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Expression of the *c-myb* Oncogene in Human Small Cell Lung Carcinoma[1]

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ABSTRACT

We have found that the oncogene *c-myb* is differentially expressed in human lung cancer cell lines and that *myb*-homologous RNA can be detected only in small cell lung cancer (SCLC) cell lines. Polyadenylic acid-RNA from 13 established cell lines was examined by Northern blotting for its ability to hybridize to a radiolabeled *v-myb* probe. A 3.5-kilobase RNA transcript homologous to *v-myb* is present in four of four lines of classic SCLC and in three of four SCLC variant lines but not in five of five non-small cell lung cancer lines tested. This transcript is the same size as that found in the immature myeloid cell lines KG1, but the amount of RNA is only about 10% of that in the KG1 line. A second transcript hybridizing to *v-myb*, 2.4 kilobases in size, is also present in the variant SCLC lines and the COLO 320 line, all of which have amplification of the *c-myc* gene and markedly increased *c-myc* messenger RNA. The presence of *myb* transcripts in SCLC suggests that the *myb* gene may have a specific role in the initiation or maintenance of an important human epithelial tumor.

INTRODUCTION

The human bronchial epithelium gives rise to 4 major types of lung cancer (squamous cell, adeno-, large cell undifferentiated, and small cell carcinomas), each of which expresses properties characteristic of particular differentiated cell types in the normal mucosa. SCLC[3] accounts for 25% of human lung cancers and is a distinct, aggressive neoplasm with neuroendocrine properties. This tumor is unusual in that it appears to have the potential to change towards other lung cancer histologies with time (4, 14, 43). An important transition step in this process may involve a variant cell form (SCLC-V) (14, 18, 22). Recently, the variant form has been shown to have amplification of the *c-myc* oncogene and increased amounts of *c-myc*-RNA in cell culture (22), suggesting a role for this oncogene in the phenotypic conversion and malignant behavior of this human lung cancer (22). The increasing evidence that cell transformation may require 2 or more oncogenes acting in concert (20, 21, 30, 37) prompted us to study expression of other oncogenes in human lung cancer. We now show that *c-myb* is differentially expressed in human lung cancer cell lines.

MATERIALS AND METHODS

Cells. All cell lines have been described previously (15, 18, 23, 33) except OH3 which was established in our laboratory from the pleural effusion of a patient with SCLC and which grows similarly to multiple established SCLC lines and expresses typical neuroendocrine markers such as L-dopa decarboxylase (15). Cells were grown in RPMI 1640 medium supplemented with 8 to 15% heat-inactivated fetal bovine serum, penicillin (100 units/ml), and streptomycin (100 μg/ml).

Nucleic Acids. Cytoplasmic RNA was prepared from confluent cell lines by a modification of the method of Favaloro *et al.* (11). Poly(A)-RNA was selected by chromatography on oligodeoxythymidylic acid-cellulose columns (3). Genomic DNA was prepared from isolated cell nuclei by digestion with proteinase K (100 μg/ml) in 1% SDS followed by phenol chloroform extraction.

Probes. The 1.2-kilobase *Kpn*I-*Xba*I fragment of the avian myeloblastosis virus genome in pBr322, containing the *v-myb* gene, was kindly provided by M. Baluda (32); the 5.5-kilobase *Bam*HI fragment of mouse *c-myc* genomic DNA in pBr322 was kindly provided by I. R. Kirsch (19), and plasmid pA1, containing a 2.0-kilobase actin cDNA, was kindly supplied by D. Cleveland (8). All probes were verified by restriction-enzyme mapping and then radiolabeled using [^{32}P]dCTP by standard nick translation procedures (35) to a specific activity of 1 to 3 × 10^8 cpm/μg. Oligolabeling (12) was occasionally used for the actin probe to a specific activity of 0.5 to 1 × 10^9 cpm/μg.

Hybridization. Gel electrophoresis of RNA was performed using 1.0 to 1.5% agarose containing 2.2 M formaldehyde. Two to 4 μg of poly(A)-RNA per lane were electrophoresed for 16 hr at 60 V with 20 mM 3-(*N*-morpholino)propanesulfonic acid (pH 7) as running buffer. DNA from pBr322 was cut with restriction enzymes to known sizes, made single stranded, and used as size markers.

RNA was blotted to nitrocellulose paper by standard methods (40) and then prehybridized at 43° for 4 to 6 hr in 50% formamide-0.05 M sodium phosphate buffer (pH 7)-0.8 M NaCl-4 mM EDTA-40 mM Tris-5× Denhardt's solution-salmon sperm DNA (0.15 mg/ml) by modification of the method of Corces *et al.* (9). Fresh hybridization solution was then used along with 1 to 3 × 10^7 cpm of probe and hybridized for 24 to 36 hr at 43°. Filters were then washed in 50% formamide-0.75 M NaCl-5 mM EDTA-50 mM Tris-0.5% SDS at 43° for 40 min followed by 0.3 M NaCl-2 mM EDTA-20 mM Tris-0.5% SDS at 65° for 40 min and finally 0.07 M NaCl-2 mM EDTA-20 mM Tris-0.5% SDS at 65° for 30 min. Filters were then exposed to Kodak XAR-5 film at −70° using Lightning-Plus intensifying screens for up to 7 days.

For Southern blots, genomic cellular DNA was digested with the restriction endonucleases *Eco*RI, *Pvu*II, *Xba*I, *Hind*III, and *Bam*HI in the manufacturers' recommended buffers at 37° overnight. DNA (10 μg/lane) was then electrophoresed through 0.8% agarose gels at 60 to 80 V for 6 to 16 hr with 0.09 M Tris-borate as electrophoresis buffer. Transfer to nitrocellulose was accomplished by standard methods (39) and then prehybridized at 65° for 2 to 4 hr in 6× SSC-10× Denhardt's solution-salmon sperm DNA (200 μg/ml). Fresh hybridization solution [1 M NaCl-10× Denhardt's solution-1% SDS-2 mM EDTA-50 mM sodium phosphate-salmon sperm DNA (200 μg/ml)] with 1 to 5 × 10^7 cpm of radiolabeled probe was then incubated with the filters at 65° for 24 to 48 hr. Filters were then washed in 2 × SSC-0.5% SDS at room temperature for 20 to 30 min followed by 4 washes of 30 min each in 0.25× SSC-0.5% SDS at 65° for the *c-myc* and actin probes and 0.5× SSC-0.5% SDS at 65° for the *v-myb* probe. Filters were exposed as above.

RESULTS

In a study of poly(A)-RNA from 13 established human lung cancer cell lines, we have found that 7 of 8 SCLC lines, including

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[3] The abbreviations used are: SCLC, small cell lung cancer; poly(A), polyadenylic acid; SDS, sodium dodecyl sulfate; SSC, standard saline citrate (0.15 M sodium chloride-0.015 M sodium citrate, pH 7.4).

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3 of 4 variant lines (Fig. 1*A*), express a 3.5-kilobase transcript homologous to *v-myb*, as does the neuroendocrine colon cell line COLO 320. This transcript is the same size as that found in the KG1 immature myeloid cell line (41) (Fig. 1*A*). No *myb*-homologous RNA was detected in 5 of 5 non-SCLC lines examined. The presence of intact RNA in all 13 cell lines was verified by subsequent hybridization of the same filters to an actin cDNA probe (Fig. 1*B*). The amount of *c-myb* mRNA detected in the SCLC line is only about 10% of the amount found in KG1 as assayed by densitometry of the autoradiogram and normalization of the *myb* signals to those for actin mRNA (data not shown). A second transcript of about 2.4 kilobases was also detected with the *v-myb* probe in the 3 variant SCLC cell lines and COLO 320 line. The size of the smaller *myb*-hybridizing transcript coincides with that described for *c-myc* mRNA (2.4 kilobases) and can be seen only in the variant SCLC lines and COLO 320. These lines all are known to have *myc* gene amplification and markedly increased *myc* transcription (2, 22). Fig. 2 shows amplified *myc* RNA in the lines which have the smaller *myb*-homologous band, with varying lesser amounts of *myc* RNA detected in all of the other cell lines tested.

We have preliminarily looked for alteration in *c-myb* genomic sequences which might explain activation of the gene in the SCLC cell lines. We did not detect gross rearrangements or amplification of the proto-*myb* gene in any of these cell lines as analyzed by Southern blotting of genomic DNA restricted with *Eco*RI, *Pvu*II, *Xba*I, *Hind*III, or *Bam*HI (data not shown).

DISCUSSION

Our present results suggest a significant difference in the levels of transcription of the *c-myb* oncogene among the 4 major histological phenotypes of lung cancer. Only one of 7 SCLC lines had *myb* RNA below our level of detection. This unusual cell line, OH-2 (18), was established from a patient with typical SCLC histology. Like other variant SCLC lines, it lacks the high levels of L-dopa decarboxylase activity which typically distinguish SCLC from non-SCLC in culture and has the 3p(−) chromosome deletion considered characteristic of SCLC.[4] OH-2 cells, however, grew as typical large cell undifferentiated lung cancer in nude mouse heterotransplants, and also, unlike other variant SCLC lines (22), we find that this cell line lacks *myc* amplification. Cell surface protein analysis showed that all clones of this cell line simultaneously expressed both SCLC and non-SCLC surface proteins (18). Thus, this SCLC variant line phenotypically contains both non-SCLC and SCLC features simultaneously.

A second transcript of about 2.4 kilobases was also detected in the 3 variant SCLC lines and the COLO 320 line using the same *v-myb* probe. Although several larger *myb* precursor RNAs have been detected in chicken tissue (17) and mouse plasmacytomas (29), only a single cytoplasmic RNA transcript has been detected in human hematopoietic cells (41). The precise source of our second, smaller transcript is currently not known. There may be several different sized *myb* poly(A)-RNA transcripts present in these cell lines. Alternatively, the smaller band might be due to cross-hybridization to another RNA species. Interestingly, the *myc* and *myb* proteins have been shown recently to have structural homology, although this homology involves conservation of related amino acids rather than identical nucleotide sequences (34). The possibility that weak homology between *c-myc* and *c-myb* RNAs might produce hybridization of the *v-myb* probe to the abundant 2.4-kilobase *c-myc* transcripts in the variant SCLC and COLO 320 lines must be considered and further explored.

Avian myeloblastosis virus, which carries *v-myb*, induces only leukemia and not carcinomas in its natural host. Because *c-myb* transcripts were initially reported only in hematopoietic cells from chickens, mice, and humans (17, 29, 36, 41) and because *c-myb* transcripts are decreased in HL-60 cells chemically induced to differentiate (41), it has been postulated that the role of this oncogene is related in some way to early stages of hematopoietic cell development (13, 41). The detection of *c-myb* transcripts in our epithelial cell lines and in occasional human carcinoma tissues (38) suggests, not surprisingly, that this gene has a function not limited to cells of hematopoietic derivation. Our data suggest at least 2 possible hypotheses concerning the expression and function of *c-myb* in epithelial cells.

c-myb could be associated with expression of a neuroendocrine phenotype. Among the human lung cancers, only SCLC consistently contains neurosecretory granules and expresses high levels of L-dopa decarboxylase (5, 6), neuron-specific enolase (24), BB isoenzyme of creatine phosphokinase (16), and bombesin (10, 28, 42). Because of these properties, this tumor has been included in the amine precursor uptake-dopa decarboxylase (4, 31) group of endocrine cells. Similar neuroendocrine properties are associated with endocrine cells located in intestinal epithelium (27). The presence of *c-myb* transcripts in SCLC cells and in an intestinal tumor (Colo 320), which has been reported to express neuroendocrine properties, then suggests a possible role for this gene in cells with a neuroendocrine phenotype. However, it is important to note that the variant SCLC cells have lost the neuroendocrine phenotype (14, 18, 22) and yet retain *c-myb* expression. Also, in our laboratory, the Colo 320 line does not contain key neuroendocrine markers such as L-dopa decarboxylase at this time. Examination of other neuroendocrine cell lines and tissues will be required to further consider this hypothesis.

Alternatively, *c-myb* expression may be important to normal or neoplastic bronchial epithelial cells at certain stages of differentiation. Increasing evidence suggests that each of the major forms of human lung cancer arises in cells of endodermal lineage and that a common bronchial mucosal stem cell may have the potential for expressing the full range of differentiated cell phenotypes seen in the bronchial mucosa, including the neuroendocrine phenotype (for review, see Refs. 4 and 14). SCLC has been observed to change with time towards the non-SCLC phenotype, both in culture (14) and in the host (1, 7, 26). If, as has been suggested, SCLC represents a neoplasm frozen at an earlier step in epithelial differentiation than that represented by the other types of lung cancers (4), then the function of the *c-myb* gene in epithelial cells may indeed correlate with early stages of cell maturation, as has been postulated initially from observations of hematopoietic cells. In turn, *myc* amplification may be able to mediate the initiation of phenotypic conversion represented by the SCLC variants only in the setting of concurrent *myb* expression, which may maintain the cells in a very early stage of differentiation. Similarly, the role of other oncogenes, such as the *raf* oncogene, which has been reported recently to

[4] J. Whang-Peng, unpublished data.

be transcribed in SCLC (25), should be considered in conjunction with *myb*.

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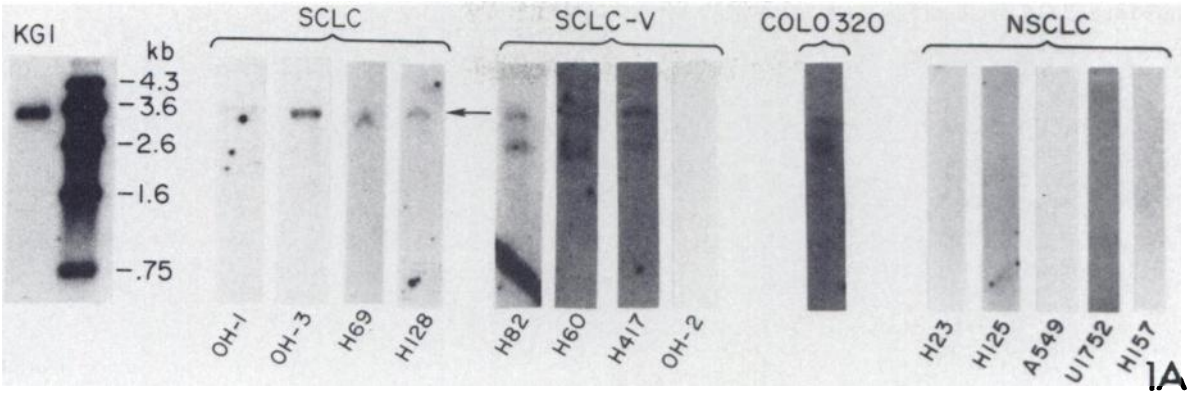
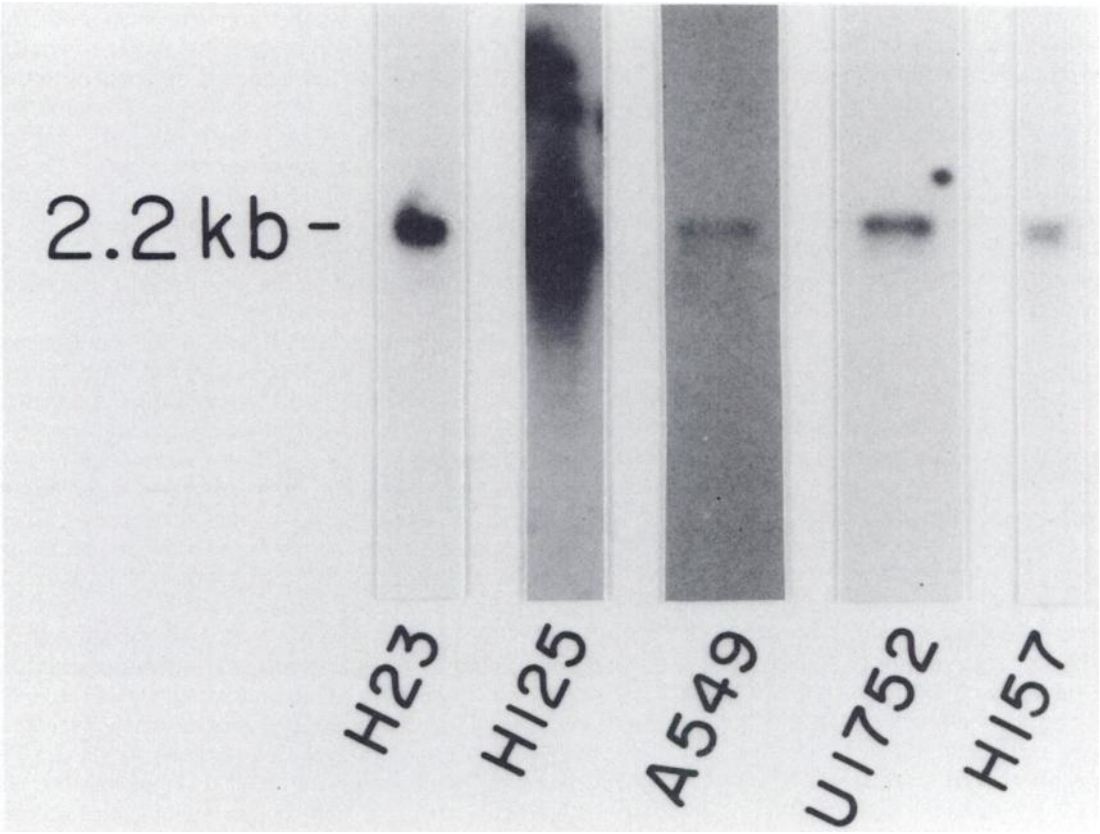
Fig. 1. *A*, Northern blots of poly(A)-RNA probed for *c-myb* in control (KG1 myeloid cell line), SCLC, SCLC variant (*SCLC-V*), and COLO 320 lines show a 3.4-kilobase band (*arrow*) present in KG1, SCLC, SCLC-V, and COLO 320 which is not detected in the non-SCLC (*NSCLC*) lines. A second band at 2.4 kilobases can also be seen in the SCLC-V and COLO 320 *lanes*. *B*, Northern blots probed with actin to verify that intact RNA in similar amounts was loaded in each *lane*. Only the NSCLC *lanes* are shown.

Fig. 2. Northern blots of the same cell lines shown in Fig. 1 probed with a *c-myc* probe and showing increased amounts of *c-myc* RNA in SCLC variant (*SCLC-V*) and COLO 320 lines, with varying amounts of much less *myc* RNA in SCLC and non-SCLC (*NSCLC*) lanes.



1A



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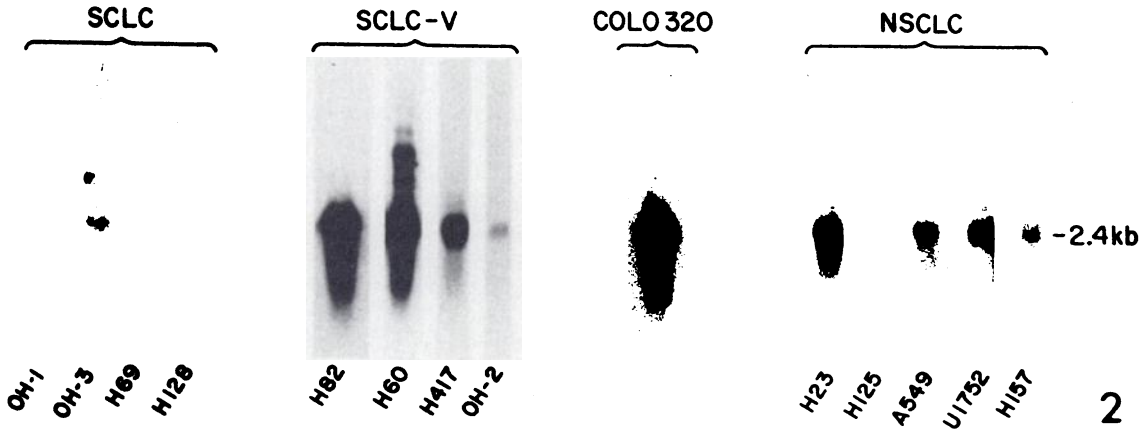

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