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An Essential Role for Polyamine Biosynthesis during Human Granulopoietic Differentiation[1]

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ABSTRACT

The role of polyamines during the process of colony-stimulating factor-induced proliferation and differentiation of human granulocyte-macrophage progenitor cells was studied using *in vitro* liquid cultures and semisolid agar cultures. Human placenta-conditioned medium, a partially purified preparation of colony-stimulating factor, rapidly and significantly increased the intracellular putrescine concentration of light-density, nonadherent, T-lymphocyte-depleted human marrow cells. Inhibition of polyamine biosynthesis with α-difluoromethylornithine, a catalytic, irreversible inhibitor of ornithine decarboxylase, resulted in an accumulation of blasts, promyelocytes, and myelocytes with a concomitant decrease in metamyelocytes and polymorphonuclear granulocytes, suggesting a granulopoietic differentiation block. The observed inhibition of differentiation could easily be reversed with exogenously added putrescine. These data indicate clearly an essential role for polyamines during the process of human granulopoietic differentiation.

INTRODUCTION

The naturally occurring polyamines putrescine, spermidine, and spermine are ubiquitous in biological systems (1, 3). The stimulation of cell growth and division, both *in vitro* and *in vivo*, is associated with an increase in the rate of polyamine biosynthesis, *e.g.*, initiation of growth in quiescent cell cultures by insulin or serum (8), after partial hepatectomy in rats (9, 19), during embryonic development of a number of animal species (2, 4, 6, 7), and after infection of cells with tumorigenic viruses (7).

ODC,[3] the rate-limiting enzyme in the polyamine-biosynthetic pathway, has been shown to increase rapidly in cells stimulated with a number of trophic hormones (20, 21, 24), suggesting a requirement for polyamines in cellular proliferation and differentiation. The availability of specific inhibitors of polyamine biosynthesis made it possible to show an essential role for putrescine and spermidine in DNA synthesis (5, 11, 22) and for cytokinesis of mammalian cells (23).

In the present study, we have investigated the role of polyamines during the process of CSF-induced differentiation of human GM-CFC into granulocytes and monocytes. Our results indicate an enhanced synthesis of polyamines, particularly putrescine and spermidine, during this differentiation process. Further, inhibition of polyamine biosynthesis by DFMO, a specific irreversible inhibitor of ODC (14), resulted in an inhibition of differentiation that could be reversed easily by an exogenous supply of putrescine. These results suggest an essential role for polyamines during granulopoietic differentiation.

MATERIALS AND METHODS

Specimen Collection. Human marrow cells, obtained from normal volunteers after their written signed consent, by posterior iliac crest puncture under local anesthesia, were collected in polystyrene tubes (Corning Glass Works, Corning, N. Y.) containing 0.3 ml preservative-free heparin (1000 units/ml) in 1.7 ml phosphate-buffered saline.

Preparation of Marrow Target Cells. Light-density (≤1.070 g/ml) mononuclear cells were obtained by density gradient centrifugation over a Ficoll-Hypaque column. The interface cells were harvested, washed twice with α-MEM plus 15% FCS, and subjected (2×10^6/ml) to adherence in 35-mm plastic tissue culture Petri dishes (Corning Glass Works) for 3 hr at 37° in an atmosphere of 5% CO_2 and air. Subsequently, the nonadherent cells were aspirated and resuspended in α-MEM plus 15% FCS at ~1×10^6/ml concentration. These cells were used for growing GM-CFC in a semisolid agar culture system. To investigate the effects of CSF-induced changes in polyamine levels and their alteration by DFMO, we used light-density, nonadherent, and T-lymphocyte-depleted marrow cells. T-lymphocytes were separated out using the nonimmune rosetting technique with neuraminidase-treated sheep erythrocytes described by Wiener *et al.* (26). After this procedure, marrow had <1% sheep erythrocyte-rosetting cells.

Culture Methods. To grow GM-CFC *in vitro*, we used the modification of the semi-solid agar culture system described by Pike and Robinson (17). Briefly, 1-ml underlayers of a mixture of 0.5% Bacto-Agar (Difco Laboratories, Detroit, Mich.) with α-MEM and 15% FCS were prepared in 35-mm Petri dishes. As a source of CSF, we used HPCM (10% v/v) instead of peripheral mononuclear cells (25). After they were solidified, the underlayers were overlaid by 10^5 or 2×10^5 light-density, nonadherent human marrow cells suspended in a 1-ml mixture of 0.3% Bacto-Agar and α-MEM plus 15% FCS. To investigate the effect of DFMO or putrescine, we incorporated their appropriate concentrations in the upper layer of the cultures. The cultures were incubated in triplicate at 37° in a fully humidified atmosphere of 5% CO_2 and air and scored after 8 days for colonies (aggregates of ≥40 cells) and clusters (aggregates of 4 to 39 cells) under an Olympus dissecting microscope. The results were reported as a mean ± S.D. of the triplicate cultures. Control cultures that did not contain HPCM grew no colonies.

Morphological Examination of Colonies and Clusters. The morphological examination of the colonies and clusters was performed to check the granulopoietic differentation pattern of each isolated cell aggregate.The method used has been described before (25).

Measurement of Polyamine Levels. The light-density, adherent cell, and T-lymphocyte-depleted human marrow cells were grown in α-MEM plus 15% FCS (5×10^5/ml) in the presence or absence of HPCM with or without DFMO. The cells were harvested on Days 1, 3, and 7 of

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[3] The abbreviations used are: ODC, ornithine decarboxylase; CSF, colony-stimulating factor; GM-CFC, granulocyte-macrophage colony-forming cells; DFMO, α-difluoromethylornithine, α-MEM, α-modified minimum essential medium; FCS, heat-inactivated fetal calf serum; HPCM, human placenta-conditioned medium; GM, granulocyte macrophage.

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culture and frozen at −20°. No differences in the cell viability were noted in different groups of cultures, as measured by the trypan blue dye exclusion method. To measure polyamine levels, we thawed the cell pellets, resuspended them in 0.2 ml of 4% sulfosalicylic acid, and then sonicated them, using a Branson Sonifier. The homogenates were then centrifuged at 10,000 × *g* in a Sorvall RO2B centrifuge at 4°. The supernatants thus obtained were used to estimate putrescine, spermidine, and spermine levels in a Durrum amino acid analyzer, according to the method of Marton and Lee (12). The results presented represent the average of 2 estimates per observation.

Statistical Methods. Statistical comparison of mean colony or cluster numbers were made by 2-sided 2-sample *t* tests.

RESULTS

HPCM-induced Changes in Polyamines in Adherent Mononuclear Cell- and T-Lymphocyte-depleted Human Marrow Cells. Table 1 illustrates the cellular levels (pmol/4 × 10^6 cells) of putrescine, spermidine, and spermine before and after the adherent cell and T-lymphocyte-depleted marrow cells were stimulated with HPCM. Over a period of 7 days in liquid culture, HPCM induced a 3-fold increase in the putrescine levels. The spermidine levels showed a 9% decline on Day 1, and by Day 7 there was an overall increase of 30%. The spermine levels, on the other hand, rose progressively to 180% of the original level by Day 7 of culture. However, DFMO (0.25 and 0.5 mM), regardless of the concomitant presence of HPCM, decreased putrescine to undetectable levels (Table 1) in the marrow cells. Spermidine levels showed an absolute decrease of 33% at 0.25 mM DFMO and 24% at 0.5 mM DFMO over a 7-day period; the decrease was even more in the presence of HPCM (61 and 70%, respectively). In contrast, spermine rose by 25 and 40% of the control level (Day 0) in the presence of 0.25 and 0.5 mM DFMO alone. In the presence of HPCM, DFMO either caused no change or a small decrease in spermine level on Day 7 (64% of control at 0.5 mM DFMO).

GM Colony and Cluster Formation. Table 2 presents the percentage of changes in the number of colonies and clusters in the presence or absence of various concentrations of DFMO. With increasing concentrations of DFMO, a progressive decline in the colony numbers was observed. This was associated with a corresponding increase in the number of clusters. However, there was no change in the total number of all types of cell aggregates. These data suggested that there was an inhibition of clonal growth of GM-CFC.

Table 1

Effect of DFMO on adherent mononuclear cell- and T-lymphocyte-depleted human marrow cell contents of polyamines

No significant change in the cellular contents of polyamines was noted over 7 days in cultures containing no HPCM or DFMO.

Treatment	Day of culture	Concentration of polyamines (pmol/4 × 10^6 cells)		
		Putrescine	Spermidine	Spermine
Untreated	0	41	741	572
HPCM alone	1	86	670	416
	3	110	700	768
	7	121	963	960
DFMO (0.25 mM)	1	0	616	486
	3	0	597	432
	7	0	493	713
DFMO (0.5 mM)	1	0	554	506
	3	0	447	624
	7	0	566	803
HPCM + DFMO (0.25 mM)	1	0	749	267
	3	0	317	460
	7	0	287	504
HPCM + DFMO (0.5 mM)	1	0	740	624
	3	0	258	511
	7	0	222	311

Table 2

Effect of DFMO on granulocyte-macrophage colony formation in agar cultures

Type of cell aggregates	No. of cell aggregates without DFMO (control)	Cell aggregates as % of control in the presence of various DFMO concentrations			
		0.125 mM	0.25 mM	0.5 mM	1.0 mM
Colonies[a]	127 ± 9[b]	40	17	9	0
Clusters[c]	180 ± 10	137	154	168	163
Total	307 ± 7	97	97	102	96

[a] Aggregates of 40 cells scored on Day 8 of culture.
[b] Mean ± S.D. of triplicate cultures per 10^5 nonadherent human marrow cells.
[c] Aggregates of 4 to 39 cells scored on Day 8 of culture.

Table 3

Effect of DFMO on differentiation pattern of GM-CFC clonal growth

DFMO concentration (mM)	Differential counts of cell aggregates (%)[a]				
	Blasts and blast-like cells	Promyelocytes	Myelocytes	Metamyelocytes + bandforms	Segmented granulocytes
0.0	1.0	3.6	27.7	27.7	40.0
0.125	3.9	10.2	49.8	22.6	13.5
0.25	8.5	15.0	51.0	17.5	8.0
0.5	11.9	19.3	53.3	11.3	4.2
1.0	18.3	23.8	48.5	7.0	2.4

[a] Mean percentage derived from cumulative data from 40 to 50 clusters/observation.

To investigate whether this inhibition of the GM-CFC clonal growth was associated with differentiation arrest, we morphologically examined 40 to 50 clusters from each observation. Table 3 shows the percentage of mean differential count from these clusters. In the absence of DFMO, the clusters showed a normal differentiation pattern of granulopoiesis with 27.7 metamyelocytes plus bands and 40% segmented granulocytes by Day 8 of culture. However, in the presence of increasing DFMO concentrations, a granulopoietic differentiation arrest was noted at progressively earlier stages of maturation. These data indicated that inhibition of polyamine biosynthesis by DFMO caused a granulopoietic differentiation block.

Reversal of DFMO-induced Clonal Differential Block of GM Progenitor Cells by Putrescine. Chart 1 depicts the data from 2 separate experiments that investigated whether the DFMO-induced differentiation block of GM colonies was due to the lack of putrescine formation in the myeloid precursor cells. Along with various concentrations of DFMO, we incorporated 50 μM putrescine in the cultures and cloned GM-CFC in semi-solid agar cultures in the presence of HPCM. In both experiments, the addition of putrescine in the absence of DFMO enhanced both colony and cluster formations significantly ($p < 0.01$) despite the fact that HPCM concentration used was in the range of the plateau of dose-response curve. It is clear from Chart 1 that DFMO caused a dose-dependent decline in the colony numbers and a corresponding rise in cluster numbers as shown before in Table 2. However, the inclusion of putrescine in the cultures resulted in a complete reversal of this phenomenon up to a DFMO dose of 0.25 mM and a partial

reversal at a dose of 0.5 mM. These data suggested that the DFMO-induced differentiation block of GM-CFC was due to the lack of endogenous production of putrescine.

Effect of Short-Term Exposure of Marrow Cells to DFMO or Putrescine on Subsequent GM-CFC Clonal Growth. To investigate whether a constant presence of DFMO was required to block GM-CFC growth and differentiation, we treated light-density (≥1.070 g/ml), nonadherent human marrow cells with either α-MEM plus 15% FCS or DFMO (2.5 mM), or DFMO plus putrescine (50 μM) for 1 hr at 37° in an atmosphere of 5% CO_2-air. Subsequently, the cells were washed thoroughly and then cultured in semisolid agar with an optimum HPCM concentration in the presence or absence of putrescine, or DFMO, or of both. As shown in Table 4, short-term DFMO preexposure of marrow cells did not significantly change ($p > 0.04$) either the growth pattern of GM colonies and clusters or the polyamine levels (data not shown). However, preexposure with DFMO plus putrescine resulted in an enhanced colony formation ($p < 0.003$). In another experiment, preexposure to putrescine alone also resulted in a similar enhancement of colony formation (data not shown). This enhancement in GM colony numbers was still higher if the agar cultures also contained putrescine. Although short-term preexposure of marrow cells to DFMO did not cause a subsequent decrease in GM-CFC clonal growth, it did sensitize the cells to the DFMO effect in agar cultures, since the decline in colony numbers was significantly more ($p < 0.002$) for marrow cells that were pretreated with DFMO than for those treated with α-MEM plus 15% FCS (control) or DFMO plus putrescine (Table 4). Similarly, putrescine plus DFMO pretreatment sensitized the marrow cells for the subsequent putrescine reversal of DFMO effect in agar cultures (Table 4).

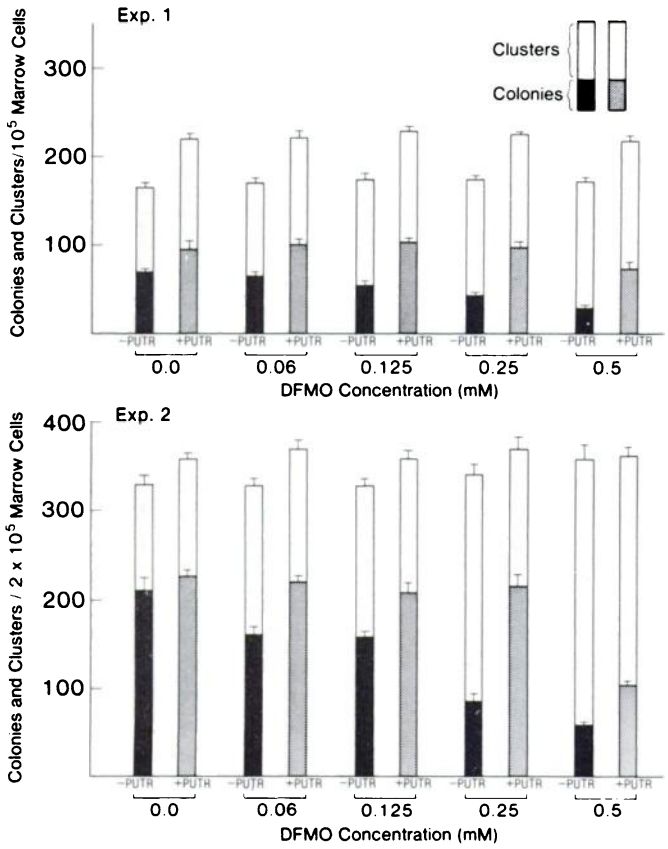

Chart 1. Reversal of DFMO-induced clonal differentiation block of GM-CFC by exogenous putrescine. We cultured light-density (≤1.070 g/ml), nonadherent human marrow cells under HPCM stimulus in the presence of varying DFMO concentrations with (+PUTR) or without (−PUTR) putrescine (50 μM). As can be seen, in both the experiments, exogenously added putrescine completely reversed the effect of up to 0.25 mM DFMO. *Bars*, S.D.

DISCUSSION

Cellular proliferation and differentiation require new DNA and RNA synthesis. Polyamines have been shown also to be essential for cell proliferation and differentiation (2, 4–9, 11, 19–22, 24). Tumor-promoting agents such as 12-*O*-tetradecanoylphorbol-13-acetate, which induces differentiation in certain animal and human cell lines (10, 13, 16, 18, 27), have been shown to elevate polyamine levels by inducing ODC (10, 16, 27). Recently, high ODC levels have been detected in cells that are undergoing differentiation into their more mature forms without proliferation (15, 24). These data suggest that polyamines may be an essential constituent of the cellular processes of proliferation and differentiation. To be able to clone human GM-CFC *in vitro* offers an extraordinary opportunity to study the role of ODC and polyamines in the physiological proliferation and differentiation induced by a humoral factor.

In this paper, we demonstrate clearly that HPCM, serving as a source of partially purified CSF, induced a 2-fold rise in putrescine by Day 1 and a 3-fold rise by Day 7. Inclusion of DFMO, a catalytic, irreversible inhibitor of ODC, along with HPCM resulted in a complete inhibition of putrescine synthesis

Table 4

Effect of short-term exposure of marrow cells to DFMO or DFMO plus putrescine on subsequent granulocyte-macrophage colony formation

Preculture treatment of marrow cells[a]	Type of cell aggregates scored	No. of cell aggregates in the presence of various agents			
		Culture medium alone (control)	Putrescine (50 μM)	DFMO (2.5 mM)	DFMO + putrescine
Culture medium alone	Colonies	180 ± 10[b]	214 ± 15	11 ± 1	85 ± 3
	Clusters	105 ± 5	85 ± 5	275 ± 1	220 ± 17
	Total	285 ± 9	299 ± 18	286 ± 8	305 ± 18
DFMO	Colonies	186 ± 8	234 ± 8	5 ± 1	60 ± 6
	Clusters	111 ± 8	77 ± 6	293 ± 15	260 ± 10
	Total	297 ± 12	311 ± 6	298 ± 15	320 ± 7
DFMO + putrescine	Colonies	228 ± 8	238 ± 8	10 ± 1	103 ± 8
	Clusters	78 ± 8	70 ± 10	303 ± 15	207 ± 6
	Total	306 ± 4	308 ± 3	313 ± 15	310 ± 9

[a] Light-density, nonadherent human marrow cells were treated with various agents for 1 hr at 37° in a 5% CO_2-air incubator prior to culture in semisolid agar.
[b] Mean ± S.D. of triplicate cultures.

and about a ≥24% reduction in the spermidine levels. The rise in spermine levels observed with DFMO alone could be a result of stabilization of *S*-adenosylmethionine decarboxylase, as observed in a number of other systems (11, 22).

With an *in vitro* semisolid agar culture system, we further demonstrated that this inhibition of polyamine biosynthesis by DFMO resulted in a proliferation and differentiation block of GM-CFC. In addition, this proliferation and differentiation block could be completely reversed by exogenous putrescine, suggesting that polyamine levels could be a limiting factor in GM-CFC proliferation and its differentiation to more mature progeny. Further, short-term exposure of marrow cells to DFMO did not affect either HPCM-stimulated GM-CFC clonal proliferation and differentiation or intracellular polyamine levels. However, 1-hr pretreatment with putrescine plus DFMO sensitized GM-CFC to the effects of CSF, which gave rise to larger and more GM colonies, despite the fact that the HPCM concentration used belonged to the plateau range of the dose-response curve. The importance of this phenomenon, however, is unclear.

Thus, our results indicate that HPCM-induced human GM-CFC proliferation and differentiation is accompanied by polyamine biosynthesis. Since inhibition of polyamine biosynthesis by DFMO, a catalytic irreversible inhibitor of ODC, resulted in an inhibition of granulopoietic differentiation that could easily be reversed with exogenous putrescine, these data indicate clearly that polyamines are essential for granulopoietic differentiation.

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REFERENCES

1. Bachrach, U. Function of Naturally Occurring Polyamines. New York: Academic Press, Inc., 1973.
2. Caldarera, C. M., and Moruzzi, G. Polyamines and nucleic acid metabolism in the chick embryo. Ann. N.Y. Acad. Sci., *171:* 709–722, 1970.
3. Cohen, S. S. Introduction to Polyamines. Englewood Cliffs, N. J.: Prentice-Hall, 1971.
4. Emanuelsson, H., and Heby, O. Inhibition of putrescine synthesis blocks development of the polychete *Ophryotrocha labronica* at gastrulation. Proc. Natl. Acad. Sci. U. S. A., *75:* 1039–1042, 1978.
5. Fillingame, R. H., Jorstad, C. M., and Morris, D. R. Increased cellular levels of spermidine or spermine are required for optimal DNA synthesis in lymphocytes activated by concanavalin A. Proc. Natl. Acad. Sci. U. S. A., *72:* 4042–4045, 1975.
6. Fozard, J. R., Part, M.-L., Prakash, N. J., Grove, J., Schechter, P. J., Sjoerdsma, A., and Koch-Weser, J. 1-Ornithine decarboxylase: an essential role in early mammalian embryogenesis. Science (Wash. D. C.) *208:* 505–508, 1980.
7. Gazdar, A. F., Stull, H. B., Kilton, L. J., and Bachrach, U. Increased ornithine decarboxylase activity in murine sarcoma virus infected cells. Nature (Lond.), *262:* 696–698, 1976.
8. Hogan, B., Shields, R., and Curtis, D. Effect of cyclic nucleotides on the induction of ornithine decarboxylase in BHK cells by serum and insulin. Cell, *2:* 229–233, 1974.
9. Janne, J., and Raina, A. Stimulation of spermidine synthesis in the regenerating rat liver: relation to increased ornithine decarboxylase activity. Acta. Chem. Scand., *22:* 1349–1351, 1968.
10. Lotem, J., and Sachs, L. Regulation of normal differentiation in mouse and human myeloid leukemic cells by phorbol esters and the mechanism of tumor promotion. Proc. Natl. Acad. Sci. U. S. A., *76:* 5158–5162, 1979.
11. Mamont, P. S., Bohlen, P., McCann, P. P., Bey, P., Schuber, F., and Tardif, C. α-Methylornithine, a potent competitive inhibitor of ornithine decarboxylase, blocks proliferation of rat hepatoma cells in culture. Proc. Natl. Acad. Sci. U. S. A. *73:* 1626–1630, 1976.
12. Marton, L. J., and Lee, P. L. Y. More sensitive automated detection of polyamines in physiological fluids and tissue extracts with *O*-phthaladehyde. Clin. Chem., *21:* 1721–1724, 1975.
13. Mendelsohn, N., Gilbert, H. S., Christman, J. K., and Acs, G. Effect of maturation on the response of human promyelocytic leukemia cells (HL-60) to the tumor promoter 12-*O*-tetradecanoyl-phorbol-13-acetate. Cancer Res., *40:* 1469–1474, 1980.
14. Metcalf, B. W., Bey, P., Danzin, C., Jung, M. J., Casara, P., and Vevert, J. P. Catalytic irreversible inhibition of mammalian ornithine decarboxylase (E.C. 4.1.1.17) by substrate and product analogues. J. Am. Chem. Soc., *100:* 2551–2553, 1978.
15. Nichols, W. K., and Prosser, F. H. Induction of ornithine decarboxylase in macrophage by bacterial lipopolysaccharides (LPS) and mycobacterial cell wall material. Life Sci., *27:* 913–920, 1980.
16. O'Brien, T. G., Simsiman, R. C., and Boutwell, R. K. Induction of the polyamine-biosynthetic enzymes in mouse epidermis by tumor-promoting agents. Cancer Res., *35:* 1662–1670, 1975.
17. Pike, B. L., and Robinson, W. A. Human bone marrow colony growth in agar-gel. J. Cell Physiol., *76:* 77–84, 1970.
18. Rovera, G., O'Brien, T. G., and Diamond, L. Induction of differentiation in human promyelocytic leukemia cells by tumor promoters. Science (Wash. D. C.), *204:* 868–870, 1979.
19. Russell, D., and Synder, S. H. Amine synthesis in rapidly growing tissues: Ornithine decarboxylase activity in regenerating rat liver, chick embryo, and various tumors. Proc. Natl. Acad. Sci. U.S.A., *60:* 1420–1427, 1968.
20. Russell, D. H., Byus, C. V., and Manen, C. A. Proposed model of major sequential biochemical events of a trophic response. Life Sci., *19:* 1297–1306, 1976.
21. Russell, D. H., and Durie, B. G. M. Polyamines as Biochemical Marker of Normal and Malignant Growth. New York: Raven Press, 1978.
22. Sunkara, P. S., Pargac, M. B., Nishioka, K., and Rao, P. N. Differential effects of inhibition of polyamine biosynthesis on cell cycle traverse and structure of the prematurely condensed chromosomes of normal and transformed cells. J. Cell. Physiol., *98:* 451–458, 1979.
23. Sunkara, P. S., Rao, P. N., Nishioka, K., and Brinkley, B. R. Role of polyamines in cytokinesis of mammalian cells. Exp. Cell. Res., *119:* 63–68, 1979.
24. Takigawa, M., Ishida, H., Takano, T., and Suzuki, F. Polyamine and differentiation: induction of ornithine decarboxylase by parathyroid hormone is a good marker of differentiated chondrocytes. Proc. Natl. Acad. Sci. U. S. A., *77:* 1481–1485, 1980.
25. Verma, D. S., Spitzer, G., Gutterman, J. U., Zander, A. R., McCredie, K. B., and Dicke, K. A. Human leukocyte interferon preparation blocks granulopoietic differentiation. Blood, *54:* 1423–1427, 1979.
26. Weiner, M. S., Bianco, C., and Nussenzweig, J. Enhanced binding of neuraminidase treated sheep erythrocytes to human T lymphocytes. Blood, *42:* 939–946, 1973.
27. Yuspa, S. H., Lichti, U., Ben, T., Patterson, E., Hennings, H., Slaga, T. J., Colburn, N., and Kelsey, W. Phorbol esters stimulate DNA synthesis and ornithine decarboxylase activity in mouse epidermal cell cultures. Nature (Lond.), *262:* 402–404, 1976.

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