Cellular Differentiation of Rat Uterine Adenocarcinoma Cells by Progesterone in Vitro

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SUMMARY

Transplantable cloned HTP/C1 culture was a stable line derived from a rat uterine adenocarcinoma that was induced by 7,12-dimethylbenz(a)anthracene in vivo and did not display density-dependent inhibition of growth. This HTP/C1 culture easily adapted to grow in a culture medium containing progesterone, 8 µg/ml. As compared with HTP/C1 culture, HTP/C1/P8 culture grown in the presence of progesterone was contact inhibited, and a cellular differentiation was observed in the tumor tissues that developed after inoculation of cells. These actions of progesterone on uterine adenocarcinoma cells were completely reversible on removal of the hormone in vitro.

These results appeared to indicate that progesterone was involved in the regulation of both cellular proliferation and differentiation. The possible mechanisms of the regulation of rat uterine adenocarcinoma cells by progesterone are discussed in relation to cellular levels of cyclic adenosine 3':5'-monophosphate in vitro.

INTRODUCTION

Recently, it has been reported that histological differentiation of human endometrial adenocarcinoma was induced by the administration of progestogens, irrespective of local or systemic application of the drugs (1-3, 5-7, 9-12, 20, 21). We reported that progesterone at concentrations of 8 µg/ml or higher was inhibitory to the growth of rat uterine adenocarcinoma cells in vitro (17). However, the mechanisms of cellular differentiation by progesterone are not known, particularly in cancer cells.

This paper describes an attempt to evaluate the regulatory mechanisms of differentiation of rat uterine adenocarcinoma cells by progesterone in vitro.

MATERIALS AND METHODS

Cells. Transplantable cloned cell line HTP/C1, which was derived from the parent culture of a Sprague-Dawley rat uterine adenocarcinoma induced by intraperitoneal administration of 7,12-dimethylbenz(a)anthracene in vivo, was used (16, 18). Cells were grown at 37°C in a humidified 5% CO₂ and cultivated in 60-mm Petri dishes (Falcon Plastics, Oxnard, Calif.). The culture medium consisted of modified Eagle’s minimum essential medium supplemented with 10% calf serum. Several biological characteristics of this cell line in vitro were described previously (16).

Hormone. The stock solution of progesterone, 8 mg/ml (Mochida Pharmaceutical Co. Ltd., Tokyo, Japan), was prepared by dissolving it in ethanol and diluting it with culture medium to give a final concentration of 8 µg/ml before use. The solvent had no effect in the test system.

Transplantation of Cells. About 10⁶ cells suspended in 0.2 ml of culture medium were injected s.c. into the interscapular region of isologous newborn rats within 48 hr after birth. The rats were sacrificed at 4 weeks after inoculation of cells, and the sex, tumor weight, and metastasis to the lung were examined. The tumor tissues obtained were fixed in 10% neutral formaldehyde solution and stained with hematoxylin and eosin, Van Gieson, and silver staining method for histological examinations.

RESULTS

Isolation of Cells Adapted to Grow in the Culture Medium Containing Progesterone. HTP/C1 culture easily adapted to grow in the culture medium containing progesterone, 8 µg/ml (HTP/C1/P8 culture). However, higher concentrations, more than 16 µg/ml, seemed to be nonspecifically toxic, and attempts to isolate cells that could grow in the culture medium containing progesterone at concentrations higher than 16 µg/ml were unsuccessful.

HTP/C1 cells grew rapidly (doubling time, 22 hr) and consisted of loosely adherent, elongated, less flattened cells that showed a marked motility (Fig. 1). HTP/C1/P8 cells, in contrast, were strongly adherent, polygonal cells with characteristic granules in cytoplasm (Fig. 2). They proliferated slowly (doubling time, 32 hr) under the influence of progesterone. On the other hand, contact inhibition of cells did not occur in HTP/C1 culture, and cells began to pile up after extensive growth on the surface. In contrast, HTP/C1/P8 cells consisted of more flattened cells, and no overlapping or multilayering of cells was seen after division ceased at confluence (Fig. 4).

The saturation density at confluency decreased from 17.4 × 10⁶ cells/sq cm in HTP/C1 culture to about 4 × 10⁶ cells/sq cm in HTP/C1/P8 culture 11 days after inoculation of about 10⁶ cells/plate.

On removal of progesterone and addition of fresh culture medium to HTP/C1/P8 culture, the cells reverted to the original morphology of cell colony within 5 days (Fig. 3).
and to their original 3-dimensional growth within 11 days (Fig. 5).

Tumorigenicity and Rate of Lung Metastasis of Cells. As shown in Table 1, the average tumor weight after 4 weeks of inoculation of 10^6 HTP/C1/P8 cells was about one-half of that after inoculation of HTP/C1 cells. In addition, the rate of lung metastasis was significantly decreased in animals given inoculations of HTP/C1/P8 cells, as compared with that produced by HTP/C1 cells.

Histology of Tumor Tissues. The histological findings of tumor tissues produced in animals given inoculations of HTP/C1 cells showed undifferentiated adenocarcinoma with large stromal reticulum fibers, and the glandular structure was scarcely detected (Fig. 6). In contrast, histology of tumor tissues produced by HTP/C1/P8 cells showed a marked glandular structure with proliferation of stromal tissues and confirmed a diagnosis of differentiated adenocarcinoma (Fig. 7).

DISCUSSION

Present results show that progesterone is involved in the regulation of both cellular proliferation and differentiation of rat uterine adenocarcinoma cells. These actions of progesterone on carcinoma cells are direct and reversible in vitro.

Histological studies of human endometrial adenocarcinomas secondary to the administration of progestational agents have been reported. Kistner et al. (9, 10) first demonstrated that synthetic progestogens produced marked changes, such as profound glandular atrophy and decidual conversion of the stroma in endometrial adenocarcinomas following prolonged progestogen therapy. Verga and Henriksen (20) also noted that the structural changes consisted of a more orderly and benign appearance of glandular pattern, secretory distension of the acini, and extensive acanthomatous transformation. The same observations as above on the histological effects of progestational agents have been reported by other investigators (1–3, 5–7, 11, 12, 21). In addition to histological alterations, the cytological changes in the cells of human endometrial adenocarcinomas following progestogen therapy were observed to be: (a) a decrease or cessation of mitotic activity, (b) pyknosis of the nuclei, (c) less piling up of the cells and a tendency toward single layering, and (d) increase in cytoplasm with prominent vacuoles, irrespective of local or systemic application of the agents (1, 5, 6, 20, 21).

On the other hand, Nordqvist (13, 14) demonstrated that human endometrial carcinomas showed a dose-dependent sensitivity to progesterone in organ culture. Kohorn and Tchao (11) also reported the effect of progesterone on endometrial carcinomas in vitro and suggested that progesterone could directly produce differentiation of carcinoma tissues, as reported in the normal human endometrium (4).

These results in vivo and in vitro suggest that progestational agents directly increase differentiation of endometrial carcinoma cells, as observed in the present study.

Although the biological role of cyclic AMP remains

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1 The abbreviation used is: cyclic AMP, cyclic adenosine 3':5'-monophosphate.
Differentiation of Uterine Carcinoma by Progesterone

Table 1

<table>
<thead>
<tr>
<th>Cell lines inoculated</th>
<th>No. of rats inoculated</th>
<th>Tumor wt/rat 4 wk after inoculation of cells (g)</th>
<th>No. of rats with metastasis to the lung</th>
</tr>
</thead>
<tbody>
<tr>
<td>HTP/C1</td>
<td>12</td>
<td>$1.1 \pm 0.1^a$</td>
<td>12</td>
</tr>
<tr>
<td>HTP/C1/P8*</td>
<td>16</td>
<td>$0.6 \pm 0.1$</td>
<td>2</td>
</tr>
</tbody>
</table>

*Mean ± S.E.
*HTP/C1 cells growing in the culture medium containing progesterone, 8 μg/ml.

obscure, it might involve the control of both cellular growth and differentiation. This is supported by the observations that cyclic AMP levels are lower in transformed cells (15, 19) and that agents that increase cellular cyclic AMP levels suppress the growth of cells (8). Therefore, whether progesterone stimulates the formation of cyclic AMP levels within rat uterine adenocarcinoma cells remains to be elucidated and is currently under investigation. Cellular levels of cyclic AMP detected by radioimmunoassay are at least 3 times higher in HTP/C1/P8 cells grown in the culture medium containing progesterone, as compared to original HTP/C1 cells grown in the culture medium without hormone (S. Sekiya, Y. Kikuchi, and H. Takamizawa, unpublished data). As a result of the increase in cyclic AMP levels in carcinoma cells by unknown mechanisms, the regulation

Fig. 4. HTP/C1/P8 culture at confluent state. At 11 days after the inoculation of $10^4$ cells. In the presence of progesterone, piling up of cells was noticeably inhibited. Phase contrast, × 100.

Fig. 5. HTP/C1/P8 culture at confluent state 11 days after removal of progesterone, at 11 days after the inoculation of $10^4$ cells. Note piling up of cells. Phase contrast, × 100.
of both cellular growth and differentiation by progesterone might occur in rat uterine adenocarcinoma cells.

ACKNOWLEDGMENTS

The authors greatly appreciate the histological examinations by Dr. K. Nagao, Department of Pathology, Chiba University.

REFERENCES


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