

Original Article

Impact of Bay 11-7085 on radiosensitivity of pancreatic cancer cell line Sw1990

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Received September 23, 2015; Accepted March 10, 2016; Epub June 15, 2016; Published June 30, 2016

Abstract: Objective: To investigate the impact of Bay 11-7085, an inhibitor of nuclear factor kappa light chain enhancer of activated B cells (NF- κ B), on the radiosensitivity of human pancreatic cancer cell line Sw1990, and to explore the possible mechanisms. Methods: The human pancreatic cancer cell line Sw1990 was cultured *in vitro* and then randomly assigned to one of five groups: the blank control group, the radiotherapy alone group, and the radiotherapy + Bay 11-7085 groups (2 μ M, 6 μ M and 10 μ M). Annexin V-FITC/PI double staining was performed to detect cell apoptosis, and western blot analysis was performed to detect the expression of NF- κ B, p65, I κ B, B-cell lymphoma 2 (Bcl-2) and X-linked inhibitor of apoptosis protein (XIAP). Results: Bay 11-7085 significantly increased the rate of apoptosis in pancreatic cancer cells ($P < 0.05$) in a dose-dependent manner. Compared with the blank control group, radiotherapy significantly increased the expression of the anti-apoptotic proteins Bcl-2, XIAP and p65 in the nucleus ($P < 0.05$) and inhibited the expression of I κ B protein ($P < 0.05$). Compared with the radiotherapy alone group, radiotherapy + Bay 11-7085 significantly inhibited the expression of Bcl-2, XIAP and p65 in the nucleus ($P < 0.05$) while increasing the expression of I κ B protein ($P < 0.05$). Conclusion: Bay 11-7085 inhibits NF- κ B activity and regulates the expression of downstream anti-apoptotic proteins, thereby enhancing the radiosensitivity of pancreatic cancer cells. Thus, Bay 11-7085 may be used as a radiotherapy-sensitizing drug for the treatment of pancreatic cancer.

Keywords: Bay 11-7085, pancreatic cancer, radiosensitivity, NF- κ B inhibitors

Introduction

Pancreatic cancer is highly malignant. Most patients are diagnosed in the advanced stages of the disease due to atypical early symptoms and thus are unsuitable for surgery and exhibit an extremely poor prognosis [1]. Advanced pancreatic cancer is less sensitive to chemotherapy, and radiotherapy is thus an important treatment. However, previous studies found that pancreatic cancer may develop a strong resistance to radiotherapy during treatment, greatly reducing the effects of treatment. Therefore, it is important to search for new methods to improve the sensitivity of pancreatic cancer to radiotherapy so as to enable effective clinical treatment of patients with pancreatic cancer [2, 3]. Studies have demonstrated that [4] nuclear factor kappa light chain enhancer of activated B cells (NF- κ B) plays an important role in the development of resistance to radiotherapy in pancreatic cancer, and the inhibition of its activity improves the sensitivity of pan-

creatic cancer to radiotherapy. In this study, we investigated the impact of the NF- κ B inhibitor Bay 11-7085 on the radio sensitivity of pancreatic cancer cells and explored its possible mechanisms to provide new ideas and methods for the clinical treatment of pancreatic cancer.

Materials and methods

Cells and reagents

Pancreatic cancer cell line Sw1990 was purchased from the Cell Bank of Shanghai Institute, Chinese Academy of Sciences; 10% fetal bovine serum (FBS; made in China) was purchased from Hangzhou Sijiqing Biotech Co.; horseradish peroxidase (HRP)-labelled goat anti-rabbit IgG secondary antibody and primary anti-I κ B, anti-P65, anti-X-linked inhibitor of apoptosis protein (XIAP), anti-H3, and anti- β -actin antibodies were purchased from Santa Cruz Biotechnology, Inc. (USA); the Annexin V-FITC apoptosis detection kit was purchased from

Table 1. Impact of different doses of Bay 11-7085 in combination with radiotherapy on the apoptosis rate of Sw1990 pancreatic cancer cells

Group	Number of wells	Apoptosis rate (%)	F	P
Blank control group	12	5.7 ± 1.12	157.612	<0.001*
Radiotherapy alone group	12	16.7 ± 2.98 [§]		
Radiotherapy + Bay 11-7085 (2 µM)	12	21.9 ± 3.21 ^{§,#}		
Radiotherapy + Bay 11-7085 (6 µM)	12	29.4 ± 4.35 ^{§,#,☆}		
Radiotherapy + Bay 11-7085 (10 µM)	12	39.2 ± 4.67 ^{§,#,☆,△}		

*P<0.05; [§]Compared with the blank control group, P<0.05; [#]Compared with the radiotherapy alone group, P<0.05; [☆]Compared with the radiotherapy + Bay 11-7085 (2 µM) group, P<0.05; [△]Compared with the radiotherapy + Bay 11-7085 (6 µM) group, P<0.05.

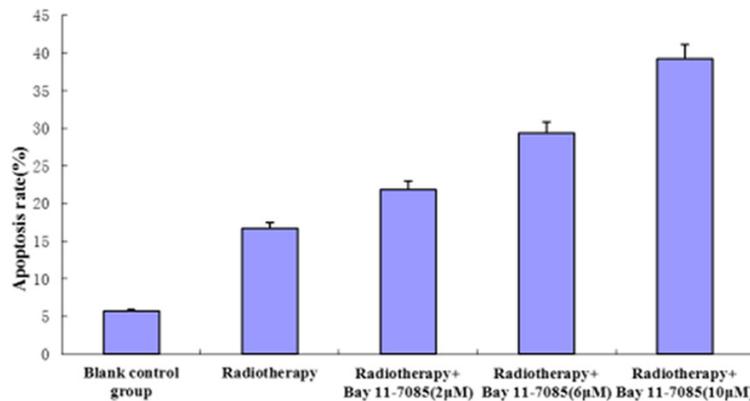


Figure 1. Apoptosis rate of pancreatic cancer cell line Sw1990 in each group.

Sigma-Aldrich (USA); Bay 11-7085 was purchased from a company in the USA; and a cellular total protein extraction kit, a nucleoprotein extraction kit and enhanced chemiluminescence (ECL) reagents were purchased from BeyotimeBiotech Co., Ltd. (China).

Cell culture and grouping

Sw1990 pancreatic cancer cells in the exponential growth phase were harvested, adjusted to 2 × 10⁵ cells/mL, seeded in 96-well plates (100 µL/well), and cultured in an incubator at 37°C and 5% CO₂. There were five groups in this experiment: blank control group (no intervention), radiotherapy alone group, radiotherapy + Bay 11-7085 (2 µM) group, radiotherapy + Bay 11-7085 (6 µM) group, and radiotherapy + Bay 11-7085 (10 µM) group. Duplicate wells were set up in each group for the analysis of relevant indicators. Radiotherapy was performed with X-ray irradiation at the dose of 5 Gy based on previous studies [5]. For the radiotherapy + Bay 11-7085 groups, Bay 11-7085 was added 4 hours before radiotherapy, and the doses of

Bay 11-7085 used were selected in reference to past literature [6].

Immunofluorescence flow cytometry for the detection of cell apoptosis

Twenty-four hours after radiation treatment, cells were washed with pre-cooled PBS (4°C) twice, followed by centrifugation. Next, the cells were re-suspended with a mixture of 250 µL of 2X binding buffer and 250 µL of sterile deionized water, and 5 µL

of Annexin V-FITC and 3 µL of propidium iodide were added into each well and mixed well. The cells were kept at room temperature for 5 minutes in the dark, and flow cytometry was performed to detect apoptosis.

Western blot analysis of the expression of B-cell lymphoma 2 (Bcl-2), XIAP, IκB and p65

Each group of cells was harvested 24 hours after radiation treatment, and the cells were placed in Eppendorf tubes for centrifugation at 12,000 rpm for 1 minute. Next, the supernatant was discarded, and the precipitate was collected for protein extraction. Nucleoprotein and total protein extraction was performed in strict accordance with the instructions for the lysis buffer. The bicinchoninic acid (BCA) protein assay kit was used for the quantitative analysis of protein concentration. Next, the protein was mixed with 5X loading buffer at a 1:4 ratio, boiled (100°C) for 10 minutes, and transferred to the membrane after polyacrylamide gel electrophoresis. The membrane was blocked in 5% skim milk for 1 hour. After the

Bay 11-7085 and pancreatic cancer

Table 2. Western blot analysis of the expression of p65, IκB, Bcl-2 and XIAP

Group	Number of wells	P65	IκB
Blank control group	12	0.67 ± 0.23	0.98 ± 0.31
Radiotherapy alone group	12	1.23 ± 0.49 [§]	0.12 ± 0.05 [§]
Radiotherapy + Bay 11-7085 (10 μM)	12	0.88 ± 0.27 [#]	0.95 ± 0.29 [#]
<i>F</i>		7.873	46.940
<i>P</i>		0.002*	<0.001*

**P*<0.05; [§]Compared with the blank control group, *P*<0.05; [#]Compared with the radiotherapy alone group, *P*<0.05.

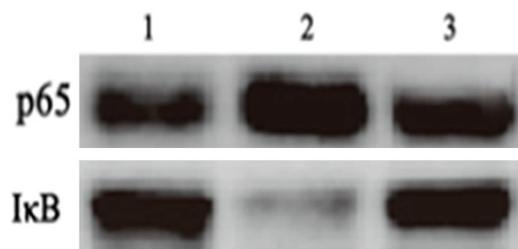


Figure 2. Impact of Bay 11-7085 on p65 expression in Sw1990 pancreatic cancer cells. 2.3. Impact of radiotherapy and Bay 11-7085 intervention on the expression of Bcl-2 and XIAP.

addition of diluted primary anti-β-actin, anti-H3, anti-IκB, anti-p65, anti-Bcl-2 and anti-XIAP antibodies, the membrane was placed on a shaker overnight at 4°C. After thorough TBST wash, the secondary antibody was added for incubation at room temperature for 1 hour. After TBST wash, ECL luminescence agent was added for gel imaging. The Image J software was used for grey-scale analysis. H3 was used as the internal reference for p65, and β-actin was used as the internal reference for Bcl-2, XIAP and IκB.

Statistical analysis

SPSS19.0 statistical software was used for data analysis. Measurement data with normal distribution were expressed as the mean ± standard deviation (SD) and were analysed using analysis of variance. An LSD-t test was performed for pairwise post hoc comparison. The significance level was set at $\alpha = 0.05$, and *P*<0.05 was considered statistically significant.

Results

Detection of the apoptosis rate of Sw1990 pancreatic cancer cells in each group

We observed a significant difference in the cell apoptosis rate between the groups (*F* =

157.612, *P*<0.001). Pairwise post hoc comparison revealed that compared with the blank control group, the apoptosis rate of the Sw1990 pancreatic cancer cells was higher in the radiotherapy alone group (*P*<0.05). Furthermore, compared with the radiotherapy alone group, Bay 11-7085 treatment before radiotherapy significantly increased the apoptosis rate of the Sw1990 pancreatic cancer cells (*P*<0.05) in a dose-dependent manner (**Table 1, Figure 1**).

Impact of radiotherapy and Bay 11-7085 intervention on the expression of IκB and p65 in pancreatic cancer cells

Based on the above findings, 10 μM Bay 11-7085 was used in the subsequent experiments to investigate the molecular mechanism. The analysis of variance revealed a significant difference in the expression levels of P65 and IκB between the groups (*P*<0.05). Pairwise post hoc comparison indicated that the expression levels of P65 and IκB were significantly higher in the radiotherapy alone group than in the blank control group (*P*<0.05), whereas they were significantly lower in the radiation + Bay 11-7085 (10 μM) group than in the radiotherapy alone group (*P*<0.05) (**Table 2; Figure 2**).

Impact of radiotherapy and Bay 11-7085 intervention on the expression of Bcl-2 and XIAP

The analysis of variance revealed that there was a significant difference in the expression levels of Bcl-2 and XIAP between the groups (*P*<0.05). Pairwise post hoc comparison demonstrated that the expression levels of Bcl-2 and XIAP were significantly higher in the radiotherapy alone group than in the blank control group (*P*<0.05), and they were significantly lower in the radiotherapy + Bay 11-7085 (10 μM) group than in the radiotherapy alone group (*P*<0.05) (**Table 3; Figure 3**).

Discussion

NF-κB, a transcription factor, is a dimer composed of two monomers. NF-κB monomers mainly include Rel, p65, RelB, p52 and p50,

Table 3. Western blot analysis of the expression of p65, IκB, Bcl-2 and XIAP

Group	Number of wells	Bcl-2	XIAP
Blank control group	12	0.31 ± 0.11	0.12 ± 0.02
Radiotherapy alone group	12	0.97 ± 0.49 [§]	0.29 ± 0.09 [§]
Radiotherapy + Bay 11-7085 (10 μM)	12	0.41 ± 0.17 [#]	0.17 ± 0.06 [#]
<i>F</i>		16.201	22.711
<i>P</i>		<0.001*	<0.001*

**P*<0.05; [§]Compared with the blank control group, *P*<0.05; [#]Compared with the radiotherapy alone group, *P*<0.05.

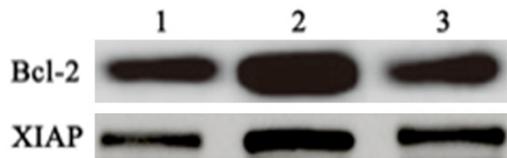


Figure 3. Impact of Bay 11-7085 on the expression of Bcl-2, XIAP and IκB in Sw1990 pancreatic cancer cells.

where the most common NF-κB dimer is composed of p65 and p50 [7]. In the resting state, IκB, the inhibitory unit of NF-κB, binds to NF-κB via its specific C-terminal ankyrin repeats and prevents the transfer of NF-κB to the nucleus, thus enabling NF-κB to exist in the cytoplasm in an inactive form. When the cell is stimulated by extracellular signals, the IκB kinase complex is activated, leading to phosphorylation and degradation of IκB and exposure of the nuclear docking site of NF-κB; thus, free NF-κB is rapidly transferred to the nucleus and binds to specific DNA sequences, thereby promoting the transcription and protein expression of target genes. Recent studies have reported that NF-κB is a key factor mediating radiotherapy resistance in a variety of tumours [8]. After radiotherapy, broken DNA in the tumour cells activates IκB kinase 3, and damaged DNA activates the ataxia telangiectasia mutated enzyme (ATM) and DNA-dependent protease (DNA-PK). In turn, these pathways promote the mitogen-activated protein kinase 1/extracellular signal-regulated kinase pathway, leading to phosphorylation and activation of the specific 90-kDa ribosomal antigen 6 kinase, up-regulation of IκB kinase 2 activity, phosphorylation and degradation of IκB, translocation of p65 to the nucleus, up-regulation of the expression of downstream anti-apoptotic proteins, and inhibition of tumour cell apoptosis, thereby reducing the radiosensitivity of tumour cells. Previous studies have found that inhibition of NF-κB activity enhances the

radiosensitivity of a variety of tumours [7], suggesting that drugs that inhibit NF-κB activity may be used as a new auxiliary therapy to enhance radiosensitivity.

Previous studies reported excessive ac-

tivation of NF-κB in pancreatic cancer tissues [9, 10], which was closely correlated with patient prognosis. Furthermore, NF-κB activation in pancreatic cancer cells enhanced the radiotherapy resistance of pancreatic cancer [11]. In addition, previous studies have found that post-radiotherapy NF-κB activation up-regulated the expression of BCL-2 and XIAP [12]. XIAP is the most potent apoptosis-inhibitory factor. XIAP acts on the initiator and effector of the apoptosis pathway directly to exert its potent apoptosis-inhibitory effect. Bcl-2 prevents the activation of caspase proteases by cytoplasmic cytochrome C, promotes the transfer of glutathione into the nucleus and inhibits the oxidative stress response in the nucleus to enact its anti-apoptotic role. Down-regulation of the expression of Bcl-2 and XIAP protein helps to enhance the sensitivity of tumour cells to radiotherapy and to induce tumour cell apoptosis. Based on these results, we believe that targeted inhibition of NF-κB activity may effectively increase the radiosensitivity of pancreatic cancer. Bay 11-7085 is a novel inhibitor of NF-κB, with specific inhibitory effects on NF-κB activity. The results of the present study demonstrated that although radiotherapy induced apoptosis in pancreatic cancer cells, it also led to the activation of NF-κB activity and increased the expression level of the anti-apoptotic proteins Bcl-2 and XIAP. Bay 11-7085 treatment before radiotherapy effectively inhibited the translocation of p65 into the nucleus and thus reduced the expression level of the anti-apoptotic proteins Bcl-2 and XIAP and significantly increased the apoptosis rate of pancreatic cancer cells, suggesting that Bay 11-7085 treatment before radiotherapy effectively enhanced the radiosensitivity of pancreatic cancer cells.

In summary, Bay 11-7085 increases the radiosensitivity of pancreatic cancer cells by inhibiting NF-κB activity, which may be related to inhibition of the expression of the anti-apoptotic

proteins Bcl-2 and XIAP. In the future, animal experiments are needed to investigate whether Bay 11-7085 enhances radiosensitivity to provide a theoretical basis for the clinical treatment of pancreatic cancer.

Disclosure of conflict of interest

None.

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