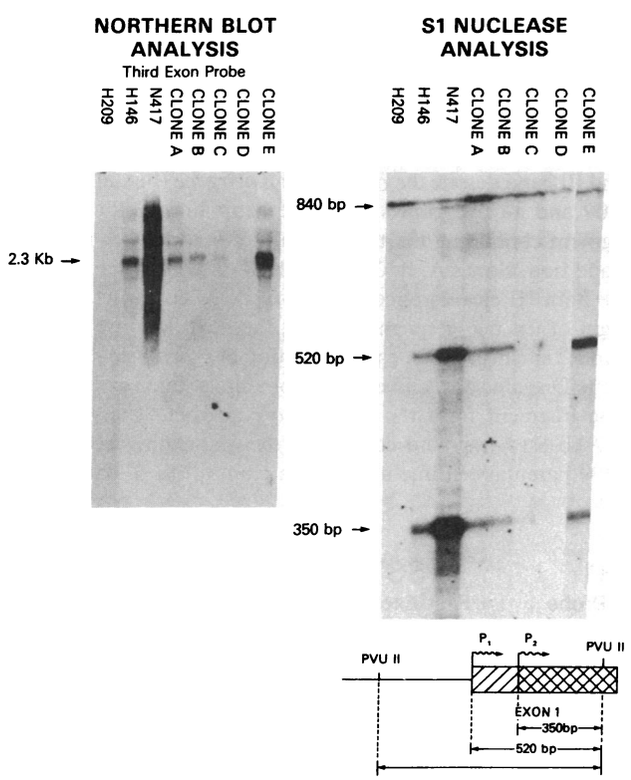

Figure 4. Northern blot and S_1 nuclease analysis of c-*myc* expression of SCLC cell lines and transfected clones. RNA was prepared from the cell lines and transfected clones. In the first panel, 10 μg of total cellular RNA was electrophoresed on agarose-formaldehyde gels, transferred to nitrocellulose paper, and hybridized to a ^{32}P-labeled third exon probe of c-*myc*. In the second panel, 20 μg of total cellular RNA from three SCLC cell lines and five transfected clones was hybridized with a 0.84-kb single strand ^{32}P-labeled Pvu II fragment generated by primer extension of an exon 1 M13 subclone essentially as described by Battey (25). Under the second panel, P_1 denotes the first promoter and P_2 denotes the second promoter. The hatched region represents the first exon of the human c-*myc* gene.

Morphology in cell culture and athymic nude mouse xenotransplants. In cell culture the parental H209 cell line grows in floating tight spheres (Fig. 5). The variant N417 cell line shows larger cells that grow in a very linear pattern. Clone E has larger cells and is growing in a more linear and open morphology than H209, resembling a variant pattern in cell culture.

The classic SCLC cell line H209 forms tumors in nude mice composed of small monotonous cells and very few mitotic figures (Fig. 6). The histology of N417 in nude mice has enlarged cells with prominent nucleoli and more mitotic figures. Clone E has histologic features between H209 and the variant, N417. Clone E cells are larger with more prominent nucleoli and mitotic figures than H209 cells. DNA was extracted from an athymic nude mouse xenograft of clone E, digested with Bam HI, and hybridized with the third exon c-*myc* fragment. Six single copies of c-*myc* in the same pattern as the original clone growing in cell culture were demonstrated (data not shown). Therefore, additional c-*myc* amplification or rearrangement in the nude mouse tumor did not account for the change in morphology. Thus, the transfected H209 clones expressing c-*myc* appear to have developed some but not all of the morphologic and histologic changes associated with the variant phenotype.

Doubling time and cloning efficiency analysis. The doubling time was inversely related to the steady state level of c-*myc* mRNA in the control cell lines, H209, H146, and N417 (Table I). The c-*myc* mRNA signal on autoradiographs was quantitated by densitometry and the amount of c-*myc* mRNA in each lane was standardized using a beta actin mRNA signal as a reference control. The cell growth curves of the c-*myc* transfected clones grew more rapidly than the parent line, H209 (Table I). Clones A, B, C, and E all grew faster than the parent line, H209. Clone D, the clone with no transfected c-*myc*, grew at a rate comparable to the original cell line, H209. Most significantly, on doubling time replicates performed 1 mo apart, clone D, with no detectable c-*myc* mRNA, maintains a mean doubling time similar to the parent cell line H209 (90 vs. 94 h). In clones A, B, and E the mean doubling time decreases to 54, 51, and 57 h, respectively. The clone which expresses the least c-*myc* mRNA, clone C, also has the longest mean doubling time, 62 h. However, the doubling times do not decrease to the time seen in the c-*myc* amplified variant cell line, N417 (33 h).

The cloning efficiency of H209 is also increased after transfection and expression of c-*myc*. Clone E, the highest c-*myc*

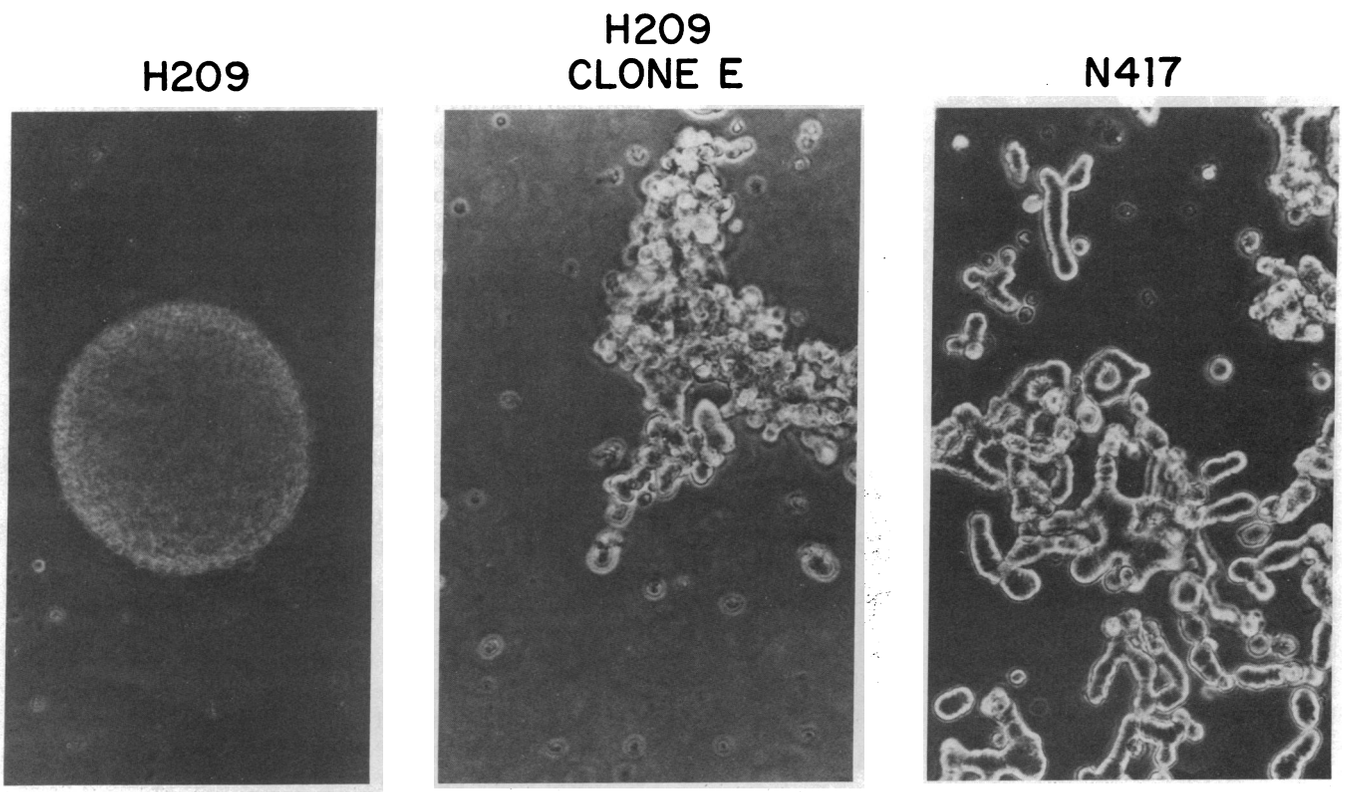

Figure 5. Cellular morphology of SCLC cell lines and a transfected clone. Cells growing in log phase in RPMI plus 10% FCS were spit 1:4 4 d before being photographed. New medium was added 2 d before, and the cells were photographed using a phase construct Nikon photo microscope. Magnification, × 100.

producing transfected clone, has a 5–10-fold increase in cloning efficiency over its parent H209 and resembles that of H146, correlating with the comparable levels of c-*myc* mRNA in these two cell lines. The cloning efficiency of clone A and D resembled that of the parent line H209. Although c-*myc* mRNA was present in clone A and the doubling time was shortened, no difference in the cloning efficiency was observed compared with the parental line H209. Possible explanations are that an early observed effect with increased c-*myc* mRNA is more rapid growth, but a higher level of c-*myc* mRNA expression is needed to observe an increase in cloning efficiency, or additional unidentified factors other than c-*myc* are present in H146 which increase the cloning efficiency.

Biochemical properties. The parent line, H209, produces large amounts of bombesin-like immunoreactivity (Table I). Both c-*myc* producing control SCLC lines, H146 and N417, yield very low levels of bombesin-like immunoreactivity (gastrin releasing peptide). Bombesin is produced in all five transfected clones at levels comparable to or greater than the parent cell line H209. L dopa decarboxylase, a marker of neuroendocrine differentiation, is produced in the control cell lines, H209 and H146, but not in the variant, N417 (Table I). L dopa decarboxylase continued to be produced in all five transfected clones.

Discussion

The small cell lung cancer system offers an opportunity for studying the role of c-*myc* in human malignancy. We have demonstrated that electroporation can introduce an exogenous normal human c-*myc* gene into a typical classic SCLC cell line, H209, and that c-*myc* mRNA can now be expressed. The clone producing the highest amount of steady state c-*myc* mRNA, clone E, shows properties analogous to the variant type of SCLC associated with c-*myc* amplification and expression (15). The morphology in cell culture became more open and linear, the atymic nude mice xenotransplants developed larger cells with prominent nucleoli, the doubling time shortened, and the cloning efficiency increased. However, the bombesin-like immunoreactivity and L dopa decarboxylase production did not markedly decrease.

Classic SCLC cells are unusual because the cell lines examined with the exception of H146 have no detectable c-*myc* mRNA expression (11, 12). It appears that this decreased expression is not associated with a translational increase in c-*myc* protein because no immunoreactive c-*myc* protein is detected in another classic SCLC line, H128 (13).

The classic SCLC cell line H209 expresses no detectable c-*myc* mRNA. When the normal c-*myc* gene is introduced by electroporation into the host genome, the clones produce detectable c-*myc* mRNA. The absence of c-*myc* mRNA in this SCLC cell line does not appear to be an unusual genetic property of the person from whom the cell line is derived. The Epstein-Barr Virus transformed lymphoblastoid cell line, H209BL, started from the same individual, produces detectable c-*myc* mRNA (data not shown). We cannot formally prove the exogenous c-*myc* gene is responsible for the expressed c-*myc* mRNA. However, the evidence of the increased c-*myc* steady state mRNA with increased c-*myc* copy number in clone E and nondetectable

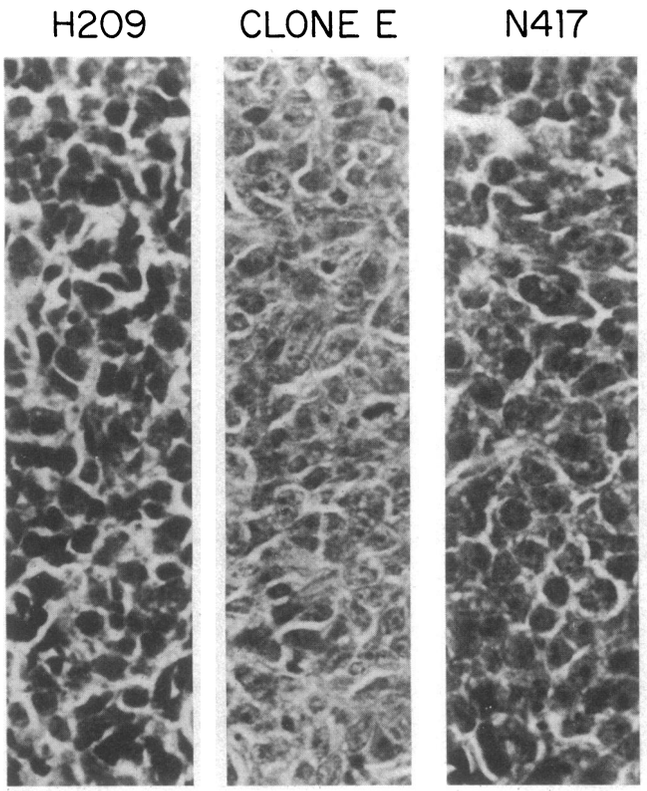

Figure 6. Histology of athymic nude mouse xenotransplants of SCLC cell lines and a transfected clone. ~2 × 10^7 cells growing in log phase were injected subcutaneously into athymic nude mice. The tumors were harvested simultaneously, fixed in formalin, embedded in paraffin, and routine sections stained with hemotoxylin and eosin. Magnification, × 550.

c-*myc* mRNA in clone D when the first and third exon are deleted, strongly suggests the exogenous c-*myc* genes are responsible for the observed increase in c-*myc* mRNA. This expression of c-*myc* in transfected SCLC lines bears some resemblance to Burkitts lymphoma where the c-*myc* gene is moved to the immunoglobulin locus and the translocated gene is nearly always expressed while the germline gene is inactive (30–33). However, the contribution of the apparent random integration of the c-*myc-neo* construct into the parent H209 genome and the SV 40 enhancer present in the c-*myc-neo* construct to the increased c-*myc* mRNA in the transfected clones is not yet determined.

The Southern blot analysis of the transfected clones shows that the 12.7-kb Eco RI fragment containing the three coding exons of c-*myc* does not usually remain intact after transfection (Fig. 2, first panel). However, the 8.8-kb Hind III fragment derived from the construct remains intact in all the clones except for the single band from clone E. (Fig. 2, second panel). It is interesting to observe that all the areas of the human c-*myc* locus which cross-hybridize with the mouse c-*myc* locus are contained in this Hind III-Eco RI fragment (25). The deletion of the most 5′ sequences of the 12.7 kb Eco RI c-*myc* fragment and the loss of the coding exons of c-*myc* in clone E suggest that the c-*myc* gene may be altered during electroporation, during integration into the host genome, or may be deleted after integration.

The S_1 nuclease analysis also shows that the promoter utilization of the c-*myc* gene in SCLC can be altered after transfection. In the SCLC cell lines producing c-*myc* mRNA, the 1:3 P_1/P_2 promoter ratio seen in Epstein-Barr Virus transformed lymphocytes is maintained (12, 33). In the transfected cell line clone E switches to make P_1 the predominant promoter. This is analogous to the Burkitt's lymphoma cell lines, BL16, BL22, BL31, and BL37, where all three coding exons of c-*myc* remain together after rearrangement and the P_1/P_2 ratio changes to approximately equimolar (33, 34). Thus, in both these systems when all three exons of c-*myc* are introduced into a new chromosomal environment, the promoter ratio is changed despite the three-exon structure of the gene remaining intact.

Morphologic changes and alterations in growth properties with *myc* transfection have been observed in other systems. NIH/3T3, Rat-2 (3), and avian embryonic fibroblasts (35) change morphology after transfection with c-*myc* or v-*myc*. In addition, more rapid growth in low serum and higher cloning efficiencies are seen with rat-2 and NIH/3T3 cells after transfection with c-*myc* (3).

Table I. Growth and Biochemical Characteristics of Control Cell Lines and Transfected Clones

	H209	H146	N417	Clone A	Clone B	Clone C	Clone D	Clone E
Relative c-*myc* mRNA (Arbitrary units*)	<0.05	1.5	65	1	1	0.25	<0.05	3
Doubling time (*h*)								
Replicate 1	92	44	35	55	45	54	90	57
Replicate 2	96	44	32	52	56	69	90	56
Cloning efficiency								
Mean percent	0.3	2.0	7.4	0.2	Not done	Not done	0.2	1.5
(SE)	(0.04)	(0.1)	(0.2)	(0.02)			(0.06)	(0.1)
Bombesin-like immunoreactivity‡	5.5	0.1	<0.01	18	6.0	9.3	5.5	5.5
L dopa decarboxylase activity§	55	116	0.4	50	85	57	55	37

* Arbitrary units were determined by densitometry tracings of autoradiograms (exposed in the linear range) normalized to the amount of beta actin mRNA detected on the same blot. Equivalent relative values are obtained if the c-*myc* signal is normalized to the amount of mRNA added to each lane. ‡ Picomoles/milligram of protein (pm/mg protein). § Nanomoles of carbon dioxide/milligram of protein (nm CO_2/mg protein).

Variant SCLC cell lines have markedly decreased or absent bombesin-like immunoreactivity and have decreased production of one of the neuroendocrine markers, L dopa decarboxylase. At the level of c-*myc* expression in the transfected clones, the biochemical markers, bombesin-like immunoreactivity and L-dopa decarboxylase, are not markedly different from those of the original clone. The steady state level of c-*myc* mRNA in the transfectants is at least 10-fold below the variant N417. This may explain the observation that the L dopa decarboxylase activity does not decrease to the level of the variant, N417 (Table I), if high levels of c-*myc* mRNA are needed to suppress L dopa decarboxylase activity. However, some of the transfectants produce steady state c-*myc* mRNA comparable to the control cell line H146 where bombesin-like immunoreactivity is decreased (Table I). Eight additional clones were examined for bombesin-like immunoreactivity and none were markedly decreased (data not shown). It is possible that the altered growth properties and morphology may be associated with c-*myc* mRNA production and the biochemical properties are independent but at this time there is insufficient data to draw any firm conclusions.

This study shows that following transfection of the c-*myc* gene in a c-*myc-neo* construct into a parent SCLC cell line, H209, not expressing c-*myc* mRNA results in c-*myc* mRNA expression. This expression is associated with the altered cellular morphology, accelerated growth rates, and increased cloning efficiencies also observed in SCLC cell lines naturally expressing c-*myc* mRNA. These observations demonstrate that increased c-*myc* expression can cause altered growth properties and altered morphology in human SCLC.

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