CORRELATION BETWEEN SIGNS OF MALIGNANCY AND RESPONSE OF TUMOR CELLS TO TRANSFORMING GROWTH FACTOR

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UDC 616-006.03-039.38:616-006.04-02:616-006-018.15

KEY WORDS: transforming growth factor; tumors; cloning in a semisolid medium.

Tumor cells are known to secrete transforming growth factors (TGF), which are proteins stimulating cell multiplication. Clones differing in their ability to react to TGF and in the degree to which they secrete this factor have been isolated from a mouse sarcoma tumor cell population [3]. Clones differing in sensitivity to TGF have also been described by other workers [7, 8]. The biological importance of these differences between the clones is not yet clear. The question arises whether these differences may be related to the expression of features of malignant transformation.

In the investigation described below the oncologic characteristics of several clones of mouse sarcoma were compared and the ability of the clones to respond to TGF (factors) and to secrete them was evaluated.

EXPERIMENTAL METHOD

The following cell lines were used: 1) clones from a culture of sarcoma cells PS-84 [2], induced by subcutaneous insertion of a polyvinyl chloride lamina into CBA mice — the clones were isolated from the solid substrate; 2) clone 384/5 from a culture of PS-103 cells [3]. Cells until the 20th passage after cloning were used in the experiments. The cells were grown at 37°C on Eagle's medium with 10% bovine serum and monomycin (100 μg/ml). The method of determination of the cloning efficiency was described previously [4].

Mitomycin C (Sigma, USA) was added in a dose of 20 μg/ml to the culture medium with serum and the cells were incubated for 2 h at 37°C in darkness. The cells were then washed 3 times with Hanks' solution and incubated for 2 h at 37°C in medium with serum and without mitomycin C. The cells were then removed from the flasks with trypsin and added to a solution of methylcellulose to determine the cloning efficiency.

The cells were transplanted into syngeneic CBA mice. TD_{50} (50% take dose) was calculated by Behrens' method [1].

EXPERIMENTAL RESULTS

From a culture of sarcoma PS-84 cells we isolated six clones and tested how they respond to TGF and secrete it. As we showed previously, the easiest method of investigating the degree of TGF secretion by clones and their response to it is the method of mixed cultures [3, 6]. In this method, a mixture of cells of the clone secreting TGF and cells responding to it is placed in medium with methyl cellulose. As cells secreting TGF we used cells of clone 31k which, depending on the results of preliminary tests, intensively secreted TGF. To make sure that cells of this clone do not themselves begin to multiply when mixed with cells of the test clone, in some experiments before they were transferred into the methylcellulose solution they were treated with mitomycin C. Treatment with mitomycin C, which arrests cell multiplication, does not prevent TGF secretion [5, 9]. It will be clear from Table 1 that 31k cells, both treated and untreated with mitomycin C, stimulate growth of clones 6k, 9k, and 31k in semisolid medium. Among the responding clones, the strongest response was given by clone 6k. Multiplication of clones 34k, 30k, and 28k was not stimulated in mixed cultures.

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 0007-4888/87/0006-0812$12.50 © 1987 Plenum Publishing Corporation

TABLE 1. Reaction of Clones from PS-84 to TGF Secreted by Clone 31k

Clone responding to TGF	Mean number of colonies		
	of clone responding to TGF, 10^4 cells per dish	of clone secreting TGF, 10^5 cells per dish	of clone responding to TGF+ clone secreting TGF, 10^4+ $9 \cdot 10^4$ ($5 \cdot 10^4 + 9 \times 10^4$)
6k	0	13,3±3,2	150,7±28,8
	0*	0*	200,6±27,6*
9k	0	1,8±0,4	43,0±8,8
	0*	0*	37,2±9,8*
31k	0*	0*	29,4±15,6*
34k	0	0 4	0
	0*	0*	0*
30k	0	0	0
	0*	0*	0*
28k	0	0	0
	0*	0*	0*

Legend. Asterisk indicates clone secreting TGF and treated by mitomycin C.

TABLE 2. Mixed Cultures 384/5 and 34k (treatment with mitomycin C)

Clone secreting TGF	Clone reacting to TGF		
	0	384/5 without mitomycin C	384/5 with mitomycin C
0	—	0	0
34k without mitomycin C	0	43,8	0
34k with mitomycin C	0	35,7	—

TABLE 3. Reaction of 384/5 to TGF Secreted by Clones PS-84

Secreting clone	Mean number of colonies		
	384/5, 10^4 cells per dish	clone secreting TGF, $5 \cdot 10^4$ cells per dish	384/5+ clone secreting 10^4 + $5 \cdot 10^4$ TGF
6k.	0,4±0,3	0	73,9±13,3
9k.	7,3±2,7	0	84,6±21,0
31k	5,3±2,3	0	313,7±72,8
34k.	4,7±2,1	0	41,9±6,2
30k.	0,5±0,3	0	130,0±10,9
28k.	0,5±0,3	0	54,5±4,0

Secretion of TGF by cells of the different clones was tested by seeding cells of these clones in mixed culture with clone 384/5 (isolated from another sarcoma — PS-103). A previous investigation showed that 384/5 cells react well to TGF secreted by cells of a PS-103 culture [3]. In fact, in our own experiments treatment of 384/5 cells with mitomycin C completely inhibited their multiplication in mixed culture with 34k cells, whereas treatment of 34k cells with mitomycin C did not abolish their effect on multiplication of the 384/5 clone (Table 2). Thus 384/5 cells responded by stimulation of multiplication also to TGF (factors) secreted by a clone of another mouse sarcoma — PS-84. All clones tested from sarcoma PS-84 secreted TGF, but they differed in the degree of secretion (Table 3). Generalized results of the study of the response of the clones to TGF and its secretion by cells of these clones, and also their oncologic characteristics, are summarized in Table 4. Cells responding to growth factor grew well in medium with methylcellulose and their take rate was higher. They evidently also stimulated their own multiplication, for when seeded in low density (10^4 cells per dish) no colonies were formed. Clones not responding to TGF virtually did not multiply in medium with methylcellulose (even when seeded at the rate of 10^5 cells per dish) and their take rate was lower (with a larger number of cells or with a longer latent period).

TABLE 4. Oncologic Characteristics of PS-84 Clones

Clone PS-84	Response to TGF	Secretion of TGF	Growth on medium with 1.2% methylcellulose per 10^5 cells per dish	$TD_{50} \cdot 10^3$	Average latent period per 10^4 cells, days
6k.	+++	+	211,0	1,1	13,5
9k.	++	+	258,5	5,0	20,2
31k.	++	++	6,4	1,5	20,3
34k.	—	+	0	3,0	32,5
30k.	—	++	0	10,0	18,0
28k.	—	+	0,3	20,0	149,0

These results show that the response of the cells to TGF secreted by them or by neighboring cells correlates with the degree of expression of two features of transformation: transplantability and loss of substrate dependence. No correlation was found between the degree of TGF secretion and expression of features of malignant transformation.

Possibly the acquisition of increased ability to respond to TGF by the cells gives a selective advantage to clones with these properties, enabling them to multiply under conditions when cells responding less strongly to TGF do not proliferate. However, as the writers showed previously [3], the population of tumor cells contains not only clones responding to TGF, but also clones not responding to it, or even clones inhibited by TGF. Interaction between the different cells in the tumor can thus create conditions preventing the preferential multiplication of particular clones. The selective advantage of these clones may be realized in cases when the organized clonal structure of the tumor is disturbed, such as during metastasization of single cells. Further research will show whether these hypotheses are valid.

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