DYNAMICS OF CHANGES IN ABNORMAL HUMAN CELLS DURING LONG-TERM CULTURE IN THE STATIONARY PHASE

INVESTIGATION OF CELLS WITH TRISOMY 7

S. G. Vorsanova

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Ontogenetic changes in normal diploid cells and cells with chromosomal aberrations (strain LTsCh-162; 47,XY, +7) during long-term culture in the stationary phase were studied. The human fibroblasts in culture were found to possess a type of ontogenetic change specific for each culture, and the dynamics of these changes depended on the density of the cell population. Two morphologically different subpopulations of cells, differing in their degree of heterochromatinization of the cell nucleus (under normal conditions), and three different subpopulations of cells in the aberrant strain (47,XY, +7) were found on investigation of the cell nucleus.
KEY WORDS: cell culture; ontogenetic changes; heterochromatinization.

A study of the dynamics of changes in human cells during long-term culture in the stationary phase showed that human fibroblast-like cells undergo regular changes while kept in this phase [1]. These changes are expressed as regular heterochromatinization of the nucleus, the accumulation of lipids, an increase in acid phosphatase activity, and a decrease in the viability (seeding efficiency) of the cells. Heterochromatinization of the nucleus is manifested as an increase in the number of cells with large chromocenters, the mean number of which may reach 20. Two subpopulations of cells are found in cultures of diploid fibroblast-like cells: one with diffuse chromatin and one with a heterochromatinized nucleus. The repetitive character of the changes in the unreproduced cell population suggests the presence of a group of ontogenetic changes in the cultured cells. It has also been shown that in the case of the "xeroderma pigmentosum" gene mutation the group of ontogenetic changes is sharply disturbed.

In this investigation the character of the ontogenetic changes was studied in an undivided population of fibroblasts of strain LTsCh-162 (47,XY, +7)

EXPERIMENTAL METHOD

Normal diploid cell strains and an abnormal strain obtained at the Laboratory of Human Cytogenetics (LTsCh), Institute of Medical Genetics, Academy of Medical Sciences of the USSR, were used. The fibroblast-like cell strains were obtained and cultured by the method usually used in the Laboratory of Cytogenetics [3]. Strain LTsCh-162, obtained from spontaneous abortion material, was described previously by Kuliev et al. [3]. Just as in the previous experiments, cells were seeded in the following three concentrations: $2.5 \cdot 10^5$, $1.5 \cdot 10^5$, and $0.5 \cdot 10^5$ cells/ml. The following properties of the cells were investigated: the state of the nucleus, the state of the cytoplasm (accumulation of lipids, acid phosphatase activity, adsorption of neutral red), and the viability of the cells. All the methods used were fully described previously [1].

EXPERIMENTAL RESULTS

Strain LTsCh-162 (47,XY, +7), like strain LTsCh-484 (xeroderma pigmentosum) described previously, differs sharply from normal cells in certain very important properties. Fibroblasts of strain LTsCh-162 are

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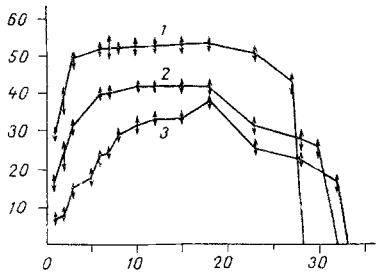

Fig. 1. Changes in number of cells per flask during culture in stationary phase (strain LTsCh-162). Abscissa, days of culture; ordinate, number of cells per flask ($\cdot 10^4$). 1) Initial concentration of cell suspension $2.5 \cdot 10^5$ cells/ml; 2) initial concentration of cell suspension $1.5 \cdot 10^5$ cells/ml; 3) initial concentration of cell suspension $0.5 \cdot 10^5$ cells/ml.

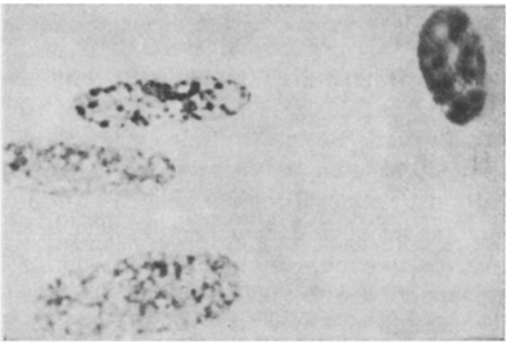

Fig. 2. Cell nuclei of strain LTsCh-162 (47,XY, +7). Cells of three types can be seen.

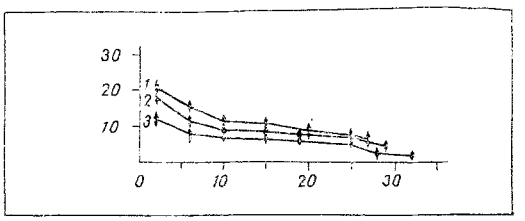

Fig. 3. Changes in number of cells with chromocenters and number of chromocenters in cells of fibroblast cultures from spontaneous abortion (47,XY, +7) during long-term culture in stationary phase (strain LTsCh-162). Abscissa, days of culture; ordinate, fraction of nuclei with chromocenters (in %). 1) Initial concentration of cell suspension $2.5 \cdot 10^5$ cells/ml; 2) initial concentration of cell suspension $1.5 \cdot 10^5$ cells/ml; 3) initial concentration of cell suspension $0.5 \cdot 10^5$ cells/ml.

more resistant to an increased population density with an initial concentration of $2.5 \cdot 10^5$ cells/ml. In this cell concentration death of the culture occurred on the 29th day, compared with the 20th day normally (Fig. 1). With an initial concentration of $1.5 \cdot 10^5$ and $0.5 \cdot 10^5$ cells/ml death of the culture occurred on the 33rd-34th days (a little earlier than normally). Death of the cells of strain LTsCh-162 took place, moreover, at a lower saturating density than normally. Morphological investigation showed that strain LTsCh-162 consists of three

subpopulations of cells differing in the degree of heterochromatinization of their nucleus (Fig. 2). Three types of cells can be seen. Type I is characterized by a pale nucleus with a delicate network of diffuse chromatin, type II by a nucleus with a definite number of large chromocenters, and type III by a nucleus with a definite number of smaller chromocenters, which are evidently more numerous in the cells. Counting the chromocenters in the course of cultivation showed that their number was constant and was about 22. The longer the cells were kept in the stationary phase, the fewer cells of type II and III (with chromocenters) were present (Fig. 3). On treatment of the cells by Arrighi and Hsu's method [2] no "karyomeres," such as are observed in normal strains, could be detected, and the cell nuclei of strain LTsCh-162 were evidently more sensitive to this treatment for they became deformed and homogeneously stained. An important feature distinguishing the cells of strain LTsCh-162 is their extremely low level of lipid accumulation. By the end of the stationary phase lipid deposits could be seen only in the form of tiny scattered granules. No significant differences from normal were found in acid phosphatase activity and adsorption of neutral red. Changes in the nutrient medium took place just as normally. Replacement of the nutrient medium did not lead to changes in the cells. The seeding efficiency of strain LTsCh-162 also was reduced, but less so than normally (from 81% at the beginning of culture to 51% at its end). The cell density in the culture 1 week before death was a little reduced, but later, just as normally, simultaneous migration of the cell layer away from the glass took place (see Fig. 1).

The results are thus further confirmation of the existence of a definite group of ontogenetic changes in cells cultured in the stationary phase, namely a group of morphological, metabolic, and physiological properties, varying with physical and biological time and with the population-ecological conditions (in this particular case, on the population density).

It will be clear from a comparison of normal cells and cells with gene and chromosome mutations that these cells differ from normal in a wide range of parameters. These differences are perhaps connected somehow with disturbance of differentiation processes in aberrant strains [3]. The most important feature of strain LTsCh-162 distinguishing it from normal strains and from strain LTsCh-484 (xeroderma pigmentosum), is the presence of three morphologically different subpopulations. The cells of type I and II are like those of normal strains, except that there are more large chromocenters (22) in the nuclei of the type II cells, evidently on account of the extra No. 7 chromosome. The subpopulation of cells (type III) with a large number of tiny chromocenters is unusual. In connection with these observations the question of the role of genetic factors in the subdivision of the cell population into morphologically different subpopulations arises. Further investigations into the life span of cells in the stationary phase, and into the structure and dynamics of undivided cell populations may help to solve this problem.

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LITERATURE CITED

1. S. G. Vorsanova, K. N. Grinberg, T. L. Éraizer, et al., Arkh. Anat., No. 11, 74 (1975).
2. F. E. Arrighi and T. C. Hsu, Cytogenetics, 10, 81 (1971).
3. A. M. Kuliev et al., Humangenetik, 17, 285 (1973).