8. T. Timonen, Y. Ortaldo, and R. B. Herberman, J. Exp. Med., 153, 569 (1981).
9. T. Timonen, C. Reynolds, Y. Ortaldo, et al., J. Immunol. Methods, 51, 269 (1982).
10. T. Timonen and E. Saksela, J. Immunol. Methods, 36, 285 (1980).
11. A. Uchida, Nat. Immunol. Cell Growth Regul., 3, 181 (1983/84).

EFFECT OF TUMOR GROWTH PROMOTOR 12-O-TETRADECANOYLPHORBOL-13-ACETATE ON PROLIFERATION OF VARIOUS MOUSE TUMOR CELL CLONES IN SEMISOLID MEDIUM

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Tumors are known to be heterogeneous with respect to their cell composition, and this heterogeneity arises as a result of clonal evolution [9]. It is not clear, however, whether different clones replace one another in the course of tumor development or whether they must coexist in the tumor in order to maintain it. It has recently been shown [10] that clones of pseudonormal rat cells differ in their response to growth-stimulating factors. The question arises whether clones of tumor cells differ in their response to exogenous agents affecting cell multiplication.

The aim of this investigation was to study the ability of different clones of tumor cells to proliferate in semisolid medium in response to the action of the tumor growth promotor 12-O-tetradecanoylphorbol-13-acetate, which induces DNA synthesis in resting monolayer cultures [7], and can also induce colony formation in semisolid medium by certain types of transformed cells [6, 8].

EXPERIMENTAL METHOD

Clone CAK-25AG^r, isolated from a spontaneously transformed CAK line of fibroblasts from AKR mice [1], and clones 103/11 and 3sb PS-103 [1], isolated from a culture of PS-103 sarcoma of CBA mice [4], were studied. The conditions of culture of the cells in the monolayer were described previously [1, 5]. A 1.2% solution of methyl cellulose (MC, from Sigma, USA) in the culture medium was used as the semisolid medium. The method of determination of the cloning efficiency (CE) in MC was described previously [5]. In most experiments CE of the cells on the substrate was determined parallel with CE in MC.

The growth promotor 12-O-tetradecanoylphorbol-13-acetate (TPA, from Sigma) was dissolved in acetone or dimethyl sulfoxide (DMSO) to a concentration of 1 μg/ml. The solution was kept at −70°C and the cells treated with it 72 h before seeding in MC (final TPA concentration 5 ng/ml). To test the effect of this treatment with TPA on gene amplification, colchicine (from Merck, USA) was used; colchicine was added to the culture medium in a concentration of 0.07 μg/ml.

EXPERIMENTAL RESULTS

Clones of tumor cells used for the investigation were characterized by low CE in MC, namely about 10^{-4}-10^{-3} (Table 1). By determining the survival rate of cells of clones CAK-25AG^r, 103/11, and 3sb PS-103 after treatment with different doses of TPA it was possible to choose the maximal nontoxic dose for preventive treatment of the cells with the compound (5 ng/ml). The clones studied did not differ in sensitivity to the toxic action of TPA. Even so, they were found to differ in their ability to form colonies in MC in response to treatment with TPA.

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TABLE 1. Effect of Pretreatment with TPA on CE of Clone CAK-25AG^r in Media with MC and Colchicine, and also on CE in MC of Clones Isolated from PS-103 Culture

Clone	No. of experiment	Selective agent	Pretreatment with TPA	CE in MC ($\times 10^{-4}$)	CE on substrate, %
CAK-25AG^r	1	MC	—	1,4±0,5	16,7±0,4
			+	3,7±0,9	18,1±0,6
	2	MC	—	6,7±0,6	22,9±1,6
			+	4,3±0,2	24,9±2,3
	3	Colchicine	—	0,3±0,1	21,0±0,7
			+	17,8±1,4	26,0±2,2
103/11	4	MC	—	80,0±20,0	H. o.
			+	17,0±3,0	H. o.
	5	MC	—	86,0±9,0	50,7±1,6
			+	13,0±9,0	43,0±1,4
3sb PS-103	6	MC	—	0,5±0,2	H. o.
			+	11,4±2,2	H. o.
	7	MC	—	1,7±0,3	50,8±0,8
			+	11,1±+,9	39,9±3,0

Legend. N.d.) Not determined.

TABLE 2. CE in MC of Subclones Isolated from Semisolid Medium in Experiments to Study the Action of TPA on Clone 3sb PS-103

Origin of subclone	No. of subclone	CE in MC ($\times 10^{-4}$)
Subclones of clone 3sb PS-103 isolated from MC —TPA	1	0,54±0,04
	2	1,22±0,2
	3	0,48±0,1
Subclones of clone 3sb PS-103 isolated from MC after pretreatment with TPA +TPA	4	2,7±0,04
	5	9,5±0,6
	6	7,0±0,8
	7	6,3±0,7

Legend. CE in MC is mean value of at least two experiments.

Pretreatment of clone CAK-25AG^r with TPA caused no change in the number of colonies formed by this clone in MC (Table 1), inducedmarked inhibition (by 5-25 times) of the number of colonies formed by clone 103/11 in MC and a marked increase (b7 6-20 times) in colony formation in MC by clone 3sb PS-103 (Table 2); the latter effect was accompanied by a marked increase in size of the colonies.

The absence of change in CE of clone CAK-25AG^r in MC in response to treatment with TPA cannot be explained by the absence of sensitivity of these cells to TPA, since pretreatment of clone CAK-25AG^r by the same scheme led to a sharp increase in the number of colchicine-resistant colonies (Table 1). This is evidence of induction of gene amplification in CAK-25AG^r cells under the influence of TPA [3]. Inhibition of colony formation by clone 103/11 in MC was not linked with increased sensitivity of this clone to the toxic action of TPA, for although it depressed colony formation in MC, TPA did not reduce the CE of these cells on the substrate (Table 1). Those workers who observed an increase in the number of colonies in semisolid medium under the influence of TPA [6, 8] showed that TPA induces variants in which increased CE in MC was inherited. In order to find out whether this is true for clone 3sb PS-103 (Table 1), colonies formed in MC by control cells (—TPA) and by a group of cells pretreated with TPA (+TPA) were isolated. These colonies were propagated on glass, and the two groups of subclones obtained from them were tested in MC. As Table 2 shows, the +TPA subclones preserved the 3-10-fold increase in CE in MC acquired under the influence of TPA, whereas the —TPA subclones were virtually indistinguishable from clone 3sb PS-103 as regards CE in MC. The data indicate that in the case of clone 3sb PS-103, we obtained an inherited increase of CE in MC under the influence of TPA, i.e., TPA can evidently not only affect CE of tumor cells in MC, but can also induce hereditary changes in them.

Clones of tumor cells can thus respond in several different ways to TPA: by stimulation, which may be inherited, by inhibition of multiplication in MC, and by no response. The possibility cannot be ruled out that this reflects differences in their general response to external environmental factors (the coordinated response norm) [2]. In fact clones of pseudonormal rat cells differ in their reactivity to a series of growth factors [10]. Differences in the coordinated response of individual tumor cells to external environmental factors may largely determine the behavior of the tumor cell population as a whole. Detailed characteristics of the response of our clones to environmental factors will show how valid these ideas are.

LITERATURE CITED

1. R. M. Brodskaya and A. A. Stavrovskaya, Byull. Eksp. Biol. Med., No. 9, 342 (1984).
2. Yu. M. Vasil'ev and I. M. Gel'fand, Interaction of Normal and Neoplastic Cells with the Environment [in Russian], Moscow (1981).
3. B. P. Kopnin and A. V. Gudkov, Genetika, No. 6, 872 (1983).
4. T. G. Moizhess, Tsitologiya, No. 2, 215 (1978).
5. A. A. Stavrovskaya, T. P. Stromskaya, R. M. Brodskaya, and Yu. M. Vasil'ev, Genetika, No. 3, 434 (1980).
6. N. H. Colburn, Carcinogenesis, 1, 11 (1980).
7. P. Dicker and E. Rozengurt, J. Cell. Physiol., 109, 99 (1981).
8. P. B. Fisher, J. H. Bozzone, and I. B. Weinstein, Cell, 13, 695 (1979).
9. G. H. Heppner, Cancer Res., 44, 2259 (1984).
10. P. L. Kaplan and B. Ozanne, Cell, 33, 931 (1983).