INVESTIGATION OF STATIONARY POPULATIONS OF GENETICALLY DIFFERENT HOMONUCLEAR STRAINS* OF HUMAN CELLS

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Morphological and cytophysiological parameters of cells and the cell population in the stationary phase were studied. By contrast with normal cell strains, in which a definite combination of ontogenetic changes is found, in cells of strains with chromosomal and gene mutations individual differences are observed, evidently due to a disturbance of differentiation processes and to the manifestation of features characteristic of malignant cells.

KEY WORDS: heterochromatinized nucleus; stationary populations; chromosomal and gene mutations; abnormal cells.

Earlier it was shown that for long-term cultivation in the stationary phase specific, repeating changes occur in human fibroblasts, which allows us to speak of the complex of ontogenetic changes in stationary cell populations [1, 3, 4]. The following morphological and cytophysiological indicators were used to evaluate the presence of the complex of ontogenetic changes: 1) morphology of the cell nucleus, 2) the fraction of cells with chromocenters, 3) the characteristics of cell-population growth, 4) the change in cell density, 5) seeding efficiency (the ratio of attached cells to seeded cells, expressed in percent), 6) the time of cell-population death. In normal diploid cell strains, these indicators were identical. It was also shown that in cell stains with chromosomal and gene mutations obtained from a patient with trisomy 7 (karyotype 47, XV, +7) and from a patient with a pigmented xeroderma, a complex of ontogenetic changes was observed which is different from that of normal cells.

In this investigation a number of cell strains with chromosomal and gene mutations were studied during long-term culture in the stationary phase.

EXPERIMENTAL METHODS

Cell strains obtained from patients with the karyotype 48, XXXX; 49, XXXXY, and 47, XX, +21 and from a patient with Marfan's syndrome, in the second phase of culture (the period of optimal growth), were used [7]. The preparation and culture of the cell strains were carried out by methods adopted in the Laboratory of Human Cytogenetics, Institute of Medical Genetics, Academy of Medical Sciences of the USSR [6]. The following three concentrations of cells were used: 2.5×10^5, 1.5×10^5, and 0.5×10^5 cells/ml. The cells were cultured without change of medium until natural death (one passage). In the course of culture the cells were periodically fixed and analyzed. Essentially cells and cell populations in the stationary phase, which is the longest in time and the most important from the point of view of functions, were subjected to morphological and cytophysiological investigation.

EXPERIMENTAL RESULTS

Cells of strains obtained from patients with chromosomal mutations (karyotypes 49, XXXXY; 48, XXXX; and 47, XX, +21), are round and larger than normal fibroblast-like cells. A layer of such cells is weakly organized on a slide. With growth they form a compact layer without any distinct orientation.

Populations of cells of strains with chromosomal mutations, like normal cells, consist of two types: cells with diffuse chromatin in the nucleus and cells with a heterochromatinized nucleus (Fig. 1). The number of chromocenters in the cell is equal to the approximate haploid number of chromosomes and remains unchanged during prolonged culture in the stationary phase. The proportion of cells with chromocenters and their dynam-

*The term "homonuclear strains" is used instead of the term "diploid strains," for as a group it also includes aneuploid strains, which preserve a stable karyotype. Instead of diploid – heteroploid strains, homonuclear – heteronuclear strains are thus taken as opposite categories [5].

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TABLE 1. Characteristics of Cell Strains (for 3 concentrations – 2.5×10^5, 1.5×10^5, and 0.5×10^5 cells/ml)

Cell strain	Time of flattening out to stationary phase (days after beginning of culture)	Time of death (days after beginning of culture)	Proportion of chromocenters at beginning of culture, %	Proportion of chromocenters at end of culture, %	Saturating density (number of cells per flask × 10^6)	Final density (number of cells per flask × 10^6)	Seeding efficiency, %		Mean number of chromocenters in cell
							max	min	
Normal diploid strain	7±2	24±2	41±2	81±6	0,70±0,08	1,25±0,11	92±3	26±2	20
	9±1	41±3	41±2	95±1	0,59±0,05	1,05±0,14	88±3	19±3	20
	14±3	44±3	38±3	87±2	0,39±0,03	0,75±0,18	86±2	21±2	20
Cell strain from patient with Marfan's syndrome	3±1	20±2	40±2	14±2	0,78±0,03	1,80±0,09	92±5	66±9	—
	6±2	31±3	35±3	12±5	0,51±0,02	1,20±0,07	91±1	69±2	—
	12±2	31±4	28±5	8±4	0,41±0,04	0,90±0,08	89±6	71±3	—
Cell strains with chromosomal mutations									
47, XX,+21	5±2	28±2	54±3	53±3	0,32±0,02	1,65±0,06	91±1	62±4	22,4±4,1
	5±1	28±3	52±3	52±4	0,61±0,02	1,40±0,05	92±2	69±2	22,3±5,2
	24±4	39±3	56±4	58±7	0,39±0,02	0,65±0,03	91±4	49±2	22,4±5,6
49, XXXXY	6±2	22±3	38±6	18±1	0,80±0,02	1,70±0,05	94±4	38±2	24,3±5,5
	8±3	23±3	40±4	10±2	0,40±0,02	1,40±0,04	89±2	56±3	24,1±4,6
	16±3	32±4	40±1	9±3	0,22±0,04	0,65±0,04	78±2	42±2	24,5±5,1
48, XXXX	6±2	29±2	39±1	61±2	0,59±0,03	0,90±0,06	86±2	61±2	23,2±4,6
	6±2	34±2	31±2	62±2	0,48±0,02	0,70±0,07	85±3	60±2	23,1±5,1
	12±3	36±2	30±1	59±2	0,32±0,03	0,55±0,05	77±2	62±2	23,4±4,3

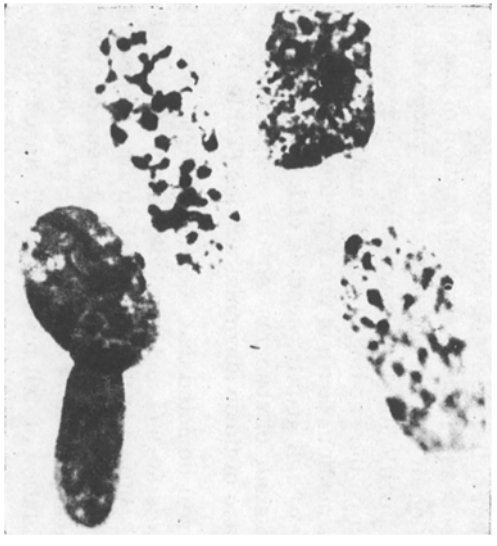

Fig. 1. Cell nuclei of strain with chromosomal mutations (from a patient with the karyotype 47, XX, + 21): Two types of cells can be seen.

ics differed for different strains (Table 1). The seeding efficiency during prolonged culture fell in the stationary phase but the minimal seeding efficiency at the end of culture was higher than normally (Table 1). Stimulation of cell growth by a change of nutrient medium led to an increase in the saturating density (the so-called final density of the cell population; see Table 1) at all times during the stationary phase. The final density in two strains with chromosomal mutations (47, XX, + 21 and 49, XXXXY), incidentally, was higher than in normal diploid strains (Table 1). Cells with chromosomal mutations, like normal cells, died critically.* The time of onset of cell death depended on the initial concentration of the cell suspension (initial density, see Table 1).

Cells of the strain obtained from the patient with Marfan's syndrome were larger, rounder, and less elongated than normal fibroblast-like cells. The population of this strain consisted of cells of two types. Cells of the first type had a pale nucleus with diffuse chromatin, cells of the second type had many small chromocenters in their nucleus. In the course of culture in the stationary phase the proportion of cells with chromocenters decreased (Table 1). The seeding efficiency decreased toward the end of culture, but by a lesser degree than normally. Stimulating cell division by changing the medium led to a marked increase in the saturating density (Table 1). The cells died critically and their death depended on the initial cell density (Table 1).

The study of abnormal cells and cell populations in the stationary phase during prolonged culture thus showed that they differed sharply from normal in all properties studied. It was previously postulated that the process of heterochromatinization of the cell nucleus in culture is connected with differentiation [2, 3]. In normal diploid cell strains two morphologically different subpopulations of cells characterized by a heterochromatinized nucleus and a nucleus with diffuse chromatin are found. It has been suggested that cells with a heterochromatinized nucleus are permanently in the I_0 or R stage of the cell cycle and are more differentiated. Cells characterized by a nucleus with diffuse chromatin, the number of which falls during culture, are probably stem or semistem cells, on account of which the culture can regenerate under certain population-ecologic conditions. The process of heterochromatinization of the nucleus in culture can be represented as the morphological expression of inactivation of a certain proportion of the genes and as a manifestation of the change-over of the cells to specific forms of synthesis. Observations on the changes in the morphology of normal cells in culture and data on the seeding efficiency and changes in saturating density during stimulation of cell division by changing the medium confirm this hypothesis. Changes in the proportion of cells with chromocenters, in the morphology of cell growth, seeding efficiency, and saturating density during stimulation of cell division observed in the study of abnormal strains suggested that the process of cell differentiation in culture is disturbed in the case of these abnormal strains (Table 1).

The fact will be noted that seeding efficiency was highest during the period of change-over to the stationary phase. It was 80-90% and was independent of the cell density. High seeding efficiency was observed toward the end of culture in the abnormal strains, especially those with gene mutations (Table 1). The high viability of the abnormal cell populations at the end of culture is attributed to the tendency toward manifestation of features characteristic of cells which have undergone malignant change. This is also confirmed by the fact that after stimulation of cell division at all stages of growth the final density of the culture in abnormal strains (especially those with gene mutation) was higher than in normal strains (Table 1).

It can tentatively be suggested on the basis of all these data that, by contrast with normal cell strains, cells of strains with chromosomal and gene mutations are characterized by individual differences connected with a disturbance of differentiation processes and with the manifestation of certain features characteristic of cells which have undergone malignant change.

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*Cell death is considered to be critical when it takes place within a short time interval, by contrast with "lytic," when it takes place gradually on account of death of individual cells.