Effects of glucocorticoid on proliferation, differentiation, and glucocorticoid receptor expression in human ovarian carcinoma cell line 3AO

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KEY WORDS dexamethasone; glucocorticoids; glucocorticoid receptors; ovarian neoplasms

ABSTRACT

AIM: To study the effects of dexamethasone (Dex), a synthetic glucocorticoid, on proliferation, differentiation, glucocorticoid receptor expression and regulation in human ovarian cancer cell line 3AO. METHODS: 3AO cells proliferation was evaluated by viable cell count, activity of alkaline phosphatase (AKP) and tumor marker CA125 level were determined; the expression and regulation of glucocorticoid receptor (GR) in 3AO cells was studied with radioligand binding assay. RESULTS: Dex inhibited the proliferation of 3AO cells accompanied by morphological changes in concentration- and time- dependent manner. AKP activity was increased and tumor marker CA125 was decreased in 3AO cells after treatment with Dex. The induction of AKP activity by dexamethasone was blocked by RU486, a potent glucocorticoid antagonist. There existed high affinity and low capacity of GR in 3AO cells, and the GR binding activity could be downregulated by Dex. CONCLUSION: Glucocorticoids play an important role in the regulation of 3AO cell proliferation and differentiation. There existed functional GR in 3AO cells and the cellular effects of dexamethasone on 3AO cells were mediated by GR.

INTRODUCTION

Ovarian carcinoma is the third frequently diagnosed malignant tumors of gynecology and the leading cause of death in women[1]. Ovary not only can secrete sex hormones, but also is a target organ for steroid hormones, such as estrogen, progesterone, androgen, and glucocorticoids (GC). In recent years, some studies suggest GC exerts profound effects on the proliferation and differentiation of many types of normal and malignant cells, such as fibroblasts, lymphatic leukemia cells[2-5]. Up to now, there are few reports about the effects of GC on biological behaviors of ovarian carcinoma. 3AO is a newly-established human serous ovarian cancer cell line. In this paper we initially evaluated the effects of dexamethasone (Dex), a synthetic glucocorticoid, on proliferation and differen-
tion of 3AO cells.

MATERIALS AND METHODS

Materials RPMI-1640 medium was obtained from Nissui Pharmaceutical Co, Ltd (Tokyo, Japan). [1,2,4-^3H]Dex was purchased from New England Nuclear (Boston, MA, USA). [α-^32P]dCTP was purchased from Amersham Corp Products (Arlington Heights, IL, USA). Dex from Sigma Chemical Co (St Louis, MO, USA). The antiglucocorticoids compound RU486 was supplied by Roussell-Uclaf (Romainville, France). All other materials for cell culture and biochemical analysis were of the highest quality commercially available.

Cells The cell line 3AO was established from a surgical specimen of human serous ovarian carcinoma with estrogen and progesterone receptors. The cultures were maintained at 37°C in a humidified atmosphere of 5% CO_2+95% air in RPMI-1640 medium supplemented with antibiotics and 5% FBS.

Determination of AKP activity AKP activity was determined as described.\(^6\)

Determination of CA125 in 3AO cells CA125 released into conditioned medium was quantitated using ELISA. The kit was purchased from Sorin Biomedica, Italy.

Determination of GR Specific binding of [1,2,4-^3H]Dex to GR was determined by a whole cell binding assay.\(^7\) Cell suspension was incubated with increasing concentrations of [1,2,4-^3H]Dex (2–26 nmol/L) in the presence or absence of a 1000-fold molar excess of unlabeled Dex. The binding reaction was continued for 2 h at 24°C. Cells were washed three times with ice-cold PBS, and radioactivity was determined by liquid scintillation counting. Data were analyzed according to Scatchard plot analysis.

Statistical evaluation All the data presented here were expressed as mean±SD of at least three independent determinations; the results were statistically evaluated by Student’s t test.

RESULTS

Hormonal effects on 3AO cell proliferation in monolayer culture Initially, the effects of Dex on 3AO cell proliferation in monolayer culture was studied. 3AO cells were cultured for 5 d in the presence of various concentrations of Dex. As illustrated in Tab 1, Dex significantly inhibited cell proliferation in a concentration-dependent way. The morphological alterations of 3AO cells changed from typical epithelial cells to fibroblast-like ones after treatment with Dex, which strongly suggested that Dex might induce 3AO cell differentiation. To clarify this possibility, alkaline phosphatase (AKP) activity and the levels of CA125 were further evaluated.

Tab 1. Effects of Dex on the proliferation and induction of AKP activity in 3AO cells. n=6 in each group. Mean±SD. \(^*P<0.05, **P<0.01\) vs control.

<table>
<thead>
<tr>
<th>Group</th>
<th>Ratio of viable cell counting/%</th>
<th>AKP activity /µmol·min⁻¹·g⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100</td>
<td>282±10</td>
</tr>
<tr>
<td>Dex/µmol·L⁻¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.0001</td>
<td>98.0±2.5</td>
<td>260±11</td>
</tr>
<tr>
<td>0.001</td>
<td>77±3(^*)</td>
<td>320±20(^*)</td>
</tr>
<tr>
<td>0.01</td>
<td>76±4(^*)</td>
<td>388±9(^*)</td>
</tr>
<tr>
<td>0.1</td>
<td>61±6(^*)</td>
<td>422±10(^*)</td>
</tr>
<tr>
<td>1</td>
<td>57±4(^*)</td>
<td>416±9(^*)</td>
</tr>
</tbody>
</table>

Effects of Dex on AKP activity AKP activity in 3AO cells could be induced at 48 h by Dex in concentration-dependent (Tab 1) and time-dependent way, reaching a maximum of about 2.1 fold at 96 h after Dex (1 µmol/L) treatment (Fig 1). RU486, a potent antiglucocorticoid, had no effects on AKP activity. Co-addition of RU486 (1 µmol/L) and Dex (1 µmol/L) to 3AO cells could almost completely reverse the stimulatory effects of Dex on AKP activity (Fig 2).

Effects of Dex on CA125 expression The levels of CA125 were decreased in a time- and concentration-dependent way after Dex treatment (Tab 2, Fig 3).

Radioligand binding assay GR was investigated in 3AO cells. Because the biological responses to glucocorticoids are thought to be mediated by GR, and the
Effects of glucocorticoids on 3AO cells could be reversed by RU486. When 3AO cells were incubated with increasing concentrations of [1,2,4-\textsuperscript{3}H]Dex, saturation of specific hormone binding occurred. Scatchard analysis of specific binding yielded apparent dissociation constant ($K_d$) of (1.37±0.19) nmol/L and maximum binding capacities of (92.3±2.3) fmol/1×10\textsuperscript{6} cells. It might be concluded that the bioresponses of 3AO cells to Dex were mediated by GR.

Regulation of GR binding activity in 3AO cells

Treatment of 3AO cells with Dex 1 µmol/L led to a significant decrease in GR binding activity in a time-dependent way (Fig 4). These results were consistent with those described in other systems. A maximum decrease occurred at 24 h at 1 µmol/L (Fig 5).

### Tab 2. Inhibition of CA125 release from 3AO cells by Dex 0.1 µmol/L. n=6 in each group. Mean±SD. \textsuperscript{a}$P<0.01$ vs control.

<table>
<thead>
<tr>
<th>Group</th>
<th>Time/d</th>
<th>CA125 release (pg/cell)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2</td>
<td>8.4±1.1</td>
</tr>
<tr>
<td>Dex</td>
<td>2.3±0.6\textsuperscript{b}</td>
<td>2.3±0.9\textsuperscript{c}</td>
</tr>
</tbody>
</table>

Effects of glucocorticoids on 3AO cells could be reversed by RU486. When 3AO cells were incubated with increasing concentrations of [1,2,4-\textsuperscript{3}H]Dex, saturation of specific hormone binding occurred. Scatchard analysis of specific binding yielded apparent dissociation constant ($K_d$) of (1.37±0.19) nmol/L and maximum binding capacities of (92.3±2.3) fmol/1×10\textsuperscript{6} cells. It
DISCUSSION

Glucocorticoids have been shown to suppress both in vivo and in vitro growth of many different types of normal and transformed cells. In the present study, it was further demonstrated that glucocorticoids could significantly suppress the proliferation of human ovarian carcinoma 3AO cell line in a concentration- and time-dependent way. The morphological appearance of 3AO cell changed from typical epithelial cells to fibroblast-like ones during Dex treatment, strongly suggested that Dex might induce 3AO cell differentiation. To clarify this possibility, alkaline phosphatase (AKP) activity and CA125 expression were further evaluated. It was demonstrated that the AKP activity was increased and CA125 expression was decreased in a time- and concentration-dependent way after Dex treatment. Therefore, it could be concluded from these results that Dex did induce 3AO cell differentiation in addition to its growth-suppression effects since AKP activity and CA125 are two independent markers of ovarian carcinoma cell differentiation.

To clarify further the molecular mechanism of glucocorticoid action, the effects of RU 486 on proliferation and differentiation were simultaneously investigated. It was clearly shown that AKP activity could be at least partially reversed by RU486 and which was consistent with the work of other reports. They strongly suggested that these effects were mediated by GR. Verification of the presence of the GR in 3AO cells was accomplished by saturation analysis of [1,2,4-3H]Dex binding in the 3AO cells and corresponding Scatchard plot analysis of the specific binding. It was demonstrated that there existed a saturable GR with high-affinity in 3AO cells, further demonstrating that the effects of glucocorticoids on these cells were mediated by their cognate receptors.

To this end, the well-documented downregulating effects of glucocorticoids on GR in 3AO cells were first studied, and it was found that the autoregulation of GR did occur in 3AO cells, reflected by a decrease on the level of glucocorticoid binding in a time- and concentration-dependent manner. Homologous downregulation of GR by glucocorticoids has been well established in various systems, the majority of evidence indicates that downregulation of GR is a very complex process involving transcriptional, posttranscriptional, and posttranslational mechanisms. The molecular mechanism of this remains open. GR is a hormone-dependent transcriptional factor, which is a major determinant of glucocorticoid responsiveness. Therefore, it is of crucial importance to understand the factors that regulate GR expression. Karlan reported the inhibition of ovarian cancer cells with the increase of HER-2/neu messenger RNA after Dex treatment; Ferrandian found epithelial growth factor receptor (EGFR) was increased in the OVCA433 ovarian cancer cells by Dex, while EGFR played an essential role in cell proliferation and differentiation. Braunschweiger et al discovered that cell proliferation cycles were inhibited in G stage by Dex and the degree was related with the amount of GR. It was found that cells with GR might not be sensitive to GC in some experiments, which might be related with variety of cells, activities of receptor, modulating factor of receptors, and content of receptor.

In conclusion, the present study now provides evidence that glucocorticoids can induce growth suppression and differentiation of 3AO cells, which were mediated by functional GR in 3AO cells, and GR in 3AO cells could be downregulated by Dex at protein levels.

REFERENCES


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