A FACTOR PRODUCED BY HUMAN CELLS IN VITRO THAT CHANGES HeLa CELL COLONIAL MORPHOLOGY

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SUMMARY

Colonies of HeLa cells cultured in media supplemented with human or bovine serum or both can be morphologically described as three types: diffuse, intermediate, and compact, with their modal distribution depending on the serum or sera added to the growth medium. We have observed that for a particular medium or serum system, the percentage of compact colonies remains fairly constant under normal culture conditions, 0.2%, whereas the diffuse and intermediate colonies vary over a much wider range. The presence of certain substances as trypsin, heparin and Darvan in the medium favor the increase of compact colonies at the expense of other types. Furthermore, we have discovered that colonial morphology is influenced by cocultivation of the HeLa cells with human fibroblast-like cells, the compact colonies increasing as the density of the fibroblast element introduced into the mixed cultures is increased. Subsequent investigation revealed that conditioned medium from confluent fibroblast and HeLa cell cultures contained a factors(s), that significantly increased the percentage of compact colonies. The factor is nondialyzable, heat-stable and can be neutralized by serum. Recorded in this presentation are preliminary observations on the kinetics of colony formation and the interaction among the three HeLa cell colony types, the diffuse, the intermediate, and the compact. The factor’s effect on HeLa cell colonial morphology is time dependent and rapidly reversed if the factor(s) is removed and fresh medium added.

Key words: fibroblast-like cells; conditioned medium; HeLa morphology; compact colonies.

INTRODUCTION

Human cells in vitro are subject to morphologic changes when propagated in human, bovine, or horse sera (8,10). Murphy et al. quantified these differences by developing a classification system of human epithelial cells including HeLa, Chang liver, and Maben. They recognized two general types of cells: a) diffuse cell variant in which the cells form a very loose arrangement, and b) compact cell variant which consists of tightly packed cells with frequent multilayering. The frequency distribution of these cell variants changes with the type of sera the cells are propagated in.

We have noted (3) that most human fibroblastic cell lines from malignancies when propagated with HeLa cells increased compact HeLa cell variants (henceforth referred to as colonies), whereas fibroblastic cell lines from human embryonic tissues increased diffuse colonies. Further study (4) indicated that the ability of a fibroblastic cell line to induce HeLa cell compact colonies did not correlate with fibroblastic collagen production, but did so with increasing production of mucopolysaccharides. Ongoing study has noted a correlation between the density of fibroblastic cell growth, and the development of compact HeLa cell colonies. When we propagated the HeLa cells with low density foreskin fibroblastic cells, there was little change in HeLa cell colonial morphology, whereas in the presence of a high density of fibroblastic cells a marked increase in HeLa cell compact colonies resulted.

We have further observed that “conditioned medium” in which fibroblastic or HeLa cells themselves proliferated, contained a factor or factors that induced changes in the colonial morphology of HeLa cell cultures.

Studies on this factor(s) constitutes the basis of this report.

MATERIALS AND METHODS

Growth Medium. Eagle’s basal medium (BME) (5) in a modified buffer and containing 50 μg/ml of Gentamycin and 2 μg/ml of Fungizone. The medium was supplemented with 10% fetal bovine serum (FBS) for stock maintenance of HeLa cells.

For the growth of fibroblastic cells from tissue explants, medium KBM/199 was used containing the above antibiotics with 10% FBS for stock cultures, and further supplemented with 10% human serum (HS) for experimental cultures. [KBM/199 represents a basal medium with lactalbumin hydrolysate in a phosphate phosphite buffer enriched with Medium 199 and having a milliosmolarity approximately 170 mOsM(9)].
HeLa MORPHOLOGY CHANGING FACTOR

FIG. 1. Effect of fibroblast inoculum size on HeLa cell compact colonies. As the inoculum size increases, the percentage of compact colonies increases also, attaining an experimental maximum of 9.6% with a $10 \times 10^3$ inoculum, and decreasing to a value of 0.2% with an inoculum of $1 \times 10^2$ fibroblasts per culture.

Sera. FBS was purchased from Hyclone Lab Inc. and from Grand Island Biological Company, Grand Island, NY; human sera (Rh+) was obtained locally.

Cells. HeLa cells, CCL-2 were supplied by the American Type Culture Collection, Rockville, MD. Fibroblastic cell lines were initiated from neonatal foreskin explants procured from local hospitals.

The cell lines were grown for 2 wk on antibiotic-free medium and monitored for bacterial and mycoplasma contaminants. Use was made of the procedure of Chanock et al. (1) using an enrichment broth with subsequent inoculation of (PPLO) agar plates for PPLO monitoring.

For the experiments, a suspension of HeLa cells was dispensed into 60-mm plastic dishes. Each dish received 350 to 500 cells, and the cultures were incubated at 36.5°C in a humidified air, 3.5% CO2 atmosphere. After 24 h the medium was removed and a graded suspension of fibroblastic cells, usually in Passages 4 to 7, was added to each dish. After 11 d in culture, the dishes were stained with Wright stain and the HeLa cell colonial morphology scored for 100 colonies as previously described (3). In brief, colonies were classified into three types: a) diffuse, in which the cells remain spread in a very loose arrangement; b) intermediate, in which the cells in the center of the colony have become packed together and frequently multilayered while the edges still contain cells in a loose arrangement; and c) compact, in which the entire colony contains cells tightly packed together with frequent formation of multilayers of cells.

Procedures

Ten culture dishes were plated with 400 HeLa cells each, in both control and conditioned media. After 11 d the dishes were stained and the colonial morphology of 100 colonies in each dish was scored. The results of comparison of control and experimental groups for compact colonies significant at level $P = 0.001$.

Values, mean ± SE.

<table>
<thead>
<tr>
<th>Colony Type</th>
<th>Control Medium</th>
<th>Conditioned Medium</th>
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<tbody>
<tr>
<td>Diffuse</td>
<td>$44.2 \pm 2.1^*$</td>
<td>$9.1 \pm 1.0^*$</td>
</tr>
<tr>
<td>Intermediate</td>
<td>$55.8 \pm 2.1$</td>
<td>$53.0 \pm 2.1$</td>
</tr>
<tr>
<td>Compact</td>
<td>$0.0$</td>
<td>$37.9 \pm 1.9$</td>
</tr>
</tbody>
</table>

TABLE 1

EFFECTS OF FIBROBLAST-CONDITIONED MEDIUM ON HELA CELL COLONIAL MORPHOLOGY

![FIG. 2. Eleven-day-old HeLa cultures stained with Wright stain. ×12. A, control medium: diffuse (d) and intermediate (i) colonies. B, conditioned medium: compact colonies.](image_url)