CLONAL STRUCTURE OF TUMORS: INTERCLONAL VARIATION IN SECRETION OF GROWTH FACTORS AND CELLULAR RESPONSE TO THEM

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Tumor cells are known to secrete growth transforming factors (GTF) [6, 8]. In this connection the hypothesis of autocrine stimulation, according to which tumor cells themselves stimulate their own multiplication and become independent of external environmental factors, has achieved popularity [7]. In fact, multiplication of lines of transformed cells can be stimulated by the addition of factors secreted by them into the culture medium [6, 8]. Tumors constitute a heterogeneous cell population. The question arises whether the concept that tumor cells maintain their own multiplication — and whether in fact each clone of a tumor is stimulated by the growth factor secreted by itself or whether a more complex system of interclonal interaction operates in the tumor — is valid for different clones of cells of the same tumor.

In this investigation an attempt was made to answer this question by studying clones isolated from a culture of mouse sarcoma PS-103. Cells of this tumor, as was shown previously [2], secrete a factor, evidently a GTF, which is a protein with mol. wt. of about 15 kilodaltons.

EXPERIMENTAL METHOD

The following cells were used in the tumor: 1) line PS-103 — a cell culture of a sarcoma induced by introduction of a polyvinyl chloride disk into a CBA mouse [3]; 2) clones from a culture of PS-103 cells, isolated from semisolid medium (clones 384/5, 384/6, and 384/9) and from a solid substrate (clone 3 sb). Cells of up to the 15th passage after cloning were used in the experiments. The conditions of cell culture were described previously [2, 4]. Conditioned medium (CM) was collected from PS-103 cells by the usual method [2, 5] and added to medium with methylcellulose (MC, 1:3) by volume), cells of the clones were introduced into semisolid medium, and the number of colonies grown to a size of 80 μ or more was counted 10-14 days later [4]. CM was collected from the clines and added to MC, and cells of the PS-103 test culture were introduced into this medium. The colonies were counted in the same way as in the previous experiment.

TABLE 1. Changes in Cloning Efficiency (CE) ($\times 10^{-4}$) of Clones and General PS-103 Culture in Semisolid Medium under the Influence of CM and General Characteristics of Clones

Cells	Response of clones to CM from PS-10 PS-103		Effect of CM of clones on multiplication of PS-103 cells		Characteristics of clone	
	–CM	+ CM from PS-103	–CM	+ CM of clone	response to factor (factors)	secretion of factor (factors)
3 sb	1,4±0,6	0,4±0,3	16,5±5,1	399,3±11,6	0	++
384/9	1,5±0,2	7,0±0,3	3,3±1,3	36,5±8,9	+	++
384/5	3,7±1,1	33,1±5,7	6,8±2,5	15,5±3,3	++	±
384/6	117,9±14,6	8,6±1,7	6,8±2,5	40,3±6,4	—	+

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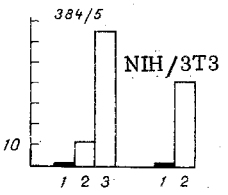

Fig. 1. Effect of CM from clones 384/5 and 3 sb on CE of 384/5 cells in semisolid medium. Ordinate, CE ($\times 10^{-4}$). 1) Without CM; 2) CM from 384/5; 3) CM from 3 sb.

EXPERIMENTAL RESULTS

Although in the previous study [2], the growth-stimulating factor secreted by PS-103 cells was concentrated and fractionated, in the present experiment unfractionated CM from PS-103 cells was used, because, besides the one factor which we found, it could also contain other forms of activity affecting multiplication of different clones. Among the 20 clones studied, three types of clones were found in the tumor (Table 1): 1) cells virtually not responding to CM from PS-103 (clone 3 sb; differences between experiment and control not significant); 2) cells responding to CM from PS-103 by stimulation of multiplication in semisolid medium (clones 384/5 and 384/9); 3) cells whose multiplication was inhibited by CM from PS-103 (clone 384/6). This clone had the highest cloning efficiency in MC, which differed from that of the general population. However, it is unlikely that inhibition of proliferation by CM and the highest cloning efficiency (CE) are directly connected. First, in the series of clones which we studied there were other clones with increased CE, multiplication of which was not inhibited by CM (results not given). Second, clone 384/5 was tested at the 7th and 37th passages during culture. CE for cells at the 7th passage was $2.75 \cdot 10^{-4}$, and for cells at the 37th passage it was $22.7 \cdot 10^{-4}$. An increase in CE did not lead to any change in the response of the clone to CM from PS-103 cells; the number of colonies increased on addition of CM for cells of both passages by about 16 times.

Multiplication of PS-103 cells was stimulated by the greatest degree by CM from clones 3 sb and 384/5 (Table 1). The ability of CM of clone 3 sb to stimulate growth in a semisolid medium by a greater degree than CM of clone 384/5 was shown also on 384/5 and NIH/3T3 (Fig. 1).

The writers suggested previously that different clones and tumors interact, as a result of which rapid replacement of cells proliferating more slowly by clones which proliferate more intensively does not take place [1]. To study possible interactions between different PS-103 clones experiments were carried out with combined culture of cells of clone 3 sb with cells 384/5 and 384/6 in MC (Table 2). The number of colonies in mixed culture increased sharply compared with cultures of each clone separately. In these experiments clone 384/6, multiplication of which was inhibited by CM from PS-103, responded by stimulation of multiplication to a factor secreted by clone 3 sb. In other experiments (results not given) multiplication of 384/6 also was stimulated by addition of CM of clone 3 sb to MC. CM from the general PS0103 culture may perhaps contain an inhibitor of proliferation, to which 384/6 cells respond by inhibition of multiplication.

The differences discovered in this investigation between the clones are evidence that, first, secretion of factors soluble in the medium by tumor cells and, second, differences in the ability of cells to respond to these factors, play a role in the maintenance of the clonal structure of a tumor. It can be imagined that cells similar to clone 3 sb in a tumor secrete a factor and, while not responding to it themselves, they have an influence on multiplication of other cells, such as clone 384/5: multiplication of this last clone may be delayed by reducing the fraction of factor-secreting cells. Proliferation of another cell fraction (clone 384/6) can be inhibited by the same or another factor secreted by one of the clones of the tumor.

TABLE 2. Growth of Clone 3 sb and Clones 384/5 and 384/6, Separately and during Combined Culture in Semisolid Medium

Cells	Number of cells per dish	Number of colonies per dish
3 sb	10^5	6,0±2,2
384/5	10^4	23,5±2,3
384/6	10^3	0
3 sb + 384/5	$9 \cdot 10^4 + 10^4$	490±76,7
3 sb + 384/6	$9 \cdot 10^4 + 10^3$	31,0±6,5

The existence of different clones may reflect different stages of tumor progression. The possibility likewise cannot be ruled out that the system of intercellular interactions enable the tumor tissue to preserve a structure that resembles that of the original normal tissue. Further investigations of interclonal interactions in tumors will show which of these suggestions is right.

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INDUCTION OF Ca^{++} TRANSPORT IN HUMAN PLATELETS BY THYROID HORMONE RECEPTOR OF MALIGNANT CELLS

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Physiological effects of thyroid hormones are determined by their influence on transcription and translation [14, 15] and also their modifying action on the permeability of biological membranes [8, 13]. Several views are currently held on the mechanism of action of thyroid hormones on biological membranes [4, 10, 11]. It has been shown that these compounds influence biosynthetic processes by modulating the protein-lipid composition of the membranes and thereby modifying protein-lipid interactions in them [9]. Meanwhile the possibility of a direct membranotropic action of thyroid hormones has been investigated [8] and it has been shown that their physiological action is the result of a change in the physicochemical properties of biological membranes, leading to a change in their permeability [10].

There is no doubt that the effects of thyroid hormones are mediated through their interaction with specific receptor proteins. The isolation of thyroid hormone receptors of normal and malignant cells, and some of their properties, have been described previously [3, 5]. It has been shown that the hormone-receptor complex of cancer cells, irrespective of the location and etiology of the tumor, induces Ca^{++} conduction in experiments on artificial bilayer phospholipid membranes (BPM), whereas in the presence of other cations (H^+, K^+, Na^+, Ba^{2+}, Mn^{2+}, Mg^{2+}, Sr^{2+}) the resistance of BPM is unchanged [2, 6]. Under analogous experimental conditions the hormone-receptor complex of normal cells selectively induces only H^+-conductance of BPM [6].

It will be evident that the conditions of model experiments do not always adequately reflect physiological processes taking place in biological systems. Accordingly, it was necessary to study the effect of thyroid hormone receptors of normal and cancer cells on permeability of biological membranes.

This paper describes a study of the effect of thyroid hormone receptor on Ca^{++} transport in human platelets.

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