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Original article

Radiosensitivity of prostate cancer cells is enhanced by EGFR inhibitor C225☆

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Abstract

Purpose: To determine the direct effects of the epidermal growth factor receptor (EGFR) inhibitor C225 on the radiosensitivity of human prostate cancer cells.

Experimental design: Human prostate cancer DU145 cells were irradiated with ^{60}Co (1.953 Gy/min) at various doses in the presence or absence of C225. The cellular proliferation and cell-survival rate were evaluated by MTT and colony-forming assays after irradiation. The cell-cycle distribution, cell apoptosis, and MAPK expression were investigated using FCM. The expression of Cyclin D1, CDK2, CDK4, and Survivin were determined by RT-PCR.

Results: The RBE in the C225 group compared with that in the control group was 1.39. Cells treated with C225 and irradiated at 4 Gy predominantly exhibited G_0/G_1 phase arrest and significant decrease in the fraction of cells in the S phase in comparison with those in the control cells, respectively. An evidently higher apoptosis rate on irradiation at 4 Gy was observed in C225-treated cells compared with that in the control cells. Decreased cell proliferation and increased cell death were further supported by the down-regulation of cyclin D1, CDK2, CDK4, and survivin in C225-treated DU145 cells, as determined by RT-PCR. Furthermore, C225 significantly inhibited the phosphorylation of P38-MAPK in DU145 cells.

Conclusions: The EGFR inhibitor C225 increased the radiosensitivity of DU145 cells through antiproliferative effect, inhibition of clonal growth, G_0/G_1 phase arrest, apoptosis induction, and inhibition of EGFR-signaling pathways by the down-regulation of MAPK activation.

Keywords: EGFR inhibitor; Prostate cancer cells; Cell proliferation; Apoptosis

1. Introduction

Growth factors control cellular proliferation and differentiation and are important for the initiation and maintenance of neoplastic transformation. Transforming growth factor-α (TGF-α) and epidermal growth factor (EGF) and its specific receptors, the epidermal growth factor receptors (EGFRs), have been implicated in the development and progression of the majority of human epithelial cancers, including prostate cancer [1–3]. TGF-α and EGF and/or EGFR are expressed at high levels in prostate cancers, which are generally associated with advanced disease and poor prognosis [4,5]. EGFR activation is not only critical for cell proliferation but EGFR-mediated signals also contribute to other processes that are crucial to cancer progression, including angiogenesis, metastatic spread, and the inhibition of apoptosis [6,7]. The high expression of EGFR is also associated with resistance to cytotoxic drugs or ionizing radiation, as determined in several preclinical models.

EGFR activation may prevent apoptosis induced by radiation in cancer cells. This may be clinically relevant because it could represent a mechanism via which cancer cells escape radiation-induced cell death. A large body of experimental and clinical work supports the view that the EGFR is a relevant target for cancer therapy. Different pharmacological and biological approaches have been developed for blocking EGFR activation and/or function in cancer cells.

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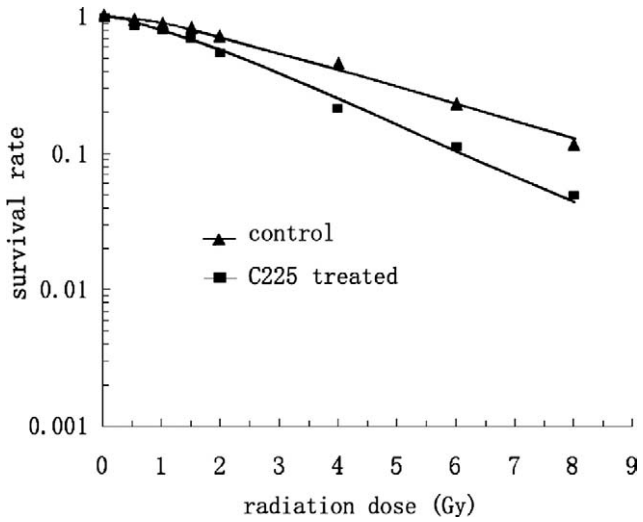

Fig. 1. Dose-survival curves of DU145 and C225-treated DU145 cells after irradiation.

Intensity-modulated radiation therapy for early-stage prostate cancer has produced similar therapeutic effect and fewer complications than those produced by radical surgery [8–10]; however, further improvements in the therapy of prostate cancer are required. It was reported that prostate cancer cells exhibited decreased requirement for exogenous growth factors compared with that exhibited by normal cells. This is attributable in part to the ability of prostate cancer cells to overexpress growth factors and/or their specific cell membrane receptors, resulting in the autonomous activation of autocrine and paracrine growth pathways. Thus, blocking these receptors by monoclonal antibodies (mAbs) or other agents may significantly impact clinical prostate cancer therapy. Several anti-EGFR blocking mAbs were recently developed. C225 (cetuximab), a chimeric human-mouse IgG1 mAb [11–13], has been clinically used for the therapy of human head and neck cancers and colon cancers [14,15]. In the present study, we directly evaluated the potential antagonistic effects of ionizing radiation and C225 by detecting cell proliferation, cell cycle distribution, and apoptotic activity in human prostate cancer cells (DU145), which express both TGF-α and EGFR. Our data showed that C225 significantly enhanced cell death induced by ionizing radiation. Our present study may have potential impacts on the clinical applications of combining C225 with irradiation therapy in patients with cancers.

2. Materials and methods

2.1. Cell lines and cell culture

The DU-145 prostate carcinoma cell line was kindly provided by the Urinary Surgery Department of the First Affiliated Hospital of Peking University. It was maintained in RPMI1640 supplemented with 20 mM HEPES (pH 7.4), 100 IU/ml penicillin, 100 mg/ml streptomycin, 4 mM glutamine, and 10% heat-inactivated fetal bovine serum (Hangzhou Sijiqing Biological Engineering Materials Co., Hangzhou, China) in a humidified atmosphere of 95% air and 5% CO_2 at 37°C. C225-treated DU145 cells were obtained by culturing cells with 100 nmol/l C225 (EGFR inhibitor; Boehringer Co., Ingelheim, Germany) for 24 h before irradiation. The concentration of cetuximab (100 nmol/l) in vitro was comparable to in vivo dose of cetuximab (3 to 8 mg/kg) in prostate cancer and NSCLC xenografts [16,17]. Phospho-P38 mitogen-activated protein kinase (MAPK) mAb (Alexa Fluor) was provided by Cell Signaling Technology (Boston, MA).

2.2. Cell-growth analysis by methyl thiazolyl tetrazolium (MTT) assay

Cell proliferation was determined by assessing the mitochondrial reduction of MTT. Cells from the control and C225-pretreated groups were exposed to different radiation dosages (0, 2, 4, 6, and 8 Gy) according to previous reports [18–20]. Cells were plated at 1×10^3 cells/well in 96-well plates containing 200 μl growth medium and allowed to attach for 24 h. The medium was removed, and the C225-treated cells were quiesced for 2 days in a medium supple-

Table 1
Growth-inhibitory effects of radiation and C225 treatment on DU145 cell line (%, $\bar{X} \pm s$)

	Radiation dose (Gy)				
	0	2	4	6	8
	Growth inhibitory rate				
Control	0	57 ± 4.6	68 ± 3.4	70 ± 4.0	73 ± 3.3
C225 group	43 ± 3.0[a]	67 ± 3.8[b]	72 ± 5.8[c]	79 ± 3.7[d]	88 ± 4.2[e]

Compared with control group t = −25.4.
[a] $P < 0.01$; t = −3.1.
[b] $P < 0.05$; t = −1.1.
[c] $P < 0.05$; t = −3.0.
[d] $P < 0.05$; t = −4.7.
[e] $P < 0.05$.