The Effect of Interleukin-6 on the Proliferation of Prostate Cancer Cells in Vitro and the Modulation of This Procedure

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Summary: The role of interleukin-6 (IL-6) in the growth of an androgen-independent prostate cancer cell line (PC-3m) was defined and the effect of dexamethasone, which was previously shown to modulate IL-6/IL-6 receptor (IL-6R) on this procedure was investigated. By using a pretty sensitive and specific enzyme immunoassay (ELISA), it was found that PC-3m produced certain IL-6, but there was no difference in IL-6 secretion between the group with or without dexamethasone treatment. It was also found that PC-3m cells could not be stimulated to grow by exogenous IL-6 \( (P>0.05) \), while it could be inhibited to grow by anti-IL-6 monoclonal antibody and dexamethasone with a dose-dependent fashion. Our observation indicated that IL-6 acted as an autocrine growth factor for PC-3m, and dexamethasone could inhibit cell proliferation by a mechanism independent of its effect on IL-6 mRNA expression.

Key words: interleukin-6; prostate cancer; dexamethasone; crystal violet

The incidence of prostate cancer is steadily increasing and is now becoming the third most common urological cancer in China. Unfortunately, the treatment of those patients was far from successful. IL-6 is a pleiotropic cytokine that has been shown to have many complex physiological and pathological functions. It can induce either growth-inhibitory or growth-stimulatory activities in several tumor cells, depending on the nature and the IL-6 receptor expressing condition of the target cells [15]. Until now, the relationship between IL-6 and prostate cancer is uncertain, and some reported that IL-6 could act as an autocrine growth factor for prostate cancer cell [21], but others pointed out that IL-6 could restrain the proliferation of LNCaP, a hormone-dependent prostate cancer cell line [22]. Dexamethasone is known to inhibit IL-6 gene expression, but its role in regulation of IL-6 production and proliferation on PC-3m has not been studied. The objective of this study was to explore the effect of IL-6 in the pathogenesis of an androgen-independent prostatic cancer cell line and to study the role of dexamethasone in this procedure with an attempt to explore effective therapies for prostate cancer.

1 METHODS AND MATERIALS

1.1 Reagents

hIL-6, anti-IL-6 antibody and IL-6-ELISA-Kit were purchased from Beijing Biotech Co. RPMI 1640 was obtained from Gibco BRL. Crystal violet was procured from the Second Biochemistry Reagent Factory of Shanghai, and dexamethasone was bought from Yichang Pharmaceutical Factory.

1.2 Cell Culture

PC-3m is a subtype of PC-3, an androgen-independent prostate cancer cell line. It was obtained from Beijing Medical University and kept in RPMI 1640 containing 10 % FCS under the routine condition of cell culture.

1.3 Quantitative Analysis of IL-6 Secreted by PC-3m Cells and the Influence of Dexamethasone on the Procedure

The presence of IL-6 protein in the supernatant of cultural tumor cells was quantitated by ELISA. Briefly, \( 5 \times 10^4 \) cells were distributed into each well and then treated by RPMI 1640 with or without dexamethasone. After 24 h, 48 h, 72 h, and 96 h incubation, the supernatants were collected, centrifuged, and then measured by ELISA by employing an IL-6-ELISA kit. At every designed time points, the supernatants were harvested in triplicate and the experiment was performed twice.

1.4 Effect of Exogenous IL-6 and IL-6 Antibody on the Growth of PC-3m Cells

1.4.1 Morphological Assess

Cells were seeded on a flat-bottom 96-well plate at \( 1 \times 10^4 \) cell/well and \( 1 \times 10^4 \) cell/well, and it would be used to determine the effect of IL-6 and IL-6 antibody respectively. After incubation overnight, recombinant human IL-6, which at a final concentration range of 20 ng/ml to 100 ng/ml, and IL-6 antibody, which at a range of 1...
500 to 1:62.5, were added into the media. The morphology of control group, IL-6 group, and IL-6 antibody group were examined after 24 h, 48 h, 72 h, and 96 h incubation by using phase-contrast microscopy.

1.4.2 Proliferative Activity As described above, cell proliferative activity and cytotoxicity were determined after two day co-culture of IL-6 and IL-6 antibody by using crystal violet assay. Cellular proliferative activity and cytotoxicity were calculated by the following formula

$$\text{Proliferative Activity} = \frac{OD_{570} \text{ of experimental group}}{OD_{570} \text{ of control}}$$

$$\text{Cytotoxicity} = (1 - \frac{OD_{570} \text{ of experimental group}}{OD_{570} \text{ of control}}) \times 100\%$$

1.5 Effect of Dexamethasone on Proliferation of PC-3m Cells

Into each well 1 x 10^4 cells/well were distributed and cultured routinely for 24 h, and then the cultural medium was removed and dexamethasone (10^-8 mol/L, 10^-6 mol/L and 10^-4 mol/L) were supplemented. The morphology and cytotoxicity was determined after 2- or 4-day co-culture of dexamethasone.

1.6 Statistical Analysis

All the data were analyzed by student t-test.

2 RESULTS

2.1 Secretion of IL-6 by PC-3m Cells

By using a sensitive ELISA, it was found that IL-6 could be detected in the 72 h and 96 h cultural supernatants of both the groups with or without dexamethasone pretreatment. The level of IL-6 in the later sample was lower than that in the former one.

2.2 Effect of Exogenous IL-6 and IL-6 Antibody on the Growth of PC-3m Cells

2.2.1 Morphological Study Cancer cells grew in media supplemented with recombinant IL-6 was not significantly different from cells grown in media in absence of IL-6 during the period of observation (P>0.05).

The morphology and arrangement of most of the cancer cells was essentially normal after 24 h co-incubation of IL-6 antibody. But after another 24 h, some cells began to show process contraction, rounding up and the disappearanace of cell boundary of cells; some tumor cells detached from the wall of the culture plate. In the 3rd and 4th day, plenty of cells shed from the plate wall and there were many dead cells floating in the medium. During this period, the morphology exhibited little alteration in the control group cells.

2.2.2 Cell Proliferative Activity Under the same conditions for the co-culture experiments, IL-6 could not play a growth-stimulatory role in PC-3m cells after 2 days. However, anti-IL-6 antibody could exert a growth-inhibitory effect on PC-3m cells in a dose-dependent fashion (P<0.05). The cytotoxicity of anti-IL-6 antibody was 14.84 %, 18.73 %, 30.47 %, and 41.40 % (fig. 1).

2.3 Effect of Dexamethasone on the Growth of PC-3m Cells

2.3.1 Morphological Assessment Phase-contrast microscopic analysis showed that 48 h after administration of dexamethasone at varied concentrations, some tumor cells began to lose their normal shape and became rounding; some other cells died and became inflated in the media. More and more cells died and deprived from the plate with time passing. However, the differences were not of significance among the groups.

2.3.2 Cytotoxicity Dexamethasone had an inhibitory effect on PC-3m cells proliferation in dose-dependent fashion (P<0.05) and the cytotoxicity was 14.06 %, 20.29 % and 31.25 %, respectively (fig. 2).

Fig. 1 The cytotoxic effect of IL-6 antibody at different titers on the PC-3m cells

Fig. 2 The inhibitory effect of dexamethasone at different concentrations on the PC-3m cells

3 DISCUSSION

IL-6 is a cytokine that is closely associated with the pathogenesis of many cancers. IL-6 may affect cancer progression through its actions on cell adhesion and motility, thrombopoiesis, tumor specific antigen expression and cancer cell proliferation. IL-6 can either inhibit or stimulate cancer cell proliferation, relying upon the cell types and the presence or absence of IL-6R. The relationship of IL-6 and the proliferation was also of concern in the recent years. It was