CYTOMORPHOLOGICAL CHANGES IN HUMAN TUMOR CELLS UNDER THE INFLUENCE OF HIGHLY POLAR COMPOUNDS

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Experimental evidence has been obtained that neoplastic properties of tumor cells are in principle reversible, and it has been shown that the tumor phenotype can be suppressed and dominance of the normal cell potential assured [8, 14]. Cells of embryonic tumors, provided with a normal microenvironment can differentiate in directions corresponding to normal differentiation processes [7, 13]. Spontaneous differentiation of tumor cells in culture [4, 5] and cases of spontaneous regression of human malignant tumors, connected with their differentiation [2, 11], have been described. Finally, it has been found that reversion of tumor cells can be induced with the aid of chemical compounds [9, 10, 15], among which the study of the group of highly polar compounds (HPC), such as dimethyl sulfoxide (DMSO), dimethyl formamide (DMFA), and others possessing high biological activity, is particularly interesting.

This paper describes the study of the effect of some HPC on the cytomorphological characteristics of human sarcoma cells in culture.

EXPERIMENTAL METHOD

Cells of line Sa-2, obtained in 1974 during culture of cells from a retroperitoneal metastasis of a synovial sarcoma of the foot, and line Sa-4, obtained in 1975 by culture of cells of an osteogenic sarcoma (both lines of sarcoma cells were obtained by E. A. Timofeevskaya) were used in the experiments. The tumor cells were grown in Eagle's medium with 10% bovine fetal serum, glutamine, and monomycin (10 units/ml). The following concentration of HPC were used: 1% and 2% solutions of DMSO, DMFA (100 mM), N-methylformamide (200 mM), and dimethylacetamide (0.1 and 10 mM). Tumor cells (2×10^4) were suspended in 2 ml medium and transferred to penicillin flasks with coverslips. The culture medium in the experimental flasks was replaced after 24 h by medium with the HPC, and in the control flasks by fresh medium. After culture for 4 and 6 days tissues from the experimental and control flasks were stained by the Romanovsky–Giemsa method for cytomorphological study and for the following enzymes: α-glycerophosphate dehydrogenase (GPDH) and succinate dehydrogenase (SDH) [3], acid and alkaline phosphatase (AcP and AlP, respectively) [12]. The degree of enzyme activity was estimated visually: low, average, and high. Acid mucopolysaccharides were stained with alcian blue by Steedman's method. To determine the doubling time of the number of cells, 2.5×10^5 sarcoma cells were suspended in 10 ml medium and cultured in Carrel's flasks with or without the HPC. Every 12 h cells in three experimental and control flasks were washed off with versine and trypsin and their number counted in a Goryaev's counting chamber.

EXPERIMENTAL RESULTS

A monolayer culture of Sa-2 cells, as regards it cytomorphological features, corresponded to a synovial sarcoma of epithelial cell type. Mainly polygonal cells were represented in it, arranged in layers, and single round cells also were present. The cell nuclei were located centrally or a little eccentrically. The cytoplasm was relatively adundant, and pale basophilic in color (Fig. 1). High AcP and AlP activity and low GPDH and SDH activity were found in the Sa-2 culture. On incubation of Sa-2 cells in medium with 1% or 2% solution of DMSO for 4 days, definite cytomorphological changes took place: most cells were found and their nuclei were eccentric, giving them appearance of "rosettes"; mucous containing acid mucopolysaccharides accumulated in the apical part of the cells (Fig. 2). These changes are evidence of definite structural and functional reorganization of the Sa-2 cells, which can be interpreted as signs of pseudoglandular differentiation of cells of a synovial sarcoma. Incubation of Sa-2 cells with DMSO had virtually no effect on activity of the cell enzymes. Similar cytomorphological changes also were found on incubation of Sa-2 cells with DMFA for 4 days. However, DMFA inhibited enzyme activity, especially activity of AcP and AlP. On incubation of Sa-2 cells with the same concentrations of DMSO or DMFA for 6 days changes identical with those described above took place. The HPC increased the doubling time

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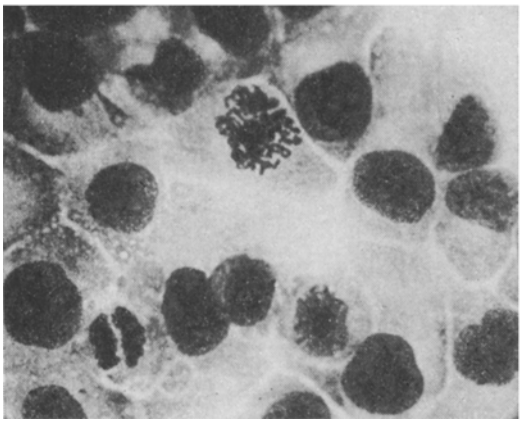

Fig. 1. Intact monolayer culture of Sa-2 cells. Here and in Figs. 2, 3, and 4 staining by Romanovsky–Giesma method, 600 X.

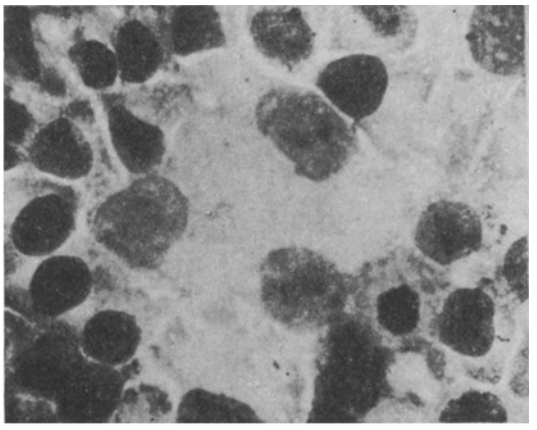

Fig. 2. Sa-2 cells after incubation for 4 days with 1% DMSO solution.

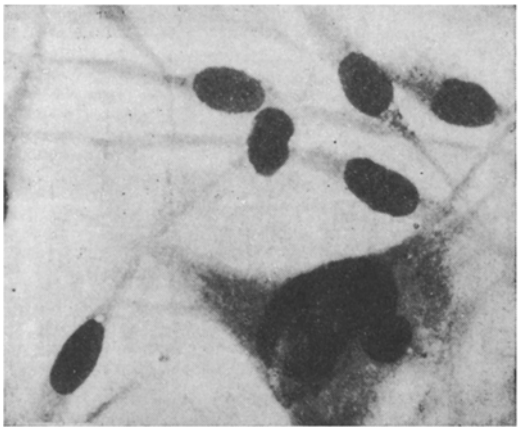

Fig. 3. Intact monolayer culture of Sa-4 cells.

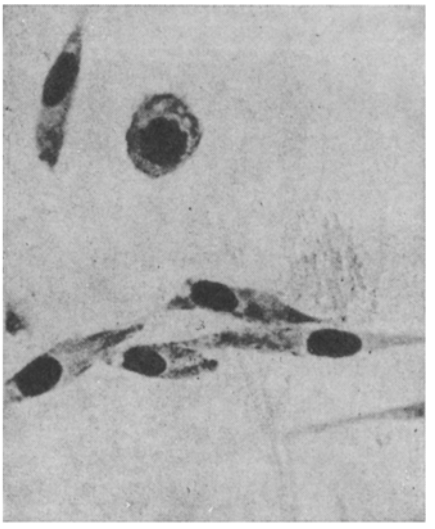

Fig. 4. Sa-4 cells after incubation for 4 days with 100 mM DMFA.

of the number of Sa-2 cells: 96 h for intact cells, 168 h for cells treated with a 1% solution of DMSO, and 192 h for cells treated with DMFA.

The monolayer Sa-4 culture is an osteogenic sarcoma consisting mainly of undifferentiated fusiform cells. The cell nuclei, oval or round in shape, are located centrally and each contains three or four large nucleoli. The cytoplasm of the cells is limited in amount, pale basophilic in color, and finely granular, Solitary polygonal and round cells also were present (Fig. 3). The Sa-4 cells have high AcP and low AlP, GPDH, and SDH activity. After incubation with DMSO or DMFA for 4 days, cytomorphological changes were found in the Sa-4 cells, in the form of a marked increase in the number of round cells (up to 20-30%). A characteristic feature of these cells was the eccentric position of their nuclei and their relatively basophilic cytoplasm with distinct outlines, evidence of their cytomorphological similarity with normal osteoblasts. About 2% of the total number of round cells divided intensively (Fig. 4). DMSO and DMFA appreciably inhibited AcP activity and the positive reaction for AlP was changed to negative. During incubation of Sa-4 cells with DMSO or DMFA for 6 days analogous cytomorphological and cytochemical changes took place in the tumor cells. DMSO and DMFA also induced an increase in the doubling time of the number of cells: 48 h for intact Sa-4 cells, 72 h for cells treated with a 1% solution of DMSO, and 96 h for cells treated with DMFA.

The action of DMSO and DMFA on the tumor cells was reversible. For instance, when cultured for a further 3 days after removal of the HPC, Sa-2 and Sa-4 cells reverted to their initial state. DMSO and DMFA, it will be noted, had no cytotoxic action on the tumor cells, for after incubation for 6 days with the HPC the tumor cells did not stain with trypan blue, and they showed no morphological evidence of degeneration.

The other HPC studied (N-methylformamide, dimethylacetamide) did not induce the changes described above in the sarcoma cells, evidence of definite selectivity of the action of DMSO and DMFA on tumor cells.

DMSO and DMFA thus induce definite cytomorphological changes in human sarcoma cells. Considering the similarity of the round Sa-4 cells to normal osteoblasts, these changes can be regarded as signs of differentiation of osteogenic sarcoma cells. Meanwhile induction of pseudoglandular differentiation in the Sa-2 cells gives no grounds for assessment of their degree of maturity. Because of relative antagonism between differentiation and proliferation of tumor cells [1] the cytostatic action of the HPC, manifested as an increase in the doubling time of the number of cells, unaccompanied by any cytotoxic effect, is interesting. Their reversible character of changes induced by the HPC in tumor cells must be emphasized. Changes in enzyme activity in the tumor cells deserve attention. In immature cells of different tissues and in tumor cells high activity of oxidoreductases is usually found, and it falls in the course of differentiation of the cells [6]. The decrease in dehydrogenase and hydrolase activity found in the present experiments can also be regarded as a possible sign of chemical differentiation of tumor cells induced by HPC. The facts described above confirm the view that HPC are inducers of reversion of neoplastic cells.

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