SPONTANEOUS DIFFERENTIATION OF HUMAN LUNG ADENOCARCINOMA CELLS IN CULTURE

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Neoplastic transformation of the cell according to the epigenome theory is connected with disturbance of function of the cell genome, and not with changes in structural genes [3, 10, 13]. It follows from this view that neoplastic changes are reversible in principle. There is experimental evidence in support of possible normalization of the tumor phenotype. Spontaneous differentiation of tumor cells has often been observed during long-term culture *in vitro* [4]. Reversal of neoplastic changes has been demonstrated during hybridization of tumor and normal cells [12], in experiments on allophene mice [9], and under the influence of certain chemical compounds and biological factors [1, 11, 14].

The object of this investigation was to study spontaneous differentiation of human lung adenocarcinoma (HLA) cells in culture.

EXPERIMENTAL METHOD

A monolayer line of HLA cells was obtained by Timofeevskaya in 1979 by the trypsinized cultures method. Original material was obtained from a metastasis in the infracla-

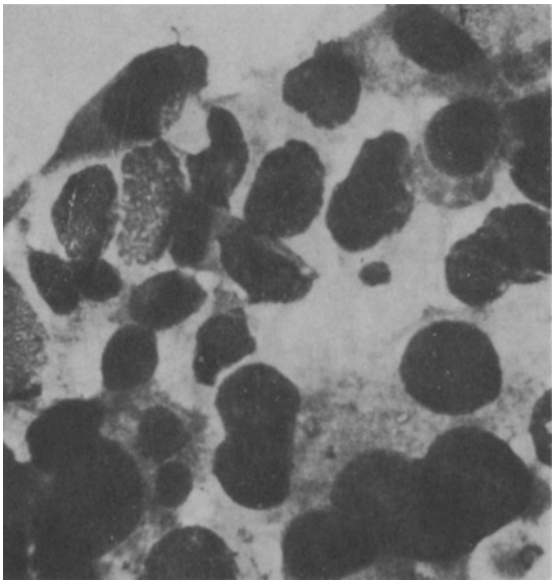

Fig. 1. Undifferentiated human lung adenocarcinoma cells in culture. Stained by Romanovsky—Giemsa method, 600 ×.

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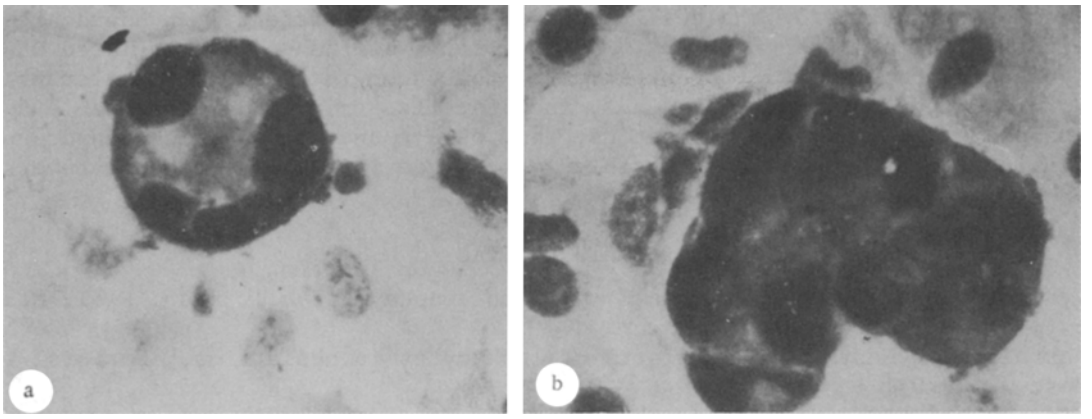

Fig. 2. Highly differentiated human lung adenocarcinoma cells in culture: a) glandular structure; b) papillary structure. Stained by Romanovsky—Giemsa method, 600 ×.

vicular lymph node of patient L. Histological examiniation of the original material revealed a dimorphic lung cancer — an undifferentiated adenocarcinoma and squamous-cell carcinoma. After trypsinization the cells were seeded into Carrel flasks in McCoy medium with the addition of 20% embryonic calf serum, glutamine, and monomycin (10 Units/ml). The cells were reseeded after 10-12 days. Tumor cells were stained by the Romanovsky—Giemsa method for cytomorphological investigation and for enzymes: α-glycerophosphate and succinate dehydrogenases, acid and alkaline phosphatases [2]. The degree of enzyme activity was assessed visually: low (+), average (++), and high (+++). Agglutination of the tumor cells under the influence of phytohemagglutinin (PHA; 0.05-0.2 ml) and concanavalin A (50-200 μg/ml) was estimated microscopically in a "hanging drop" preparation on a 3-point system [5].

EXPERIMENTAL RESULTS

Marked growth of polygonal cells, forming separate layers on the surface of the glass, was observed 5-7 days after the beginning of culture. After 3-4 weeks the cells formed a continuous monolayer. Cytological investigation revealed a monolayer consisting mainly of polygonal cells, less frequently accompanied by round or cylindrical cells; the nuclei in the cells were eccentrically arranged, and their cytoplasm was strongly basophilic (Fig. 1). The cell picture corresponded to that of an undifferentiated HLA.

Individual glandular structures began to appear 9-10 months after the beginning of culture, and thereafter their number increased. Another 6 months later (about 40 subcultures) predominantly round and cylindrical cells were observed in the culture, less frequently polygonal cells and single giant cells. The cells were arranged in multicellular structures, which were glandular and papillary complexes. The nuclei were strictly oriented and situated in the basal part of the cells. Nuclear chromatin was identified in the form of uniformly distributed granules, against the background of which the greatly enlarged 1-3 nucleoli were examined. The peripheral part of the cytoplasm was distinguished by its strong basophilia; its center was pink in color, evidence of the secretory function of the cells. Pink secretory masses also were present in the center of the glandular structures (Fig. 2). These cytomorphological features of glandular differentiation thus enabled this culture to be identified as a highly differentiated human lung adenocarcinoma.

Cytochemical investigation of the tumor cells revealed high activity (+++) of α-glycerophosphate and succinate dehydrogenases and of acid and alkaline phosphatases. In some cases high activity of oxidation—reduction enzymes has been observed in tumor cells and immature cells of various tissues, and it decreased in the course of their differentiation [6].

Marked agglutination (++ and +++) of HLA cells in culture was observed under the influence of PHA and concanavalin A. There is evidence that agglutination under the influence of lectins reflects changes in the architecture of the surface membrane characteristic of tumor cells and correlates with their oncogenic properties [7]. Increased ability to agglutinate under the influence of lectins has been demonstrated with many types of tumor cells of man and animals. It has also been found that on reversion of tumor cells their ability to agglutinate diminishes [8].

In the course of culture of the cells of a dimorphic undifferentiated adenocarcinoma and squamous-cell carcinoma of the human lung, growth of cells of the undifferentiated HLA predominated, with gradual spontaneous differentiation of the tumor into a highly differentiated HLA. Cells of the highly differentiated HLA, incidentally, exhibited considerable ability to agglutinate under the influence of lectins; they were found to have high dehydrogenase and hydrolase activity, so that it is not possible to speak definitely of the true reversion of these cells.

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INHIBITION OF TUMOR GROWTH IN SYNGENEIC MICE FOLLOWING PROCEDURES AFFECTING T LYMPHOCYTE ACTIVITY

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It is now increasingly evident that responses of the immune system of the host to tumor growth affect different levels. Processes mediated by T lymphocytes may suppress or, in general, eliminate a tumor, and they may also stimulate tumor growth and metastasization. Immunodepressive influences in some cases accelerate, in others inhibit tumor growth [4]. According to abundant evidence, the principal role in suppression of antitumor immune responses of the body is played by a subpopulation of T lymphocytes, namely T suppressors. These cells may specifically and nonspecifically suppress the response to various antigens, including tumor-specific antigens [11, 12]. It must be emphasized that these results were obtained mainly by the use of immunogenic tumors, i.e., tumors against which transplantation immunity can be induced in syngeneic animals [4]. Yet the majority of "truly spontaneous" tumors in mice and rats and also, evidently, many human tumors are weakly immunogenic in a syngeneic system [8, 9].

The aim of this investigation was to study the effect of factors modifying the immune system on growth of a spontaneous, weakly immunogenic tumor in a syngeneic mouse system.

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