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Coordinated Expression of c-*myc* Gene and a Multigenic Set May Modulate the Malignant Phenotype in Human Haemopoietic Cell Lines*

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A. Introduction

We previously showed that about 2000 non repeated DNA sequences of 3 Kbs each, designated as tumor-activated DNA (Ta-DNA), are transcriptionally activated in human neoplasias [1, 2]. A growing body evidence supports the concept that oncogenic transformation may depend on abnormal activation of cellular oncogenes (see general reviews [3, 4]), resulting in gene amplification, chromosomal rearrangements [5], or point mutations [6] and possibly involving cooperative oncogenes [7].

The aim of the present study was to search for possible correlations between tumor-activated DNA (TaDNA) activation and abnormal activation of oncogenes. For that purpose, we investigated the effects of various differentiation inducers in three lines of malignant hemopoietic cells characterized by translocation and/or abnormal expression of c-*myc*.

B. Cell Cultures and Treatment with Different Inducers

Raji and Namalwa cells (two Burkitt's lymphoma-derived lines), Epstein-Barr virus (EBV)-immortalized normal lymphocytes, the Priess cells, and HL-60 cells (a myeloid leukemia-derived cell line) were grown in suspension in conditions already described [1, 2]. They were seeded at cell concentrations of $2.5–3 \times 10^5$ cells/ml, treated with different inducers 24 h after seeding, and collected 24 h later for measuring the levels of TaDNA or *myc*-transcripts, or at different times for analysis of cell multiplication and differentiation. The HEL cells (normal skin fibroblasts) were cultured in monolayers and treated before attaining confluence. We used various agents known to be efficient differentiation inducers for HL-60 cells: DMSO, retinoic acid, mezerein, TPA [8, 9] and teleocidin [10]. Phorbol esters induce macrophage characteristics whereas DMSO or retinoic acid favor the appearance of granulocytic or megakaryocytic cells [8, 9]. TPA is of particular interest because this potent tumor promoter for skin cancer can modulate cell phenotypes in opposite directions, depending on the cell line studied [9]. In particular, TPA can induce various different markers in Burkitt's lymphoma lines or, in normal cells, provoke pleiotropic changes which mimic those associated with oncogenic conversion. A chemical analog of TPA, 4α-phorbol-12,13-didecanoate (4α-PDD) which is almost devoid of activity [10] was used as control.

I. Hybridizations Liquid

Cellular RNA and Raji cell DNA were prepared as described [1]. A single-stranded DNA fraction, greatly enriched in transcribing sequences, was labeled with ^{125}I, and depleted of sequences shared with Priess cells by successive cycles of hybridization with RNA from Priess and Raji cells,

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both cells proliferating with similar growth rates [11]. Centrifugations in cesium sulfate gradients allowed the separation of DNA–RNA hybrids from nonhybridized DNA sequences [1, 2].

II. Dot Blot Hybridizations

Dot blot hybridizations were performed following Thomas [12]. The c-*myc* probe, consisting of a cloned 1.5 kilobases Sac I restriction fragment containing most of the two exons (plasmid obtained from Dr. D. Stehelin) was nick-translated as described by Maniatis et al. [13] with ^{32}P-labeled dCTP (NEN) (3000 Ci/mmol) to obtain specific activities in the range of $5–8 \times 10^8$ cpm/μg.

C. Results

I. Cell Differentiation

When treated with potent inducers like TPA in the range of 15–20 n*M*, 80%–90% of the HL-60 cells became attached to the plastic support and the activities of two enzymatic markers (an esterase and an acid phosphatase) were increased four- to six-fold. The same treatment in Raji or Namalwa cells caused a significant decrease in the amounts of two antigenic markers (cALL and HLAdr) of the lymphoid precursor cells.

II. Cell Multiplication

Similar growth rates, at least for 48 h after seeding, were observed in all hemopoietic cell lines studied. Low concentrations (15–20 n*M*) of TPA, mezerein, or teleocidin caused an almost complete arrest of multiplication of malignant cells, but did not affect that of Priess cells. Greater concentrations of DMSO (1.3% = 60 n*M*) and retinoic acid (0.1–1 μ*M*), which are known to induce the differentiation of HL-60 cells, resulted in a 50%–55% reduction in the multiplication rates. In control assays, 4α-PDD (10–100 n*M*) had no effect on cell differentiation and growth rates.

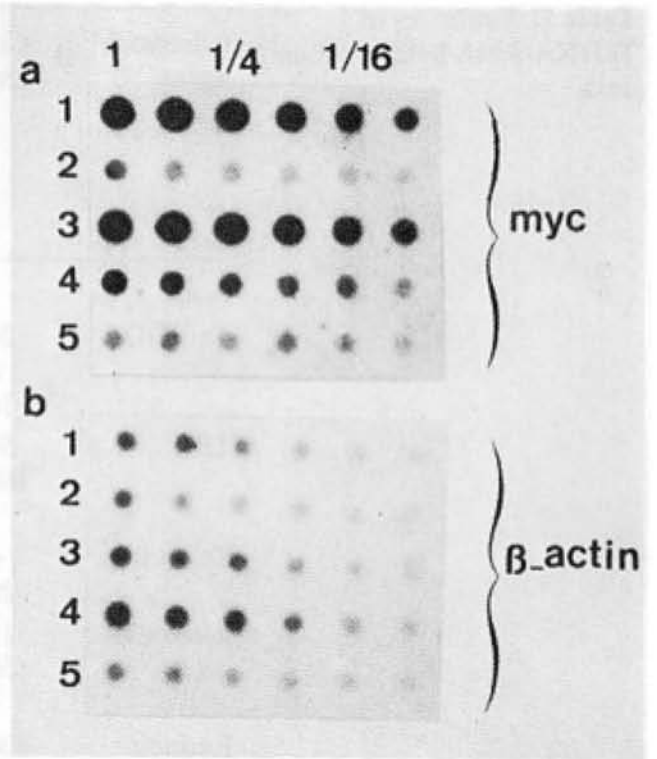

Fig. 1 a, b. Dot blot hybridizations showing the level of c-*myc* RNA in HL-60 cells treated with various agents. From left to right, the dots contained decreasing amounts (by half) of total cellular RNA: 2.5 μg, 1.25 μg, etc. *Row* 1: no treatment; *row* 2: 15 n*M* mezerein; *row* 3: 20 n*M* 4α-PDD; *row* 4: 20 n*M* teleocidin; *row* 5: 1 μ*M* retinoic acid. **a** the blots were prehybridized overnight at 42 °C in 50% formamide (v/v), 0.75 *M* NaCl–0.075 Na-citrate–0.05 *M* Na-phosphate buffer pH 6.5, containing 125 μg/ml sonicated, denatured salmon sperm DNA and 0.02% each of bovine serum albumin, Ficoll, and polyvinylpyrrolidone. They were thereafter hybridized for 24 h with the c-*myc*-specific probe (10^6 cpm/ml) in the same medium, **b** internal controls: the same blots were dehybridized (by boiling 30 min in 0.015 *M* NaCl, 0.0015 *M* Na-citrate, 0.1% SDS) and rehybridized with β-actin-specific recombinant DNA probe (obtained from Dr. A. J. Mirty, Institut Pasteur, Paris)

III. Effects on TaDNA Transcripts

The percentages of TaDNA forming DNA–RNA hybrids at saturation levels, with an excess of total RNA from the various treated or untreated cells are summarized in Table 1. It is clear that, in malignant cells, the various differentiation inducers caused a roughly dose-dependent reduction in the population of TaDNA transcripts. It was also noticed that a tenfold higher concentration of mezerein than TPA is required to

Table 1. Summary of TaDNA-RNA hybridization data

Chemical agent	Concentration	Percentage TaDNA hybridized[a] to RNA from				
		Malignant cells			Normal cells	
		HL-60	Raji	Namalwa	Priess	HEL
None		74±3	81±5	76±4	5±1.5	5±2
4α-PDD	20 n*M*	70	76	73	–	–
	3 n*M*	51	44.5	46	–	–
	16 n*M*	10.5	14	15.5	31	42.5
TPA	50 n*M*	–	–	–	40.5	58
	160 n*M*	–	–	–	58	–
	1.6 μ*M*	–	–	–	85	–
Teleocidin	4 n*M*	48	–	42.5	–	–
	20 n*M*	13	–	13	–	–
Mezerein	15 n*M*	68	–	–	–	–
	150 n*M*	11.5	–	–	28.5	–
	1.5 μ*M*	9	–	–	45	–
Retinoic acid	400 n*M*	40	–	–	–	–
	1 μ*M*	12	–	–	22.5	–
	10 μ*M*	–	–	–	35	–
DMSO	60 m*M*	16	–	–	–	–

[a] The percentages indicated are those obtained by the use of S1 nuclease at apparent saturation levels [1, 2]. In some crucial experiments, isopyknic centrifugation (in cesium sulfate gradient) confirmed that the S1 nuclease-resistant molecules exclusively consisted of DNA-RNA duplexes [1, 2]

obtain the same level of inhibition of TaDNA activity, although these agents are equipotent differentiation inducers. On the contrary, in normal cells TPA treatment caused the occurrence of RNA species, hybridizable up to 85% of the Raji cell TaDNA, in a dose- dependent manner, whereas only 4%–5% of the latter could form hybrids with RNA from untreated normal cells. At much higher concentrations, mezerein and retinoic acid produced partial enhancing effects.

IV. Effects on c-*myc* RNA

The treatment of HL-60 cells with optimal amounts of each differentiation inducer resulted in a drastic reduction (at least tenfold) in the level of c-*myc* RNA (Fig. 1). This reduction in the level of c-*myc* RNA was also found in Namalwa cells treated with TPA whereas this level was not modified in Priess cells treated with higher amounts of TPA (not shown). We have established with an actin gene-specific probe that none of the agents studied caused a significant reduction in the level of actin-specific RNA.

D. Conclusions

We have shown that the treatment of three distinct malignant cell lines, characterized by translocation and/or abnormal activation of the c-*myc* gene, with several chemically unrelated differentiation inducers, produces a drastic reduction in the levels of both d-*myc* RNA and TaDNA-specific RNA. This cannot be simply due to the cell growth inhibition caused by the differentiation inducers, for several reasons, in particular because the majority of TaDNA sequences, which were silent in normal lymphoid cells and in normal fibroblasts,

both growing exponentially, became transcriptionally active within 24 h of treatment with TPA which did not change their proliferation rates. These opposite effects of TPA suggest that this agent (and probably other agents as well) may operate either as a tumor promoter or a differentiation inducer through a common control mechanism of the TaDNA transcripts. Finally, our results suggest that TaDNA corresponds to a multigenic set which modulates the malignant phenotype, possibly in cooperation with c-*myc* or other oncogenes implicated in the initiation and genotypic maintenance of cancer. If one considers that TaDNA corresponds to a common genomic domain consisting of many distinct transcription units which are coordinated in their activation or regulation, it is unlikely, although not impossible, that the unique role of this domain is to control the cancer phenotype. The same is true for cellular oncogenes and, in fact, recent data support the hypothesis of normal functions for oncogens. This is the case for c-*myc* which appears to be involved in control of the cell cycle (for example see [14]). It is tempting to assume that TaDNA is normally implicated at early stages of embryogenesis or in the regulation of certain differentiation pathways. Further advances in the understanding of TaDNA must await the construction of a library of TaDNA sequences which is under way in our laboratory.

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