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Identification of a *c-myc* oncogene lacking the exon 1 in the normal cells of a patient carrying a thyroid carcinoma

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In this paper we describe an alteration of the *c-myc* oncogene present in the white blood cells and normal as well as neoplastic thyroid cells of a subject carrying a thyroid carcinoma. Restriction enzyme mapping and hybridization to human *c-myc* probes specific for different regions of this gene demonstrate that this subject carries, in addition to the normal one, a *c-myc* oncogene lacking the first exon and part of the first intron. The levels of the *c-myc* mRNA in thyroid cells of this subject do not show differences with respect to thyroid cells from other subjects. Taken together, these findings indicate that the deletion of the first exon of the *c-myc* oncogene, in itself, does not produce overtranscription of this oncogene nor hematopoietic malignancies.

c-myc *oncogene* *DNA alteration* *RNA level* *Neoplastic transformation*

1. INTRODUCTION

Much evidence indicates that cellular oncogenes are implicated in the genesis of cancer [1,2]. Alterations of the structure and expression of these genes are indeed frequently observed in neoplastic, but not in normal cells [1–3].

The *c-myc* oncogene, for instance, was found amplified, translocated and rearranged in tumors of different origins, such as leukemias, lymphomas and carcinomas [4–6]. In normal cells this gene is organized in 3 exons, the first of which represents a non-coding leader sequence and contains two promoter sites [5,7]. In some Burkitt lymphomas and leukemias a complete or a decapitated *c-myc* oncogene (lacking both the first exon and part of the first intron) is translocated to transcriptionally active regions of the human genome such as those containing the immunoglobulin heavy chain locus [5,7]. The rearranged *c-myc* oncogenes are overexpressed, while the normal gene is silent or expressed at very low levels [5]. Recent experiments reported by a number of laboratories suggest that there are probably multiple steps in the transcriptional activation of this oncogene including its translocation near enhancer elements [5], point mutations or the deletion of regulatory sequences involved in the repression of its expression [8,9].

Here, we describe a partial deletion of the *c-myc* oncogene identified in a subject carrying a thyroid carcinoma. The alteration of the *c-myc* oncogene was found to be present in the peripheral white blood cells, and neoplastic thyroid cells as well as the corresponding normal thyroid cells, being therefore presumably fixed in the genome of this subject.

2. MATERIALS AND METHODS

2.1. *DNA preparation*

DNA was isolated from peripheral blood cells and thyroid tissues by proteinase K treatment and phenol-chloroform extraction as described [10].

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2.2. *Southern blotting*

Approx. 10 μg of each DNA sample were digested with one or two restriction enzymes following experimental conditions specified by the supplier (Boehringer-Mannheim). Restriction enzyme generated fragments were electrophoresed on 0.8% agarose gels and transferred to nitrocellulose filters (Gene Screen-NEN) [10].

2.3. *Cytoplasmic dot hybridization*

Cytoplasmic preparations were obtained from white blood cells or tissue slices and treated with formaldehyde as in [10].

2.4. *Filter hybridization*

The filters were prehybridized and hybridized in the presence of 50% formamide and dextran sulfate as reported [10] with nick-translated [^{32}P]-DNA probes (spec. act. 10^8 cpm/μg) [10]. After 40 h of hybridization for DNA and 24 h for RNA, the filters were washed [10] and exposed to Kodak X Omat AR5 with intensifying screens (Dupont).

3. RESULTS

DNAs from the white blood cells of patients carrying different solid tumors were digested with *Eco*RI restriction enzyme, which recognizes DNA sequences outside of the *c-myc* oncogene, as indicated in the map shown in fig.1.

Fig.2 shows the autoradiographic patterns obtained after hybridization of the 3′ *c-myc* DNA probe to the *Eco*RI-restricted DNAs isolated from 13 different subjects. The 12.5 kb band expected as for normal DNA is present in all the samples. In case 6, an additional band, 8.5 kb in size, is present (fig.2, left). A similar double band pattern is also present in the normal (N) as well as neoplastic (C) thyroid tissues of this subject (fig.2, right). The abnormal *c-myc Eco*RI pattern could be compatible with (i) a DNA deletion of about 4 kb or (ii) an *Eco*RI polymorphism which introduces a new *Eco*RI site in one of the two *c-myc* genes of this subject.

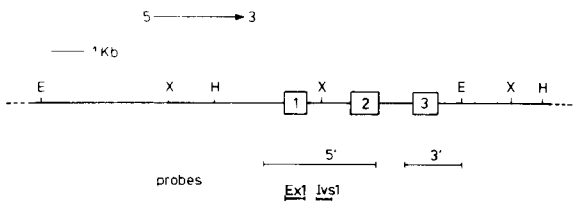

Fig.1. *c-myc* gene probes employed: pMC415PP, 5′ probe [3]; pMC413RC, 3′ probe [3]; the exon 1 probe is an *Xho*I-*Pvu*II fragment [14] and the intron 1 probe an *Sac*I-*Sac*I fragment (kind gift from Dr F. Mavilio, Lab. Ematologia, Ist. Superiore di Sanità, Rome). The restriction enzyme map of the human *c-myc* oncogene was derived from [3]. X, *Xba*I; E, *Eco*RI; H, *Hin*dIII. Boxes represent exons 1-3 of the oncogene.

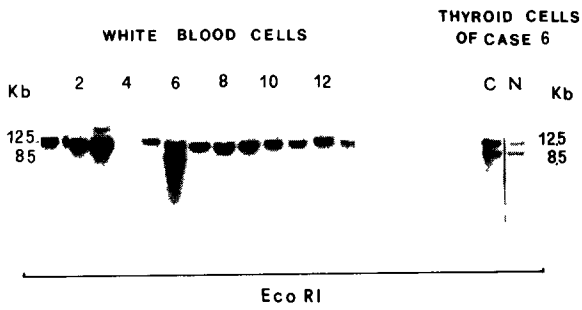

Fig.2. Left: autoradiographic pattern of the *c-myc* specific fragments obtained after *Eco*RI digestion of DNA from subjects carrying different solid tumors, including lung, thyroid and colon carcinomas. Nitrocellulose filters were hybridized to the 3′ *c-myc* probe. The faint band in case 3 is due to incomplete digestion. Right: Southern blot of the DNA from normal (N) and carcinomatous (C) thyroid samples of case 6 are also shown.

To distinguish between these two possibilities the DNA of case 6 was digested with *Xba*I and *Hin*dIII restriction enzymes, used alone or in combination with *Eco*RI.

In addition to the normal fragment as expected from the map in fig.1, an abnormal band is obtained after *Xba*I, *Eco*RI and *Hin*dIII digestion and hybridization to the 3′ *c-myc* probe (fig.3.). After *Eco*RI and *Hin*dIII digestion, the additional DNA fragment is smaller in size, whereas it is bigger after *Xba*I digestion.

These results clearly indicate that the abnormal *c-myc Eco*RI pattern is not caused by an *Eco*RI polymorphism, but might be due to a deletion of at least 4 kb which starts after the first *Hin*dIII site (see the map in fig.1) and eliminates the *Xba*I site located in the first intron.

To analyse more accurately this putative 4 kb deletion within the *c-myc* gene, we have hybridized the *Eco*RI-generated fragments of case 6 with

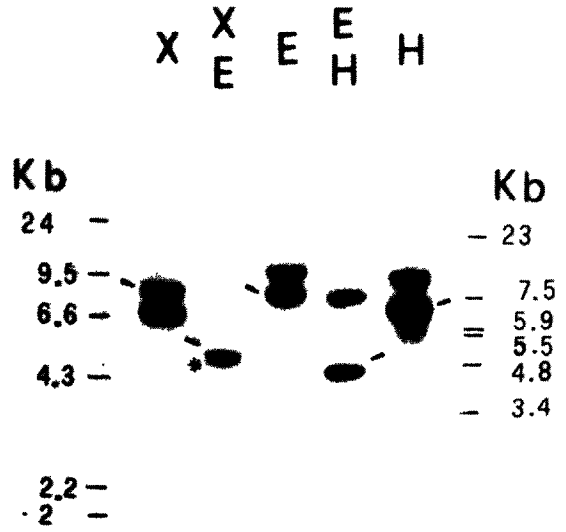

Fig.3. *c-myc* specific fragments of carcinomatous thyroid DNA from case 6 after digestion with *Xba*I (X), *Xba*I-*Eco*RI (X-E), *Eco*RI (E), *Eco*RI-*Hin*dIII (E-H) and *Hin*dIII (H). Fragments not expected for normal DNA are indicated by arrowheads. The asterisk indicates the presence of a double band barely visible in the photograph. This double band contains the abnormal fragment slightly separated from the normal expected one. On the left and right are shown *Hin*dIII (left) and *Eco*RI (right) digested λ phage DNA.

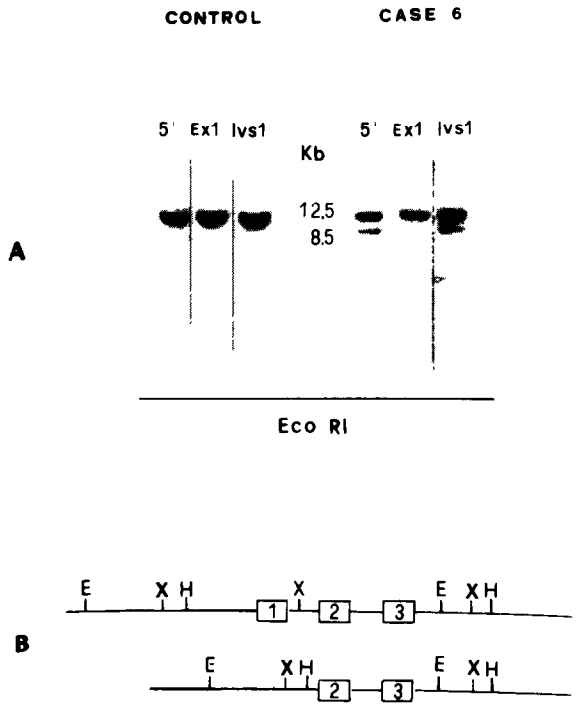

Fig.4. (A) *c-myc* specific fragments in the white blood cell DNA from a normal subject (control) and case 6. The DNA was digested with *Eco*RI restriction enzyme, and the nitrocellulose filters hybridized with *c-myc* probes specific for the 5'half, exon 1 (Ex1) and intron 1 (Ivs1) of the gene. (B) Proposed restriction enzyme map of the DNA of subject 6, containing the normal as well as truncated *c-myc* oncogene as deduced by data from fig.3 and this figure.

DNA probes specific for different regions of the gene such as the 5′ half (5′), exon 1 (Ex1) and intron 1 (Ivs1) (see fig.1.). The results are presented in fig.4A.

The additional *Eco*RI band of 8.5 kb hybridizes with the 5′ probe, which includes exon 1, intron 1 and part of exon 2, and with the Ivs1 probe, but does not do so with the Ex1 probe. Accordingly, only normal *c-myc* DNA fragments were obtained after re-hybridization of the Southern blot shown in fig.3 with the Ex1 *c-myc* probe (not shown).

Taken together the results obtained after hybridization with the Ex1 probe and by using 3 restriction enzymes (*Xba*I, *Hin*dIII and *Eco*RI) strongly indicate that the deletion includes the first exon leaving intact the second and third exon (see map in fig.4B).

The possible overexpression of this truncated oncogene was investigated in both the normal as well as carcinomatous thyroid samples of this subject by using cytoplasmic dot blot hybridization as described elsewhere [10].

The results obtained in the normal thyroid samples from a control individual and subject 6 are shown in fig.5.

The levels of *c-myc* mRNA, hybridized to the 3′ *c-myc* probe were normalized with respect to their ribosomal RNA content, evaluated after hybridization with the plasmid pXCR7, carrying sequences specific for 28 S and 18 S rRNAs.

These data indicate that in the cells of case 6, carrying the abnormal oncogene in addition to the normal one, no significant increase in overall *c-myc* mRNA levels is appreciable. Similar results were obtained studying *c-myc* RNA expression in the carcinomatous thyroid of subject 6 (not shown). By contrast, much higher levels of *c-myc*

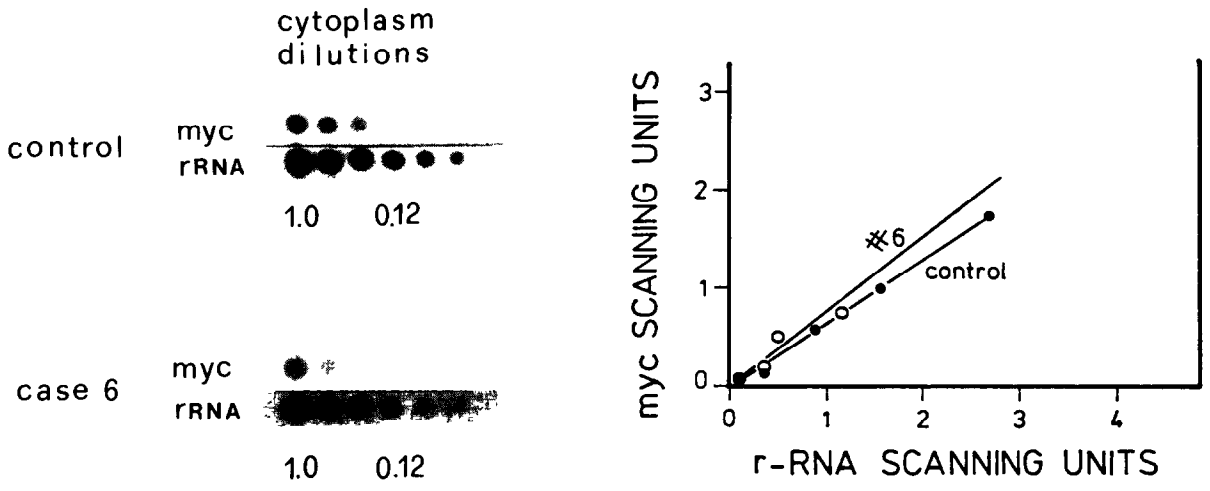

Fig.5. Cytoplasmic dot blot hybridization of normal thyroid samples from a subject carrying a goiter (control) and subject 6, carrying a papillary carcinoma. The autoradiographic spots after filter hybridization with 3′ *c-myc* probe and pXCR7 ribosomal probe are shown (left) as well as the plots of their densitometric analysis (right).

mRNA were detected in other tumor cells such as HL-60 or cells isolated from a colon carcinoma and a kidney carcinoma (not shown).

4. DISCUSSION

Alterations in the structure of the *c-myc* oncogene such as duplications, translocations, or point mutations have been reported by several authors in different tumors such as leukemias, lymphomas and carcinomas, but not in normal cells [2–5].

After the analysis of *Eco*RI *c-myc* specific fragments from the white blood cells of 50 subjects carrying solid tumors, we found only one subject showing alteration of the *c-myc* oncogene. This alteration was present in both the blood cells and the thyroid side of the tumor. This subject might therefore be considered heterozygous for a rare variant of the *c-myc* gene.

The alteration observed was further characterized and turned out to be a deletion involving the first exon, which does not codify for the *c-myc* protein [5–7] but does contain promoter sites, being therefore important for the correct transcription of the *c-myc* gene [5,7,8].

Since after hybridization with the exon 1 probe no *Eco*RI fragment was observed in addition to the 12.5 kb band (fig.4), we exclude, in this case, the presence of a translocation. In addition, the analysis of the chromosome map of this subject does not reveal any rearrangement and in situ hybridizations with the 3′ *c-myc* probe show appreciable radioactivity only on the long arm of chromosome 8 (not shown).

The isolation and characterization of *c-myc* specific clones from a genomic library of this subject would be useful to define the extent of the deletion of DNA sequences upstream of the first exon of the *c-myc* oncogene.

Expression of the *c-myc* oncogene in the thyroid cells of the case presenting the *c-myc* gene alteration is at the same level of other normal thyroid samples examined.

These results suggest that exon 1 is nor primarily involved in the negative regulatory functions during the transcription of the *c-myc* gene, as contrastingly proposed by Leder et al. [8] and Kelly et al. [11].

In addition, it is remarkable that no tumor cells are present in the peripheral blood cells of this patient.

Our data therefore are of interest when related to the studies proposed by Saito et al. [12] and Darveau et al. [13] which indicate that in both mouse and man the translation of full-length *c-myc* mRNA is much less efficient compared to that of the *c-myc* mRNAs carrying deletions of the

5′-non-coding region. Similar post-translational control was hypothesized to cause several Burkitt lymphomas [12].

Taken together, the present data demonstrate that (i) the presence of a truncated *c-myc* oncogene lacking exon 1 might be uncoupled with hematopoietic malignancies and (ii) the deletion of the first exon, in itself, does not cause over-transcription of this oncogene.

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REFERENCES

[1] Bishop, J.M. (1983) Annu. Rev. Biochem. 52, 301–354.
[2] Land, H.L., Parada, L.F. and Weinberg, R.A. (1983) Science 222, 771–778.
[3] DAlla Favera, R., Martinotti, S., Gallo, R.C., Erikson, J. and Croce, C.M. (1983) Science 219, 963–967.
[4] Collins, S. and Groudine, M. (1982) Nature 298, 679–681.
[5] Croce, C.M. and Nowell, C.P. (1984) New Trends in Experimental Hematology (Serono Symposia) 7, 240–253.
[6] Little, C.D., Nau, M.N., Carney, D.N., Gadzar, A.F. and Minna, J.D. (1983) Nature 306, 194–196.
[7] Battey, J., Moulding, C., Taub, R., Murphy, W., Steward, T., Potter, H., Lenoir, G. and Leder, P. (1983) Cell 34, 779–787.
[8] Leder, P., Battey, J., Lenoir, G., Moulding, C., Murphy, W., Huntington, P., Stewart, T. and Taub, R. (1983) Science 222, 705–770.
[9] Rabbits, T.H., Hamlyn, P.H. and Baer, R. (1983) Nature 306, 760–765.
[10] Gambari, R., Del Senno, L., Barbieri, R., Viola, L., Tripodi, M., Raschellà, G. and Fantoni, A. (1984) Cell Differ. 14, 87–97.
[11] Kelly, K., Cochran, B.H., Stiles, C.D. and Leder, P. (1984) Cell 35, 603–610.
[12] Saito, H., Hayday, A.C., Wilman, K., Hayward, W.S. and Tonegawa, S. (1983) Proc. Natl. Acad. Sci. USA 80, 7476–7480.
[13] Darveau, A., Pelletier, J. and Sonenberg, N. (1985) Proc. Natl. Acad. Sci. USA 82, 2315–2319.
[14] Watt, R., Nishikura, K., Sorrentino, J., Ar-Rushdi, A., Croce, C.M. and Rovera, G. (1983) Proc. Natl. Acad. Sci. USA 80, 6307–6311.