THE NUMBER OF NUCLEOLI AS AN INDICATOR OF PROLIFERATIVE ACTIVITY OF CELLS IN VITRO

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The number of nucleoli in the cells of the body is usually small [1, 2]. It may be increased in many normal (regenerations) and pathological processes; the latter include malignant transformation of cells, which is often accompanied by amplification of r-DNA genes [10, 14]. An increase in the number of nucleoli has also been demonstrated in psoriasis [7], the pathogenesis of which is connected with hyperproliferation of cells of the epidermis. It has been suggested that the number of nucleoli may be an informative characteristic of several diseases connected with activation of immune processes in the body, and also for monitoring their treatment [8].

However, it is not yet clear whether an increase in the number of nucleoli in the cell is a specific feature of a particular disease or whether this increase is simply due to a change in the proliferative activity of the cells.

Cell proliferation in vitro is based on stimulation of proliferation by serum growth factors [2]. A primary culture of human diploid cells is a good model with which to study stimulated cell proliferation. In this case the factor causing the cells to commence the mitotic cycle is the conditions of cultivation themselves, including the composition of the nutrient medium.

The aim of this investigation was to study the proliferative characteristics of cells of a primary culture and the early stages of culture, changes in the number of nucleoli during stimulation of entry of the cells into the mitotic cycle, and manifestation of features of differentiation depending on long-term culture.

EXPERIMENTAL METHOD

Experiments were carried out on diploid cells obtained from various tissues (skin, muscle tissue, lung, pigmented epithelium of the eye) of fetuses aborted on medical grounds at the 8th–12th week of pregnancy. The technique of obtaining primary cultures was the same as that usually adopted [1]. The conditions of culture and organization of the cloning experiment were described previously [6]. The index of labeled cells was determined autoradiographically [9]. The cells were stained by Unna's method [5] with Water blue, orcein, and eosin. By this method it is possible to demonstrate the morphological features of the cells in vitro. Considering that it is not a recognized method for specific staining of nucleoli, it was compared with the method that is accepted for this purpose, namely staining with methyl green and pyronine, by Unna's method [3]. By the specificity of staining of the nuclei, the two methods were found to be identical in this model. The results were subjected to statistical analysis by the usual methods [4].

EXPERIMENTAL RESULTS

Most primarily trypsinized cells adhered to the culture surface in 1–2 h. During the first day of culture the number of cells did not increase. The first mitoses could be observed after 40 h. After a dense monolayer had been obtained (7–10 days) the cells were trypsinized and transferred to a vessel of greater area (first passage). Later, the cultures underwent passage every 5–6 days.

The suspension of primarily trypsinized cells was divided into three parts. One part was used to determine the index of labeled cells, the other for continuing their culture, while the third part was maintained under clonal conditions. At the 2nd and 5th passages the strains were analyzed again with respect to these parameters.

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TABLE 1. Proliferative Characteristics, Features of Differentiation, and Number of Nucleoli in Cultures Obtained from Various Human Embryonic Tissues

Tissue	Passage	Index of labeled cells	Cloning efficiency, %	Per cent of differentiated colonies	Mean number of nucleoli per cell
Skin	Primary culture	28	0,2	—	—
	30 h	—	—	—	1,90±0,063
	96 h	—	—	—	2,87±0,060
	2nd	86	53	—	3,61±0,037
	5th	99	72	—	3,65±0,042
Lung	Primary culture	47	2	—	—
	30 h	—	—	—	1,95±0,077
	96 h	—	—	—	2,84±0,085
	2nd	89	47	—	3,73±0,049
	5th	98	56	—	3,74±0,051
Muscle tissue	Primary culture	42	0,8	17	—
	30 h	—	—	—	1,93±0,058
	96 h	—	—	—	2,76±0,097
	2nd	91	59	0	3,86±0,042
	5th	98	65	0	3,83±0,049
Pigmented epithelium of the eye	Primary culture	35	8	93	—
	30 h	—	—	—	1,79±0,033
	96 h	—	—	—	2,66±0,043
	2nd	87	42	36	3,68±0,065
	5th	96	51	0	3,71±0,059

The study of the number of nucleoli depending on the length of time in culture (the number of passages) showed that, irrespective of the nature of the tissue (skin, muscle tissue, lung, pigmented epithelium of the eye) transfer of the cells to in vitro conditions and associated stimulation of proliferation led to an increase in the number of nucleoli per cell (Table 1). All changes in this parameter took place before the second passage during culture, and thereafter the number of nucleoli did not change.

The study of the proliferative characteristics of the cultures and manifestation of features of tissues showed that cells of the primary culture have the lowest proliferative characteristics. They increased during culture to reach a maximum at the 5th passage. However, it was cells of the primary culture which had the most marked signs of differentiation, characteristic of cells in vivo. This conclusion applies to muscle cells and cells of the pigmented epithelium of the eye. As regards other tissues, it is more difficult to make a similar statement because of the absence of signs of differentiation clearly detectable in vitro. In the course of culture the signs of differentiation disappeared and by the 5th passage it was impossible to determine the tissue to which the strain belonged.

Hence, judging from the intensity of proliferation, the first stages (passages) in culture are the period of adaptation, but they are most favorable (especially the primary culture) for manifestation of signs of cell differentiation. This is probably due to the fact that the original tissue is a heterogeneous population and consists of cells with different proliferative characteristics and with a different degree of stability of the signs of differentiation. In the course of culture, selectkon takes place in favor of cells with high proliferative potential and a large number of nucleoli. The existence of clonal selection has now been conclusively proved [11]. Special attention must be paid to the fact that, according to the results of this study, the change in the number of nucleoli precedes a change in proliferative characteristics (Table 1).

It is a remarkable fact that the time of culture (hours) of primarily trypsinized cells also affects the number of nucleoli. A significant difference can be observed in cells fixed after 30 and 96 h are compared. The time of the first fixation (30 h) was chosen because in the earlier stages of culture it is difficult to count nucleoli. The time required for complete adherence and spreading of the primarily trypsinized cells is 30 h.

Differences in the number of nucleoli between the 30th and 96th hours of culture must evidently be explained by selection of the cells. In the writers' view, this increase in the number of nucleoli is connected with transfer of the quiescent cells into the mitotic cycle and maintenance of cell proliferation which has already begun. Cell systems in vivo are populations consisting of proliferating and nonproliferating cells; the latter,

moreover, include not only highly differentiated, but also stem cells [2]. Stem cells spend a large part of their life cycle in a resting state. Electron-microscopic investigations have shown that nucleoli of differentiated cells are characterized by certain definite morphological features (circular nucleoli). On transition of the cells from the resting state into the cell cycle and, in particular, during PHA-dependent stimulation of proliferation of human peripheral blood leukocytes, the fine structure of the nucleoli undergoes certain changes [15]; the order of these changes, moreover, is opposite to that observable during erythroid cell differentiation [13]. It is striking that the nucleolus, as a morphological structure, undergoes specific changes first in all these cases [12]. When the data given in Table 1 are analyzed, a similar relationship can be observed.

The data described above suggest that activation of the nucleoli and an increase in their number cannot be specific characteristics of any particular pathological state. Any disease connected with disturbance of the "proliferative homeostasis" of a tissue will lead to a change in the ratio between proliferating and quiescent cells and to an increase in the number of nucleoli.

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PRECURSORS OF TRANSIENT SPLENIC COLONIES IN THE MOUSE EMBRYONIC LIVER

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Colonies in the spleen of irradiated mice are known to be produced by polypotent, highly self-supporting hematopoietic precursor cells [5]. In this connection the identity of hematopoietic stem cells (HSC) and of cells forming colonies in the spleen (CFU_S) appeared evident. The two terms are often used as synonyms. Nevertheless, no strict proof of this dogma has yet been obtained; indeed, on the contrary, there is weighty, although indirect, evidence of differences between HSC and CFU_S [1]. It has recently been shown experimentally that some splenic colonies are produced by bone marrow cells incapable of self support [2]. These colonies are transient in nature, i. e., they can be detected 7–9 days, but not 10–12 days after injection of the cells. Transient colonies also are distinguished by the fact that they contain no polypotent precursors capable of forming mixed colonies of erythroid and myeloid cells in culture (CFU_{em}), and also unipotent precursors of granulocytes and macrophages (CFH_c) and of erythrocytes (PFU_e) although more mature erythroid precursors (CFU_e)

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