

Clinical Cancer Research



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Clin Cancer Res 1996;2:119-126.

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Block of *c-myc* Expression by Antisense Oligonucleotides Inhibits Proliferation of Human Thyroid Carcinoma Cell Lines¹

Janete Cerutti, Francesco Trapasso,
Caterina Battaglia, Li Zhang,
Maria Luisa Martelli, Roberta Visconti,
Maria Teresa Berlingieri, James A. Fagin,
Massimo Santoro, and Alfredo Fusco²

Dipartimento di Biologia e Patologia Cellulare e Molecolare, c/o Centro di Endocrinologia ed Oncologia Sperimentale del CNR, Facoltà di Medicina e Chirurgia di Napoli, Università degli Studi di Napoli "Federico II," via Pansini, 5, 80131 Napoli [J. C., L. Z., R. V., M. T. B., M. S.]; Dipartimento di Medicina Sperimentale e Clinica, Facoltà di Medicina e Chirurgia di Catanzaro, Università di Reggio Calabria, via Tommaso Campanella, 88100 Catanzaro [F. T., C. B., M. L. M., A. F.], Italy; and Cedars-Sinai Medical Center, Los Angeles, California 90048 [J. A. F.]

ABSTRACT

Although elevated *c-myc* expression seems to be related to an unfavorable prognosis of human thyroid neoplasias, the role of *c-myc* overexpression in the process of thyroid carcinogenesis is still unknown. We analyzed *c-myc* expression in 7 human thyroid carcinoma cell lines, originating from different histotypes, and in 50 fresh thyroid tumors and found a higher level of *c-myc* mRNA in all the thyroid carcinoma cell lines and in several fresh thyroid tumors compared with normal thyroid. The highest increases occurred in the most malignant cell lines and in undifferentiated human thyroid carcinomas. The block of c-MYC protein synthesis with *myc*-specific antisense oligonucleotides reduced the growth rate of the thyroid carcinoma cell lines significantly. Our results indicate that *c-myc* overexpression plays a critical role in the growth of thyroid cancer cells, which supports the hypothesis that the *myc* proto-oncogene might be involved in the neoplastic progression of thyroid carcinogenesis.

Received 5/31/95; revised 7/28/95; accepted 8/7/95.

¹ This work was supported by grants from the Associazione per la Ricerca sul Cancro (Progetto Speciale Oncosoppressori), Progetto Finalizzato Applicazioni Cliniche della Ricerca Oncologica, and the Progetto Finalizzato "Biotecnologie e Biostrumentazione" of the Consiglio Nazionale delle Ricerche. J. C. was a recipient of a fellowship by the Conselho Nacional de Desenvolvimento Científico e Tecnológico. L. Z. was a recipient of a fellowship awarded by the Associazione Partenopea per la Ricerca Oncologica.

² To whom requests for reprints should be addressed, at Dipartimento di Biologia e Patologia Cellulare e Molecolare, Facoltà di Medicina e Chirurgia di Napoli, Università degli Studi di Napoli "Federico II," via Pansini, 5, 80131 Napoli, Italy. Phone: 39 81 7463056; Fax: 39 81 7463037 or 7701016.

INTRODUCTION

Elevated expression of MYC proteins can induce oncogenic transformation (1, 2) and apoptosis (3, 4) and can block cell differentiation (5, 6). The proteins belonging to the MYC protein family are localized in the nucleus (7-9) and can activate transcription when brought into contact with DNA by heterologous DNA-binding domains (10-12). Recently, two proteins that form heterodimers with MYC proteins have been isolated: Max, a human protein; and Myn, its murine homologue (13, 14). The *c-myc* gene was found to be rearranged, amplified, and overexpressed in a wide variety of human cancers (15). It was also shown to be involved in the progression of various cancers (16, 17). In the thyroid systems, *c-myc* can cooperate with some viral oncogenes in inducing the neoplastic phenotype in the rat thyroid cell line PC Cl 3 (18). Furthermore, there is a positive correlation between elevated levels of *c-myc* expression and the stages of human thyroid neoplasia (19). However, the role of *c-myc* overexpression in the process of thyroid carcinogenesis is still unknown.

We have analyzed *c-myc* expression in 7 cell lines originating from different histotypes of human thyroid carcinomas and in 50 fresh thyroid carcinomas. Levels of *c-myc* were observed in all thyroid carcinoma cell lines; the highest increase was detected in the more malignant, undifferentiated cell lines. Interference with c-MYC protein synthesis by antisense oligonucleotides inhibited the growth of thyroid carcinoma cell lines.

MATERIALS AND METHODS

Cell Culture and Assay of the Transformed State. The human thyroid carcinoma cell lines studied were TPC-1 (20), WRO (21), NPA (22), ARO (22), FRO (23), NIM 1 (24), and B-CPAP (25). They were grown in DMEM containing 10% fetal bovine serum. HPCs³ were established as described (26). Tumorigenicity of the cell lines was tested by injecting 2×10^6 cells into athymic mice. Soft-agar colony assays were performed as described elsewhere (27).

RNA Isolation and Northern Blot Analysis. Thyroid tumors were obtained from the Laboratoire d'Histologie et de Cytologie, Centre Hospitalier (Lyon Sud, France). The tumor samples were frozen in liquid nitrogen and stored frozen until RNA was extracted. The procedures for total RNA extractions, Northern blots, and hybridizations have been described previously (28). The *c-myc* probe used in this study was the 1.3-kb *Pst*I-*Pst*I fragment of the pRyc 7.4 plasmid, which is specific for the human *myc* gene (29). The probe for the *max* gene was obtained by PCR amplification using the *max*-specific primers (13) 5'-CCTGGGCCGTAGGAAATGAGCGATAAAC-3' and

³ The abbreviations used are: HPC, human primary culture; NFD, nonfat dried milk; TBS, Tris-buffered saline.

3'-TGCCAGTGGCTTAGCTGGCCTCCA-5'. A mouse β -actin probe was used to ascertain the equal RNA loading (30).

Protein Isolation and Western Immunoblot Analysis.

The protein isolation procedure has been described elsewhere (31); 5×10^6 cells were washed twice with PBS, then lysed in a buffer containing 10 mM sodium phosphate (pH 7.4), 0.1 M NaCl, 1% Triton X-100, 0.1% SDS, sodium deoxycholate, 1 mM phenylmethylsulfonyl fluoride, 10 mg aprotinin/ml, 10 mg leupeptin/ml, and 10 mg pepstatin/ml. Extracts were clarified by sedimentation in a microcentrifuge at low speed for 5 min at 4°C. The sample of normal thyroid tissue was ground to a fine powder in a mortar with a pestle and sand. The powdered tissue was then resuspended in an equal volume of ice-cold protein extraction buffer. Thereafter, the proteins were extracted using the procedure used for the cell lines. The protein concentration of the supernatant was determined by the Bio-Rad protein microassay as specified by the manufacturer (Bio-Rad, Warford, England).

Western immunoblot analysis was performed according to a standard procedure (32). Briefly, protein fractionation was carried out by SDS-PAGE using 80 μ g (or 40 μ g) cell protein samples/lane of a 10% polyacrylamide gel. Before loading, proteins were diluted in loading buffer containing 10% glycerol, 2% SDS, 62.5 mM Tris-HCl (pH 6.8), 5% β -mercaptoethanol, and 0.5% bromophenol blue. Gels were transferred to nitrocellulose in transfer buffer (390 mM glycine, 480 mM Tris, 0.37% SDS, and 20% methanol). The filters were incubated for 1 h at room temperature in 1% NFDM and TBS buffer [200 mM Tris-HCl (pH 7.9) and 1.5 M NaCl]. After washing, the filters were incubated for 1 h in TBS buffer with Tween 20 [200 mM Tris-HCl (pH 7.9), 1.5 M NaCl, and 0.5% Tween 20] plus 1% NFDM containing 10 μ g/ml antibody *c-myc* (Ab-1; Oncogene Science, Inc., Uniondale, NY). After four washes, blots were incubated with antimouse antibodies conjugated with horseradish peroxidase diluted 1:1000 for 1 h in TBS buffer with Tween 20 plus 1% NFDM. Parallel gels were run to ascertain that equal amounts of protein in fact had been loaded by staining with Coomassie blue. Revelation was performed with Enhanced Chemiluminescence (Western blot detection kit; Amersham, Amersham Place, United Kingdom).

Effects of Antisense *c-myc* on *c-MYC* Protein Expression and Cell Growth. The oligonucleotides used were identical or complementary to sequences at the beginning of the coding region of *c-myc* in exon 2. The sense oligomer used was 5'-ATGCCCTCAACGTT-3'; the antisense oligomer was 3'-TACGGGGAGTTGCAA-5'; the missense oligomer was 3'-AAGCTTGGAGGCGAT-5' (33). The missense oligonucleotide was complementary to only a few reading frames of human T-cell lymphotropic virus, type I, and a portion of the neural cell adhesion molecule (34). Phosphorothioate oligonucleotides were purchased from Eurogentec (Liegi, Belgium). They were synthesized by a modification of the H-phosphonate procedure (35), purified by ion-exchange chromatography, and ethanol precipitated.

To analyze the capability of the antisense oligonucleotides to block the abundance of the *c-MYC* protein, the cells were treated with 4 μ M oligonucleotide 24 h after cell plating. Proteins were extracted 48 and 72 h after oligonucleotide treatment,

Table 1 Expression of the neoplastic phenotype of human thyroid carcinoma cell lines

Cell line	Carcinoma histotype	Colony-forming efficiency in agar (%)	Tumorigenicity in athymic mice
TPC-1	Papillary	0	NO
WRO	Follicular	8	YES
NPA	Papillary	18	YES ^a
NIM 1	Papillary	0	YES ^a
B-CPAP	Papillary	0	NO
ARO	Anaplastic	70	YES ^b
FRO	Anaplastic	38	YES ^b

^a Small tumors appeared after about 2 months.

^b Large tumors appeared after 2 weeks.

and the levels of the *c-MYC* protein were measured by Western blot (see above).

Southern Blot Analysis. The Southern blot analysis was performed according to a standard procedure (28).

DNA Synthesis Assay. To evaluate the effects of the oligonucleotides on the cell growth rate, 10^5 cells were plated, and 15 h later, they were treated with sense, missense, and antisense oligomers. After 24 and 72 h, DNA synthesis was measured by incorporation of [³H]thymidine (2 μ Ci/ml, 40 Ci/mmol; Amersham) into trichloroacetic acid-insoluble material as described elsewhere (36). The results are expressed as percentage of inhibition of thymidine incorporation in comparison with the untreated cells. Each value is the mean of at least three experiments in duplicate. Thymidine uptake values were analyzed by one-way ANOVA. Differences were considered statistically significant at $P < 0.05$.

Cellular Uptake of Oligonucleotide. Five $\times 10^5$ HPC, FRO, and ARO cells in 100 μ l culture medium with heat-inactivated fetal bovine serum were exposed to 5 μ M 5' end-labeled oligonucleotide for 16 h at 37°C. Fetal bovine serum was heated to 65°C for 30 min to inactivate DNases. The cells were washed twice, and the supernatants were saved. The percentage of uptake by the cells was the number of counts in the cell pellet/total number of counts in the supernatant. The results reported are the average of two duplicate experiments.

RESULTS

***c-myc* Expression in Human Thyroid Carcinoma Cell Lines.** Seven thyroid carcinoma cell lines (four derived from papillary carcinomas, two from anaplastic carcinomas, and 1 from a follicular carcinoma) were analyzed for *c-myc* expression by Northern blot. The origin of each cell line and their tumorigenic properties are listed in Table 1. Levels of *myc*-specific transcripts were higher in the thyroid cell lines than in normal human thyroid primary culture cells (Fig. 1). There was a 5-fold increase in NIM1 and B-CPAP cells, a 10-fold increase in WRO, NPA, and TPC-1 carcinoma cell lines, and a very high increase (between 50- and 100-fold) in the ARO and FRO cell lines that originated from anaplastic carcinomas. It is noteworthy that the ARO and FRO cell lines induced tumors in athymic mice and had the highest colony-forming efficiency in agar among the cell lines used in this study (Table 1). Because the *myc* gene product is known to form heterodimers with the

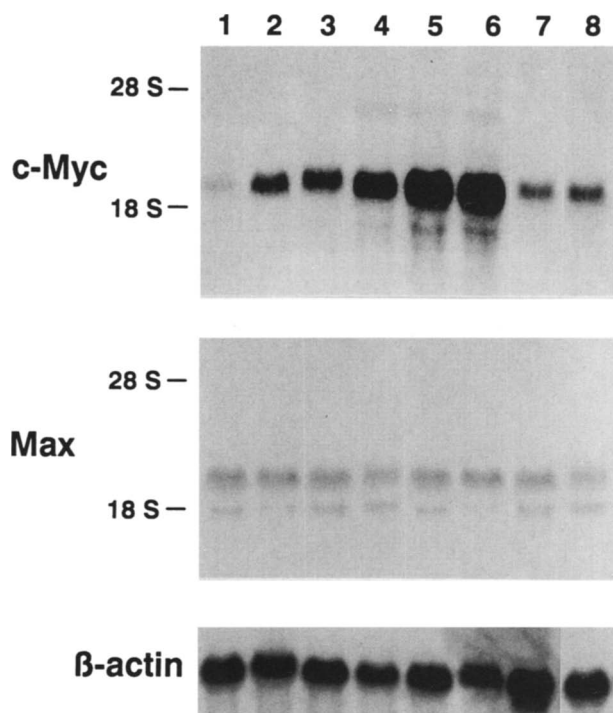


Fig. 1 Expression of the *c-myc* and *max* genes in normal and neoplastic thyroid cells. Ten μg total RNA for each cell line were size fractionated on a denaturing formaldehyde agarose gel, blotted onto nylon filters (Hybond-N; Amersham), and probed with a DNA for *c-myc*, *max*, and β -actin, as indicated. RNA was extracted from the following sources: Lane 1, normal thyroid primary culture cells; Lane 2, TPC-1 cells; Lane 3, NPA cells; Lane 4, WRO cells; Lane 5, ARO cells; Lane 6, FRO cells; Lane 7, NIM 1 cells; and Lane 8, B-CPAP cells. Actin was used as an internal control for uniform RNA loading.

product of the *max* gene, we also analyzed *max* gene expression in the same cell lines. There was no difference in the abundance of *c-max* mRNA between tumor cell lines and normal thyroid (Fig. 1).

To investigate whether the increased *c-myc*-specific mRNA expression was also associated with an increased level of the MYC protein in the thyroid carcinoma cell lines, we analyzed the MYC protein levels in six cell lines (ARO, FRO, B-CPAP, NPA, WRO, and NIM1) in HPCs and normal thyroid by Western blot. The levels of the MYC protein were higher in all six cell lines, particularly the ARO and FRO cell lines (see Fig. 2, Lanes 2 and 3) than in normal thyroid tissue or primary culture cells (Fig. 2). A parallel gel stained by Coomassie blue confirmed that the amounts of proteins loaded were equal (data not shown).

The *c-myc* gene was amplified in the ARO and FRO cells (5–6- and 3–4-fold, respectively) compared with normal thyroid cells (Fig. 3). The same Southern blot was hybridized with a β -actin probe, and there was no difference among the same DNA.

***c-myc* Proto-oncogene Expression in Thyroid Tumors.** We analyzed 44 human thyroid neoplasias of different histological types (5 adenomas, 25 papillary carcinomas, 8 follicular carcinomas, and 6 anaplastic carcinomas) and 6 nodular goiters

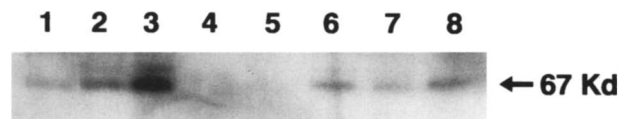


Fig. 2 Western blot analysis of MYC protein accumulation in normal and neoplastic thyroid cells and tissue. The monoclonal antibodies directed against the MYC protein were used for the Western blot analysis. The proteins were extracted from the following sources: Lane 1, WRO cells; Lane 2, ARO cells; Lane 3, FRO cells; Lane 4, normal thyroid tissue; Lane 5, normal thyroid primary culture cells; Lane 6, B-CPAP cells; Lane 7, NPA cells; and Lane 8, NIM-1 cells. Eighty μg proteins were loaded for each sample.

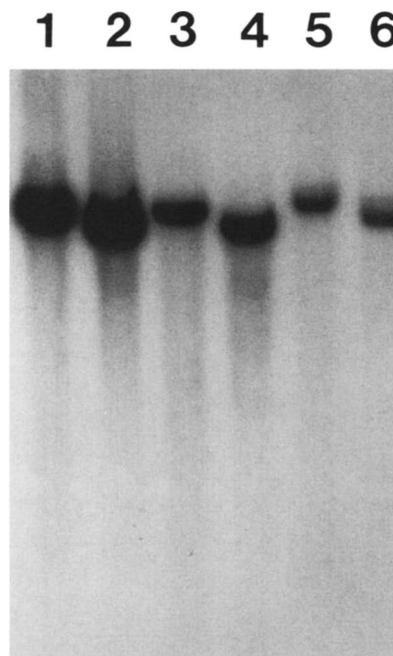


Fig. 3 Analysis of *c-myc* amplification in human thyroid carcinoma cell lines. Ten μg DNA were digested with *EcoRI* (Lanes 1, 3, and 5) and *HindIII* (Lanes 2, 4, and 6) and hybridized with a cDNA for human *c-myc* (the 1.3-kb *PstI-PstI* fragment of pRyc 7.4). The sources of DNA were: Lanes 1 and 2, ARO cells; Lanes 3 and 4, FRO cells; and Lanes 5 and 6, HPC cells.

for *c-myc* proto-oncogene expression (see Table 2). No significant increase in expression of *c-myc* was observed in adenomas or in follicular carcinomas; expression was increased slightly in 4 of 25 papillary carcinomas. Five of the 6 anaplastic carcinomas expressed very high amounts of *c-myc* mRNA. Some representative data are shown in Fig. 4. In four papillary carcinomas, the levels of *c-myc* expression are comparable with those observed in normal thyroid, whereas 2 anaplastic carcinomas showed almost 50-fold increases in *c-myc* expression. The RNAs were normalized according to the levels of β -actin expression.

Effect of Blockage of MYC Protein Synthesis on Growth of Thyroid Carcinoma Cell Lines. Next, we determined the role of the enhanced *c-myc* expression in the process of cell transformation and growth by blocking the synthesis of

Table 2 Expression of *c-myc* proto-oncogene in human thyroid neoplasias

Histological type of thyroid specimens	No. of patients with elevated level of <i>c-myc</i> mRNA/no. of patients analyzed ^a
Papillary carcinomas	4/25
Follicular carcinomas	1/8
Anaplastic carcinomas	5/6
Adenomas	0/5
Goiters	0/6

^a *c-myc* RNA levels were considered elevated when they were at least 3-fold higher versus the level found in normal thyroid. The expression of the β -actin gene was used to normalize the levels of RNA loaded on the gel.

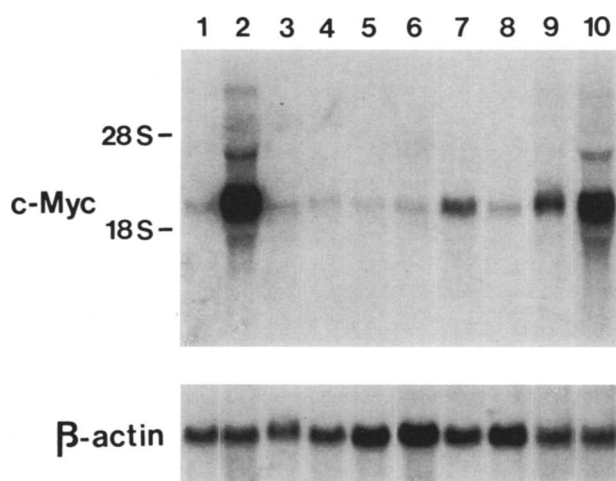


Fig. 4 Analysis of *c-myc* expression in normal and neoplastic thyroid tissues. Ten μ g total RNA for each sample were size fractionated on a denaturing formaldehyde agarose gel, blotted onto nylon filters (Hybond-N; Amersham), and probed with either *c-myc* or β -actin cDNA, as indicated. RNA was extracted from the following sources: Lane 1, normal thyroid; Lanes 2, 7, 9, and 10, anaplastic carcinomas; Lanes 3, 4, 5, and 6, papillary carcinomas; and Lane 8, a follicular carcinoma. Actin was used as an internal control for uniform RNA loading.

the *c-MYC* protein with an antisense oligonucleotide against the translation initiation region of the *c-myc* mRNA located at the beginning of exon 2. The efficiency of this antisense oligonucleotide to block MYC protein synthesis was evaluated by treating the ARO cells with the oligonucleotides and measuring MYC protein levels by Western blot 48 and 72 h later. MYC protein levels were significantly (at least 5-fold) lower in the ARO cells treated with the *myc* antisense-specific oligonucleotide (Fig. 5A, Lanes 2 and 3) than in ARO cells (Lane 1) or in cells treated with a sense *myc*-specific oligonucleotide (Lane 4). To ascertain that equal amounts of protein had been loaded on the well, parallel gels were run and stained with Coomassie blue (Fig. 5B). Almost identical results were obtained with the FRO cell line (data not shown).

The effects of the antisense *myc* oligonucleotide on the growth of the ARO, FRO, and NPA cells were analyzed by evaluating the DNA synthesis measured as [³H]thymidine in-

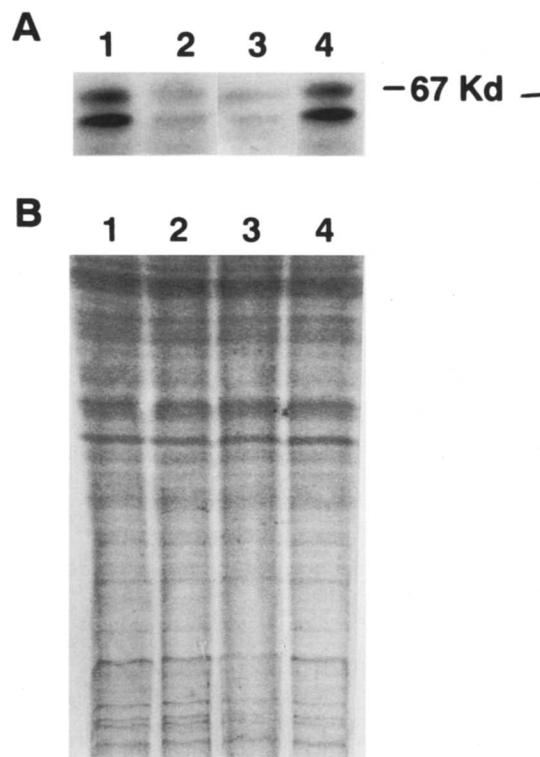


Fig. 5 Western blot analysis of the MYC protein accumulation in ARO cells treated with sense and antisense *myc*-specific oligomers. A, the proteins were extracted from the following sources: Lane 1, untreated ARO cells; Lane 2, ARO cells treated for 48 h with the antisense *myc*-specific oligomer (4 μ M/ml); Lane 3, ARO cells treated for 72 h with the antisense *myc*-specific oligomer (4 μ M/ml); Lane 4, ARO cells treated for 72 h with the sense *myc*-specific oligomer (4 μ M/ml). B, Coomassie blue staining of the parallel gel with the same samples as shown in A. Forty μ g proteins were loaded for each sample.

corporation after exposure to the oligonucleotides for 24 and 72 h. ARO and FRO thyroid carcinoma cell lines were used for these experiments, because they express high levels of *c-myc*-specific mRNA and MYC protein (Table 1).

Treatment with antisense *myc* oligonucleotides reduced the thymidine incorporation by the NPA, ARO, and FRO cells significantly (Table 3). The most striking inhibitory effect (about 80% compared with the untreated cells after 72 h) was exerted on the FRO cell line, which also had the highest expression of the MYC protein. These effects were specific, because no or only a very slight effect (inhibition of thymidine incorporation was not more than 10%) was detected in the experiments with sense or missense oligonucleotides. Moreover, inhibition of thymidine incorporation decreased with increasing concentrations of antisense *c-myc* oligonucleotides (data not shown). *c-myc* phosphorothioate antisense oligonucleotides exerted no inhibitory effect on a human thyroid primary culture (Table 3). These differences were not due to a different uptake of the antisense oligonucleotides by normal and neoplastic cells, because both cell types showed comparable uptake of the oligonucleotide (Table 4).

Sense and antisense oligonucleotides did not exert a significant toxic effect (<5%), measured by counting trypan blue-

Table 3 Inhibition of [³H]thymidine incorporation by *c-myc* antisense, sense, and missense oligonucleotides

Cell type	<i>c-myc</i> oligomer treatment ^a	% inhibition after 24 h ^b	% inhibition after 72 h
HPC	Sense	11 ± 3	10 ± 2
HPC	Antisense	12 ± 4	12 ± 2
ARO	Sense	8 ± 2	7 ± 2
ARO	Antisense	35 ± 5 ^c	50 ± 6 ^d
ARO	Missense	6 ± 3	8 ± 2
FRO	Sense	6 ± 2	9 ± 2
FRO	Antisense	65 ± 8 ^d	80 ± 10 ^d
FRO	Missense	6 ± 2	8 ± 4
NPA	Sense	9 ± 2	10 ± 3
NPA	Antisense	20 ± 5 ^c	34 ± 6 ^c
NPA	Missense	10 ± 3	12 ± 4

^a Subconfluent cells were treated with *c-myc* sense, antisense, and missense oligonucleotides, then [³H]thymidine incorporation was assayed after 24 and 72 h as described in "Materials and Methods."

^b Each value is the mean ± SE of at least three independent experiments performed in duplicate.

^c *P* < 0.05 versus the respective values obtained using the *c-myc* sense and missense oligonucleotides.

^d *P* < 0.01 versus the respective values obtained using the *c-myc* sense and missense oligonucleotides.

Table 4 Cellular uptake of labeled *myc* antisense oligonucleotide

Cell type	% oligonucleotide uptake ^a
HPC	0.80 ± 0.20
ARO	0.70 ± 0.23
FRO	0.93 ± 0.27
NPA	0.85 ± 0.15

^a The percentage of the oligonucleotide uptake was calculated by the number of counts in the cell pellet/total number of counts in the culture medium supernatant. Each value is the mean ± SE of three independent experiments performed in duplicate.

viable cells after the oligonucleotide treatment, even at a concentration of 40 μM, *i.e.*, 10-fold higher than the oligonucleotide concentration used in this study. The 40 μM *myc* antisense oligonucleotide concentration has been shown to be nontoxic in other cell types (34). The inhibitory effect of the *c-myc* antisense oligonucleotides was confirmed by a growth curve of the ARO and FRO cells in the presence of the sense or antisense *myc*-specific oligonucleotides (Fig. 6).

Moreover, colony-forming efficiency was evaluated for FRO and ARO cells treated with sense, missense, and antisense *c-myc* oligonucleotides; a significant reduction of the capability of both the cell lines to grow in a semisolid medium is induced by treatment with the antisense oligonucleotide (Fig. 7).

DISCUSSION

It is well known that high expression of the *c-myc* proto-oncogene correlates with an undifferentiated phenotype. The expression of *c-myc* is down-modulated rapidly on induction of differentiation pathways in mouse erythroleukemia cells, human promyelocytic HL-60, monoblastic U-937, and proerythrocytic K-562 cells, murine primary keratinocytes, and F19 embryonal carcinoma cells (37). Moreover, repression of *c-myc* expression by antisense *c-myc* oligodeoxyribonucleotides induces differen-

tiation of HL60 cells (33). Thyroid neoplasias are a useful model for studying whether *c-myc* expression is related to the expression of an undifferentiated phenotype. In fact, they include a broad spectrum of tumors with different phenotypic characteristics and different biological and clinical behavior: from the benign colloid adenomas through the slowly progressive, differentiated papillary and follicular carcinomas to the fatal anaplastic carcinomas (38).

We found higher expression of the *c-myc* proto-oncogene in seven human thyroid carcinoma cell lines than in normal thyroid cells or in normal thyroid tissue. This expression was much higher in the cell lines that expressed a higher degree of malignancy. Also, in fresh human tumors, *c-myc* overexpression was correlated closely with the anaplastic histotype. In contrast, *c-myc* expression was not enhanced in papillary and follicular carcinomas. The discrepancy between these data and those obtained from the carcinoma cell lines may be due to contamination of neoplastic tissue by normal thyroid tissue and tumor stroma and to the possibility that expression of *c-myc* transcripts might increase during their passages in culture. Moreover, the thyroid carcinoma cell lines were probably established from the most malignant thyroid carcinomas. Immunohistochemical analysis of paraffin blocks may reveal an increased level of MYC protein in papillary and follicular carcinomas, as has been demonstrated in 10 of 19 papillary thyroid carcinomas (39).

If this nuclear proto-oncogene represents the final pathway of the regulation of cell proliferation and differentiation, inhibition of nuclear oncogene expression should influence these processes. The results obtained in this study indicate clearly that *c-myc* overexpression is a very important event in the growth regulation of thyroid carcinoma cell lines, because *c-myc* antisense oligonucleotides inhibited the proliferation of carcinoma thyroid cell lines significantly. Moreover, the introduction of a dominant negative *myc* reduces the doubling time of the FRO cell line.⁴ Similarly, treatment of the CACO 320 cell line with *c-myc* antisense oligonucleotides inhibits their growth in semisolid medium (34). All these results indicate that the increase in the expression of the product of *c-myc* is an important event in the process of transformation of thyroid cells.

A number of mechanisms can be envisaged to explain the possible role of *c-myc* in thyroid cell transformation. It has been demonstrated that the protein coded for by the *Rb* gene and the *c-MYC* and *N-MYC* proteins can bind *in vitro* (40). Although such an association has not been demonstrated *in vivo*, the comicroinjection of *c-myc* and *Rb* suppresses the ability of *Rb* to arrest cell growth (40). Moreover, a dominant negative *myc* can block transformation by *ABL* oncogenes, indicating that *myc* overexpression is essential for the process of cell transformation induced by the *v-ABL* oncogene (41). Furthermore, the depletion of the *c-MYC* protein with specific antisense sequences reverses the transformed phenotype in *ras* oncogene-transformed NIH 3T3 cells, suggesting that a certain minimum level of *c-myc* is required to maintain *ras* transformation in NIH 3T3 cells (42). Indeed, our group has demonstrated that the *myc* oncogene cooperates with polyoma middle *T* and *v-ras* onco-

⁴ J. Cerutti, R. Visconti, M. Fedele, M. Santoro, and A. Fusco, manuscript in preparation.

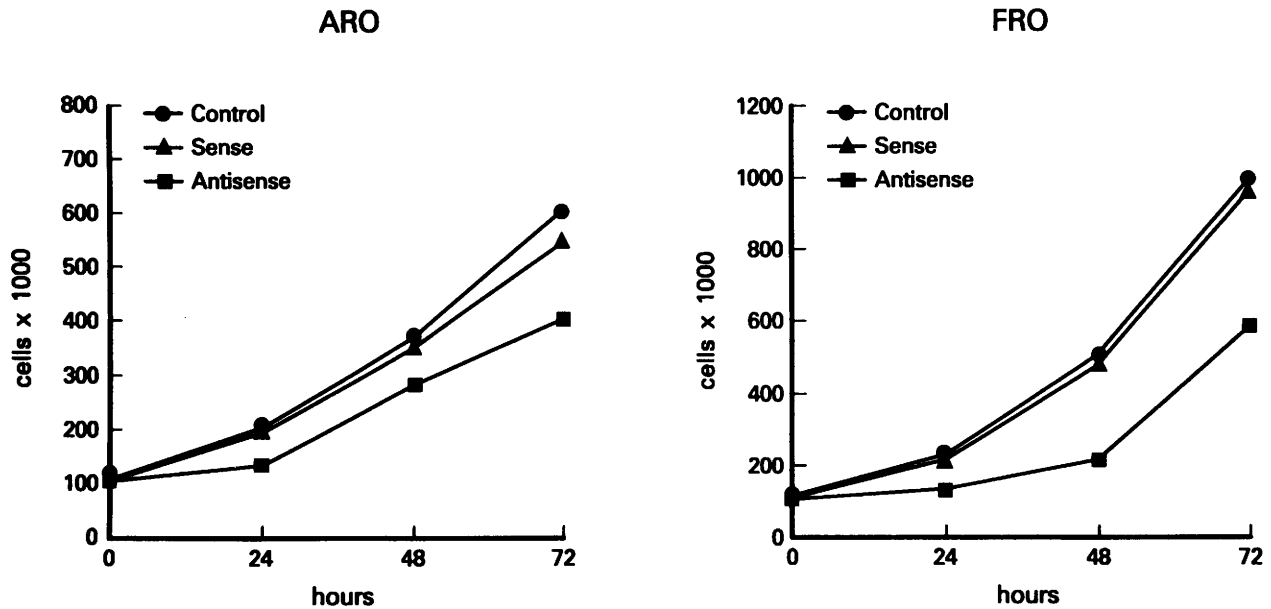


Fig. 6 Growth curves of ARO and FRO cells in the presence of *myc* sense- and antisense-specific oligonucleotides. One $\times 10^5$ cells/60-mm dish were treated with sense and antisense *c-myc*-specific oligomers (4 μ M/ml) and counted every 24 h.

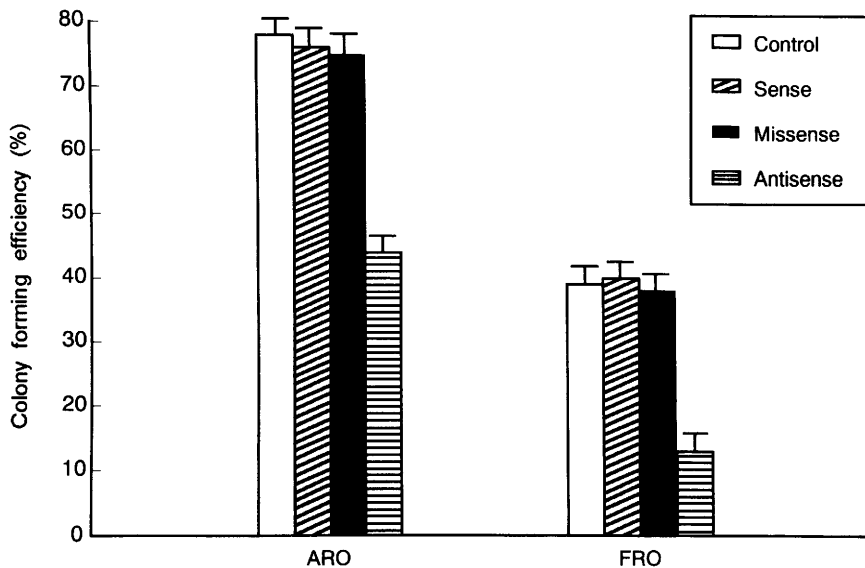


Fig. 7. Colony-forming capacity of the ARO and FRO cells in the presence of *myc* sense- and antisense-specific oligonucleotides. The cells were exposed to 8 μ M antisense, sense, or missense oligonucleotide before plating in soft agar. The colonies were counted after 10 days.

genes in the neoplastic transformation of rat thyroid cells (43, 44). Analogously, the overexpression of the *c-myc* gene could be essential for the human thyroid cell transformation induced by some oncogenes activated in thyroid neoplasias, including the *ras* genes detected with high frequency in the follicular histotype of human thyroid carcinomas (45–47) and the *RET/PTC* oncogene, found activated in about 20% of the thyroid papillary carcinomas (48). The activation of *ras* and *RET/PTC* must be considered an early event in the

process of thyroid carcinogenesis, because both were detected in benign adenomas (49) and in occult thyroid papillary carcinomas (50), whereas *c-myc* gene overexpression might be involved in the progression stage of thyroid carcinogenesis.

The inhibition of the proliferation of human thyroid carcinoma cells lines by *myc*-specific antisense oligonucleotides indicates a role of *c-myc* overexpression in the process of human thyroid carcinogenesis strongly.

ACKNOWLEDGMENTS

We are grateful to Drs. N. Fabien and C. Paulin for the B-CPAP cell lines. We thank Dr. Nicole Berger (Hôpital de l'Antiquaille de Lyon) for providing thyroid tumor samples. We are indebted to Dr. Nina Dathan for her help in writing the manuscript.

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