EFFECT OF ADENOVIRUS-MEDIATED p53 GENE TRANSFER ON APOPTOSIS AND RADIOSENSITIVITY OF HUMAN GASTRIC CARCINOMA CELL LINES

ZHANG Shan-wen 张珊文*, XIAO Shao-wen 肖绍文, LÜ You-yong 吕有勇

Department of Radiation Oncology, Beijing University School of Oncology, Beijing 100036

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ABSTRACT

Objective: To evaluate the effect of adenovirus-mediated p53 gene (Adp53) on apoptosis and radiosensitivity of human gastric carcinoma cell lines. Methods: Recombinant adenovirus expressing wild-type p53 gene was transferred into four human gastric carcinoma cell lines with different p53 genetic status. p53 protein expression was detected by immunohistochemistry assay and western blot assay. Cell survival was assessed using a clonogenic assay. TUNEL assay was used in determination of apoptosis. Four human gastric carcinoma cells infected with Adp53 were irradiated with 4Gy and cell cycle distribution and Sub-G_1 peak were assayed by flow cytometry. Results: G2/M arrest, apoptosis and inhibition of tumor cell proliferation were induced by infection at Adp53 at 100 MOI which caused high transfer rate of wild-type p53 and strong expression of p53 protein in four human gastric carcinoma cells. The radio-enhancement ratio of Adp53 at 4Gy were 3.0 for W cell, 3.6 for M cell, 2.2 for neo cell and 2.5 for 823 cell *in vitro*. Conclusion: This study demonstrated that Adp53 transfer increased cellular apoptosis and radiosensitivity of human gastric carcinoma cell lines *in vitro* independently on cellular intrinsic p53 status thus supporting the combination of p53 gene therapy with radiotherapy in clinical trials.

Key words: Gastric carcinoma; Radiosensitivity; Apoptosis; Adenovirus-mediated p53 gene

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*Author to whom correspondence should be addressed.
Phone: (0086-10)-88122454; E-mail: zhangshw@yeah.net
Biography: ZHANG Shan-wen (1946-), male, professor, Department of Radiotherapy, Peking University School of Oncology, majors in radiation oncology.

Wild-type p53 plays a key role in cell cycle control and apoptosis, especially in stressed cells by irradiation, cytotoxicity agent or hyperthermia and inhibits proliferation of tumor cell. Irradiation kills tumor cells by inducing cell cycle arrest and apoptosis. Wild-type p53 promotes cell cycle arrest and apoptosis of tumor cells following irradiation, while mutated p53 abrogate this response. p53 gene mutation occurs in more than 50% of all human tumors, inducing tolerance to irradiation[1]. Thus restoring wild-type p53 gene into tumor cells which have p53 abnormality enhances radiosensitivity of tumor cells[2, 3]. These results support the use of p53 gene therapy as a new adjuvant to radiation in treatment of malignant tumors[4, 5]. Replication- deficient adenovirus are most widely used vectors in gene therapy, because this vector can accept a large size of foreign gene (up to 7.5 kb) and high titers of viruses generally can be obtained. Adenovirus can be purified and concentrated with simple method without loss of infectivity. The high efficacy of infection of most epithelial cell lines, which leads to up to 100% gene transfer in cell's nuclear without heredity toxicity on host, makes adenovirus highly advantageous compared with other transfection agents[6-8]. We have previously demonstrated that wild-type p53 promotes G_1 arrest and apoptosis of tumor cells following irradiation, therefore, enhances intrinsic radiosensitivity of gastric carcinoma cells lines[1]. In the current study, wild-type p53 gene transfer into the four gastric carcinoma cell lines with different p53 genetic status was performed using adenovirus-mediated p53 gene (Adp53) to evaluate the effect of wild-type p53 on apoptosis and radiosensitivity compared to that obtained with X-radiotherapy (XRT) alone.

MATERIALS AND METHODS

Adp53 Generation, Purification and Concentration

Recombinant E1-deleted Adp53 was based on

adenovirus serotype 5, expressing human wild-type p53 under the control of the cytomegalovirus promoter. Adp53 vector was generated in 293 cells, a human embryo kidney cell line, which contains the adenoviral E1, thus permissive for adenovirus replication. Purification and concentration of adenovirus was performed by sequential centrifugation in CsCl step gradients. Adp53 vector was quantified according to their plaque-forming ability on 293 cells. Titers of adenovirus were quantified by plaque-forming units (pfu)/ml [7-9].

Cells and Culture Condition

Four human gastric carcinoma cell lines with different p53 status, BGC823-wtp53 cell (abbreviated W) containing wild-type p53, BGC823-mutp53 (abbreviated M) containing mutant p53, BGC823-vect cell (abbreviated neo) without p53 gene and parents BGC823 cell (abbreviated 823), were used in this study and 293 cells were cultured with DMEM medium with 10% calf serum and antibiotics.

Wild-type p53 Detection

The four cell lines were infected by Adp53 at 100 MOI (multiplicity of infection), and 48 h later, p53 protein expression was detected by immunohistochemistry assay and Western blot assay according to standard procedures[7, 8].

Apoptosis Assay

TUNEL assay (transferase-mediated dUTP-biotin nick end labeling assay) was used to label apoptotic cells[8].

Tumor Inhibition Assay

The four cell lines infected by Adp53 at 100 MOI were cultured for 14 days and cell survival was assessed using a clonogenic assay[1]. Adp53 inhibit rate for the four cell lines was accounted.

Irradiation Dose and FCM Analysis

The four gastric carcinoma cell lines were infected with Adp53 (100 MOI), 48 h later they were irradiated at 4Gy and 24 h later, cell cycle distribution and Sub-G_1 peak (means apoptosis) were assayed by flow cytometry using a model FACS240[1, 8].

RESULTS

The titer of purified adenovirus stock reached 3.0×10^{12} pfu/ml. Cultures of the four human gastric carcinoma cell lines were infected with Adp53 at 100 MOI, with a higher than 90% infection rate.

Forty-eight h after infection the wild-type p53 gene was strongly expressed in each cell line as shown by immunohistochemistry staining (Figure 1) and Western blotting (Figure 2). Infection of Adp53 induced G_2/M arrest and apoptotic Sub-G_1 peak in each cell line as demonstrated by FCM (Figure 3) and obvious apoptotic cells in each cell line as showed by TUNEL staining (Figure 4). More obvious G2/M arrest and Sub-G_1 peak induced by infection with Adp53 at 100 MOI following XRT 4Gy than either Adp53 or 4Gy alone was seen by FCM (Figure 5). Table 1 showed the survival rate of Adp53-infected cells following XRT 4Gy. Tumor cell inhibition rates of Adp53-infection were 37.5% for W cell, 55.9% for M cell, 48.1% for neo cell and 46.3% for 823 cell, respectively. Survival rate of Adp53+4Gy group was significantly lower compared than either Adp53 alone or 4Gy alone in each cell line ($P<0.001$). Radio-enhancement ratios of Adp53 for 4Gy were 1.3 for W cell, 2.1 for M cell, 1.4 for neo cell, and 1.4 for 823 cell, respectively. If evaluating radio-biologic efficacy by apoptosis, as the results of TUNEL staining showed in Table 2, radio-enhancement ratios of Adp53 for 4Gy were 3.3 for W cell, 16.0 for M cell, 6.5 for neo cell and 7.0 for 823 cell, respectively; while, as FCM's results shown in Table 3, radio-enhancement ratios of Adp53 for 4Gy were 3.0 for W cell, 3.6 for M cell, 2.2 for neo cell and 2.5 for 823 cell, respectively.

Table 1. Survival rate of Adp53-infected cells following XRT 4Gy($\bar{x}\pm s$)

Cell	Survival rate				P^*
	Control	Ad-p53	4Gy	Adp53+4Gy	
W	0.72±0.06	0.45±0.02	0.12±0.01	0.09±0.00	0.001
M	0.84±0.04	0.37±0.02	0.17±0.00	0.08±0.01	
Neo	0.79±0.03	0.41±0.02	0.15±0.00	0.11±0.01	
823	0.80±0.05	0.43±0.02	0.15±0.01	0.11±0.02	

*Hotelling T^2 examination model was used. Survival rate of Adp53+4Gy is significantly lower ($P<0.001$) than either 4Gy only or Adp53 only in each cell line.