SUBSTRATE INDEPENDENCE IN NONCLONED AND CLONAL TUMOR CELL POPULATIONS

R. M. Brodskaya and A. A. Stavrovskaya

UDC 616-006-018.15.092.4

KEY WORDS: clonal analysis; substrate independence of proliferation; cloning efficiency in semisolid medium.

Substrate independence, or the ability of cells to multiply without attachment to a substrate, in a semisolid medium is one of the principal distinguishing features of malignant transformation [6, 10]. Various lines of transformed cells can form colonies in semisolid medium with a frequency that varies from tens of per cent to one colony per 10,000, or even 100,000 cells transplanted into semisolid medium (methylcellulose, semisolid agar, agarose) [2, 9]. A population of transformed cells may thus contain both cells that give rise to colonies in semisolid media and cells which will not multiply under those conditions. The question arises: Do the cells of the first type differ from those of the second type genetically? In a previous analysis of a clone of spontaneously transformed mouse cells CAK-25 AG^r, characterized by low cloning efficiency in semisolid medium (10^{-5} per cell transplanted into 1.2% methylcellulose) the writers found that most subclones of this clone, isolated from semisolid medium, preserved a frequency of clone formation characteristic of the parental cells [2]. Cells forming a colony evidently did not differ from the remaining cells of the population in the degree of substrate dependence of proliferation.

The aim of this investigation was a clonal analysis of cultures of tumor cells induced in mice by implantation of a foreign body in order to verify whether the rules discovered for clone CAK-25 AG^r also extend to other tumor cell populations.

EXPERIMENTAL METHOD

Experiments were carried out on cell cultures from sarcoma PS-103 obtained by T. G. Moizhess (Laboratory of Mechanisms of Carcinogenesis, All-Union Oncologic Research Center, Academy of Medical Sciences of the USSR) in response to subcutaneous implantation of plastic film into CBA mice [1], and on clones isolated by the writers from this culture. PS-103 cells at the 3rd-13th passages and cells of clones and subclones from them (up to the 15th passage after cloning) were used. The cells were cultured in medium RPMI 1640 with the addition of bovine serum (10%) and monomycin (100 U/ml). The technique of determination of the cloning efficiency of the cells was as follows: A certain number of cells (usually 10^4 per dish) in semisolid medium was transferred into a 1.2% solution of methylcellulose, made up in the usual culture medium for these cells [2, 3], and 2 weeks after seeding the number of colonies at least 80 μm in diameter was counted under an inverted microscope. The method of isolation of the clones was described previously [2].

EXPERIMENTAL RESULTS

PS-103 cells were characterized by low cloning efficiency in medium with methylcellulose (Table 1). After seeding with 10^4 cells per dish only single colonies appeared. This low frequency of clone formation was reproduced consistently from one experiment to another at different passages (from the 3rd to the 13th) during culture of the tumor cells in vitro. With a tenfold increase in the number of seeded cells (10^5 per dish) the frequency of clone formation in methylcellulose was increased by almost two orders of magnitude. This may be due to secretion of a certain growth factor by the cells into the medium, stimulating their own growth [9, 11]. Later 10^4 cells per dish was used as the working concentration.

Laboratory of Genetics of Tumor Cells, Research Institute of Carcinogenesis, All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR N. A. Kraevskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 98, No. 9, pp. 342-344, September, 1984. Original article submitted December 22, 1983.

0007-4888/84/9809-1253$08.50

TABLE 1. Mean Number of Colonies Appearing in 1.2% Methylcellulose after Seeding of Sarcoma PS-103 Cells and Clones and Subclones Isolated from This Strain

Origin of clones	Name of culture or clone	Mean number of colonies arising per five dishes in one experiment
—	PS -103	1,2/0,3/0
Clones isolated with solid substrate from PS-103	1 sb	0,2/0/0/0
	2 sb	0/0/0
	3sb	0
Clones isolated from methylcellulose from PS-103	4 mc	25,1
	5 mc	155,0/151,5
	6 mc	12,8/10,8/25,2
	7 mc	501,5/315,0
	8 mc	24,8
Subclones isolated with solid substrate from clone 1sb	9 sb	1,8
	10 sb	0,3/0,6
	11 sb	1,4/1,5
	12 sb	0/0,6
	13 sb	0,8/0
	14 sb	0/0,4
Subclones isolated from methylcellulose from clone 1sb	15 mc	0,4
	16 mc	1,0/0,6
	17 mc	0
	18 mc	5,8/7,8
	19 mc	1,6/0,5
	20 mc	1,7/16,0
	21 mc	83,8/30,5

Legend. Strokes separate results of repeated experiments.

In the first part of the work clones isolated from the PS-103 culture, i.e., from a noncloned population, were analyzed. PS 103 cells were cloned by isolation of colonies from methylcellulose. All the clones isolated were propagated, and the cloning efficiency in methylcellulose was determined for each of them (Table 1). Three clones grown on solid substrate (clones 1st-3sb) retained the low cloning efficiency characteristic of PS-103 cells. This cloning efficiency was consistently reproduced in repeat experiments. All five clones isolated from medium with methylcellulose (clones 4mc-8mc) differed from the original line by a cloning efficiency in semisolid medium that was tens or hundreds of times higher.

In the second part of this investigation one of the clones isolated from solid substrate (clone 1sb) was recloned, subclones were isolated from methylcellulose and from the substrate, and the efficiency with which the different subclones grew in medium with methylcellulose was then tested (Table 1). In these experiments both subclones 1sb isolated from glass (9sb-14sb) and subclones growing in semisolid medium (15mc-21mc) did not differ significantly in the overwhelming majority of cases from the parental population in ability to form clones in medium with methylcellulose. Only one of the eight subclones isolated from semisolid medium had higher substrate independence than the 1sb cells (clone 21mc).

Our experiments with clone 4mc, characterized by relatively high cloning efficiency in methylcellulose, also supported the stable inheritance of frequency of clone formation in semisolid medium. Subclones of this clone isolated from semisolid medium retained high efficiency of clone formation in methylcellulose. This confirms yet again the conclusion drawn by the writers previously: All cells of a clonal line are characterized by a definite probability (frequency) with which they can form colonies in semisolid medium.

How can the difference in the results obtained by analysis of the noncloned and clonal populations be explained? Evidently at least two types of clones coexist in the PS-103 culture: a clone or clones characterized by low, and clones (or a clone) characterized by higher probability of colony formation in methylcellulose. Long-term coexistence of clones differing in ability to metastasize has recently been described in tumors [8, 12]. In the PS-103 population cells with low ability to form clones in semisolid medium evidently predominate. This fully explains the fact that during cloning on glass clones with low cloning efficiency in methylcellulose are isolated initially. Meanwhile, under selective conditions (in medium with methylcellulose), cells with a 10-100 times greater probability of colony formation are selected from the noncloned population. When clones are

maintained in culture, their characteristic frequency of colony formation in methylcellulose is preserved so long as the cell population constituting the clonal line remains homogeneous. In investigations in which cells are seeded in semisolid medium, heterogeneous cell populations (noncloned), subjected to different transforming procedures, are usually used [4, 5]. In these investigations, therefore, clones isolated from semisolid medium usually differ in efficiency of clone formation from the parental cells.

Most cells giving rise to colonies in semisolid medium are thus genotypically identical with cells of the original homogeneous population or with individual clones that make up the heterogeneous population.

LITERATURE CITED

1. T. G. Moizhess, Tsitologiya, No. 2, 215 (1978).
2. A. A. Stavrovskaya, T. P. Stromskaya, R. M. Brodskaya, et al., Genetika, No. 3, 434 (1982).
3. T. P. Stromskaya, B. P. Kopnin, and A. A. Stavrovskaya, Vopr. Onkol., No. 3, 24 (1980).
4. J. C. Barrett and P. O. Ts'o, Proc. Natl. Acad. Sci. USA, 75, 3297 (1978).
5. A. J. D. Bellett and H. B. Younghusband, J. Cell. Physiol., 101, 33 (1979).
6. J. McPherson and L. Montagnier, Virology, 23, 291 (1964).
7. B. Ozame, T. Wheeler, and P. L. Kaplan, Fed. Proc., 41, 3004 (1982).
8. G. Post, J. Doll, and I. J. Fidler, Proc. Natl. Acad. Sci. USA, 78, 6226 (1981).
9. R. Risser and R. Pollack, Virology, 59, 477 (1974).
10. M. Stoker, C. O'Neill, S. Berryman, et al., Int. J. Cancer, 3, 683 (1968).
11. G. I. Todaro, J. C. DeLarco, and C. M. Fryling, Fed. Proc., 41, 2996 (1982).
12. M. F. Woodruff, J. D. Ansell, et al., Nature, 299, 822 (1982).