CLONAL HETEROGENEITY OF FIBROBLASTS FROM DIFFERENT HUMAN EMBRYONIC TISSUES *IN VITRO*

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KEY WORDS: diploid cells; man; clonal heterogeneity.

In the course of ontogeny cells pass along a definite pathway of realization of genetic information, which leads them to acquire special differential functions. Each stage in ontogeny is characterized by a definite ratio of stem cells to nonstem cells. This is the basis of existence of phenotypic clonal heterogeneity of cell populations both *in vivo* and *in vitro* [3]. A promising approach to the study of the structure of the cell population and its changes in ontogeny and in pathological states is clonal analysis of cell cultures *in vitro* [9]. Heterogeneity of the cell population has been demonstrated with respect to incorporation of [^{3}H]thymidine [12], the time to pass through phases of the cell cycle [13], the size of colonies [14], DNA content, and size of cells [12]. However, most of these investigations were undertaken without clonal analysis of the strains, and even if the clonal method was used, cells of only one strain, obtained from human embryonic lung (strain wi-38) were used as the object, and most attention was paid to the study of cell aging processes.

The aim of this investigation was to study proliferative parameters and cytochemical characteristics of human embryonic cells of different tissue origin under clonal conditions.

EXPERIMENTAL METHOD

Six strains obtained from two 8-10-week fetuses obtained from medical abortions were used. From each fetus three strains were isolated. Strains IMG-841 and IMG-845 were derived from skin, IMG-842 and IMG-834 from muscle tissue, and IMG-843 and IMG-798 from lung tissue. The method of isolation of the strains from these tissues was the same as that usually adopted [1]. The conditions of culture of the cells and of the cloning experiments were described previously [5]. The index of labeled cells was determined autoradiographically from the number of cells capable of incorporating [^{3}H]thymidine [11]. Cell morphology was studied by Unna's method [4]. Acid and alkaline phosphatases were determined by the azo-coupling reaction, lipids by the method of Shiney and Storey [2].

EXPERIMENTAL RESULTS

The results given in Table 1 demonstrate high proliferative indices of all the strains studied, whether determined by the index of labeled cells or by cloning efficiency.

Independently of genotype (two different aborted fetuses) and of epigenesis (different tissue origin), all strains thus had similar parameters of proliferative activity and proliferative potential. Parameters of cloning efficiency are important not only as values of proliferative potential, but also as proof that subsequently the majority of the cell population could be characterized by clonal analysis. There is evidence in the literature that the number of clonogenic cells differs in different tissues [10] and may change in the course of embryogenesis [7]. Probably these differences have not yet been observed at the stage of embryogenesis which we studied (8-10 weeks).

In all strains, marked clonal heterogeneity could be observed for the intensity of cell proliferation, so that the clones differed in size (Fig. 1). Colonies consisted either of several tens, hundreds, or thousands of cells or of several tens of thousands of cells;

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TABLE 1. Proliferative Characteristics of Strains Obtained from Various Human Embryonic Tissues

Strain	Passage	Index of labeled cells, %	Cloning efficiency, %
IMG-841	5	99	73
IMG-842	5	99	67
IMG-843	5	95	54
IMG-845	7	99	53
IMG-834	6	98	65
IMG-798	6	96	57

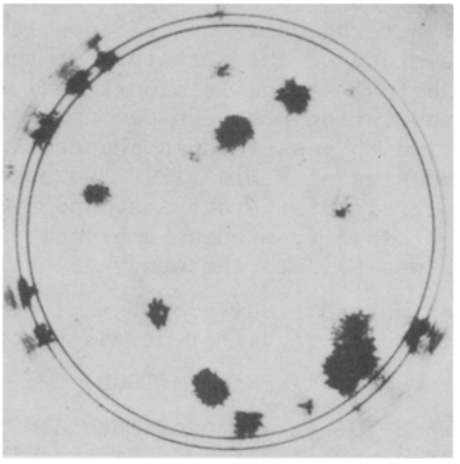

Fig. 1. Clonal heterogeneity of cells for intensity of proliferation.

colonies consisting of a smaller number of cells (<100) were found quite infrequently. As regards the morphology of the colonies and of their component cells, the clones as a whole could be divided into three types (Fig. 2). Type I were colonies characterized by the histotypical structure of fibroblasts in the stationary stage, consisting of small, fusiform, mutually oriented cells, with high mitotic activity. Type II were colonies consisting of large cells, spreading out extensively, and without any mutual orientation. These cells were characterized by sail-like or pleiomorphic morphology and low mitotic activity. Type III were mixed clonies consisting both of small fusiform and large sail-like or pleiomorphic cells.

In strains obtained from skin and muscle tissue all three types of colonies could be observed. In strains obtained from the lung of the same embryos, however, all colonies irrespective of the number of cells composing them belonged to type I only.

Cytochemical analysis after staining for lipids and acid phosphatases revealed no differences between the clones irrespective of both tissue origin and size of colony. Staining for alkaline phosphatases showed that three groups of colonies exist in strains obtained from skin and muscle tissue: those consisting entirely of positively staining cells, those consisting of both positively and negatively staining cells, and those with no cells whatever staining positively for alkaline phosphatases. In strains of muscular origin most colonies (>90%) belonged to groups 1 and 2. In strains obtained from skin the frequency of discovery of colonies of the first and second groups did not exceed 3%, but in strains obtained from lung, all colonies consisted of cells staining negatively for alkaline phosphatases.

The morphocytochemical characteristics of the colonies indicate no correlation between the rate of proliferation or the intensity of the reaction for lipids and acid and alkaline phosphatases. In both large and small colonies these parameters could remain constant (acid phosphatases, lipids). In the case of their clonal distribution (morphology, alkaline phos-

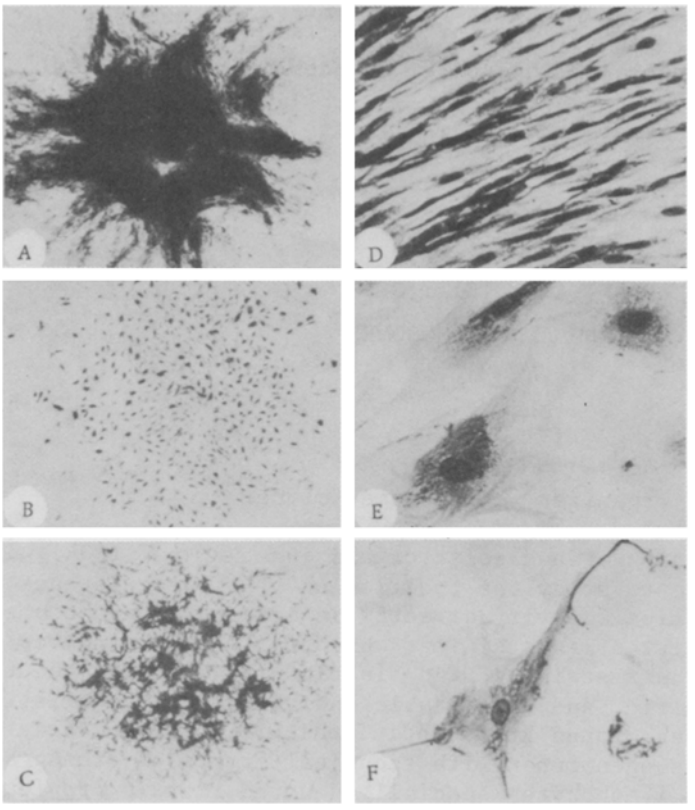

Fig. 2. Clonal heterogeneity with respect to growth and morphologic characteristics of cells. A) Colonies of type I, B) II, C) III. 30 ×. D) Fusiform, E) sail-like, F) pleiomorphic cells.

phatase), however, they were independent of colony size and were found in colonies with both high and low proliferative potential. This is evidence that postmitotic cells in phase III of growth ("old" cells), characterized by loss of proliferative activity, a high level of acid phosphatases, accumulation of lipids [8], and diminution of the reaction for alkaline phosphatases [15], may possess characteristics that differ from those of postmitotic cells in the early stages of culture. Similar results were obtained during a study of the ability of cell clones to bind concanavalin A [13].

The results are evidence that clonal differences in proliferation and morphocytochemical characteristics must be taken into account, for example, in prenatal diagnosis, when growth of cells from the amniotic fluid is essentially clonal, and also in biochemical studies of cells using micromethods. A further study of clonal heterogeneity and its change during ontogeny and in hereditary diseases may prove useful as a step toward the understanding of mechanisms of development of some human hereditary diseases.

LITERATURE CITED

1. R. Adams, Cell Culture Methods of Biochemists [Russian translation], Moscow (1983), p. 60.
2. M. S. Burstone, Enzyme Histochemistry and Its Application in the Study of Neoplasms, Academic Press (1962).
3. L. I. Korochkin, Interaction between Genes in Development [in Russian], Moscow (1977), p. 147.
4. G. I. Roskin, Microscopic Technique [in Russian], Moscow (1946), p. 215.
5. S. M. Terekhov, Tsitologiya, 23, 717 (1981).
6. S. Aizava and Y. Mitsui, Exp. Cell Res., 139, 146 (1982).
7. T. Beug and T. Graf, Exp. Cell Res., 107, 417 (1977).
8. V. J. Cristofalo, in: Senescence, New York (1977), p. 13.
9. S. Hauschka, in: Regulation of Cell Proliferation and Differentiation, New York (1977). p. 126.
10. T. B. Kirkwood and T. Cremer, Hum. Genet., 60, 101 (1982).

11. V. I. Kukharenko, K. N. Grinberg, and A. M. Kuliev, Hum. Genet., 42, 157 (1978).
12. E. L. Schneider and B. J. Powlkes, Exp. Cell Res., 98, 298 (1976).
13. J. E. Sisken and S. V. Bonner, Mech. Ageing Dev., 11, 191 (1979).
14. J. R. Smith, O. M. Pereira-Smith, and E. L. Schneider, Proc. Natl. Acad. Sci. USA, 75, 1353 (1978).
15. A. Vorbrodt, R. Charpentier, and V. J. Cristofalo, Mech. Ageing Dev., 11, 113 (1979).

CLONAL ANALYSIS OF INTERCELLULAR VARIABILITY OF NUCLEOLAR ORGANIZER ACTIVITY IN HUMAN CHROMOSOMES

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By the selective staining of nucleolar organizing regions (NOR) with silver (Ag staining) it has become possible to study the functioning of ribosomal genes by determining the product of their transcription activity directly in metaphase chromosomes. It has recently been shown that the character of Ag staining, which includes the number of NOR stained with silver, the intensity of their staining, and distribution with respect to 20 acrocentric chromosomes, is a stable, specific, and inheritable individual characteristic [12, 14]. Meanwhile most investigators have found intra-individual intercellular variability for the number of active NOR and link this phenomenon with technical errors of the Ag staining method [10]. In investigations on blood lymphocytes specially devoted to this problem, intercellular variability was shown to be a truly existing phenomenon [4, 15]. Essentially it consists of the presence of several subpopulations of cells differing in their number of active NOR. These differences arise on account of a change in the ability of one or two NOR of acrocentric chromosomes specific and characteristic for each individual, to stain with silver. It has also been found that intercellular variability does not depend on the order of cell division after the cells had emerged from the G_0 phase [3]. The observations described above can be explained in two ways: 1) In the early stages of ontogeny a definite character of activity

TABLE 1. Characteristics of Individual Chromosomes Varying in Ag-Staining of Parental Cultures and of Clonal Lines Isolated from Them

Individual	Culture	Varying chromosome	Relative percentages of two cell subpopulations
1	Parental	13	31:69
	Clone I	13	33:67
	» II	13	35:65
	» III	13	30:70
	» IV	13	36:64
2	Parental	14	37:63
	Clone I	14	50:50
	» II	14	37:63
	» III	14	39:61
3	Parental	15	52:48
	Clone I	15	54:46
	» II	15	48:52

Note. Number of metaphase plates studied for each case is shown in Fig. 1.

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