INDIVIDUAL VARIATION IN PROLIFERATIVE POTENTIAL OF HUMAN DIPLOID FIBROBLASTS *IN VITRO*

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The study of human diploid cells *in vitro* has established the identical values of parameters of proliferative potential in monozygotic twins and their significant difference in dizygotic twins [4], a decrease in the proliferative potential of cells obtained from patients with hereditary diseases, the principal phenotypic manifestations of which are signs of premature aging [2], and negative correlation between the proliferative potential of cells and the donor's age [6]. These findings are evidence in support of genetically determined control over this parameter and the presence of correlation between the proliferative potential of cells *in vitro* and *in vivo*. The percentage of cells capable of forming colonies consisting of 16 cells or more is a reliable indicator of the proliferative potential of a strain [7]. Of all strains studied in this respect only two are embryonic, and they have been obtained from lung tissue (strains Wi-38 and IMR-90). There are some doubts about the validity of comparing these strains with postnatal strains obtained from skin biopsy material [5]. There is sufficient information in the literature on the character of proliferation of human fibroblasts *in vitro* in connection with a study of aging problems. Results obtained by the study of each group are often averaged, and for that reason there are no data on individual variation within each group of donors.

The aim of this investigation was to study the scales of individual variation of proliferative potential of strains obtained from skin biopsy material from embryos obtained at therapeutic abortions and from physically healthy blood donors belonging to the same age group.

EXPERIMENTAL METHOD

Altogether 31 strains of human diploid fibroblasts were used: 16 strains were obtained from 8-12-week old fetuses from abortions on medical grounds, and 15 strains from skin biopsy material obtained from donors aged 25-35 years. The cells were cultured in a mixture of 85%

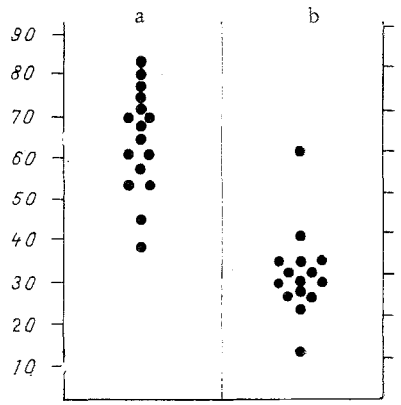

Fig. 1. Cloning efficiency of human diploid cells: a) embryonic strains; b) postnatal strains; ordinate, cloning efficiency (in %)

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Eagle's medium, 10% bovine serum, and 5% human umbilical serum. The proportions of these components for cell cloning were rather different: 80, 10, and 10% respectively. The conditions of trypsinization of the cells and of the experiments to determine cloning efficiency (percentage of cells capable of forming colonies consisting of 16 cells or more) were described previously [1]. Considering that one of the main factors affecting cell proliferation is the serum component of the nutrient medium [1], previously selected batches of bovine serum and human umbilical serum were frozen (—20°C) and thawed immediately before required for the experiments. The sera were chosen on the basis of high ability to maintain clone formation by the cells. All experiments were carried out on the same batches of medium and serum. Each value of cloning efficiency was the mean from 15-30 measurements. Mathematical analysis of the results by HP-9815A computer (USA) showed that the distribution of the individual values of this parameter obeys the law of a normal distribution. The error for each mean value of cloning efficiency did not exceed 3% (at the $P < 0.05$ level of significance).

EXPERIMENTAL RESULTS

Cloning efficiency within both embryonic and postnatal groups of strains was quite variable, but was constant for each strain. All strains were studied after five to seven subcultures. The cloning efficiency of the postnatal strains was between 12 and 60% (Fig. 1). Two of the postnatal strains had cloning efficiency of 40% or more, one strain had very low cloning efficiency (12%), and values for the other 12 strains were closely similar, between 20 and 35%. Values of cloning efficiency of the embryonic strains were distributed more uniformly over the whole range from 38 to 82%. All measures were adopted to reduce the influence of technical procedures connected with taking the biopsy material, cell culture, and performance of the cloning experiments. These precautions are essential because the proliferative potential of the strain *in vitro* may depend on the site of the biopsy and the conditions of culture of the cells [3]. Nevertheless, these results are evidence of the existence of quite wide variability of the proliferative potential of embryonic and postnatal strains of human diploid fibroblasts. It is very probable that these differences are hereditary in nature and are determined by the genotype of the individual from whom the particular strain of cells was obtained, since cloning efficiency is a parameter which characterizes each separate strain and is highly reproducible from experiment to experiment.

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