The effect of rAd-p53+cisplatin on gastric cancer cell growth and KAI1/CD82 expression

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Abstract: Objective To investigate the effects of rAd-p53 and cisplatin (DDP)alone or in combination on the proliferation, apoptosis, and KAI1/CD82 protein expression of human gastric cancer SGC7901 cells. Methods SGC7901 cells were treated with rAd-p53, DDP, or their combination for 24,48, and 72 hours. The proliferation activity of SGC7901 cells was measured using the CCK-8 assay. Apoptosis rates were determined by flow cytometry, and the expression of KAI1/CD82 protein was detected by immunohistochemistry. Results Both rAd-p53 and DDP alone, as well as their combination, inhibited the proliferation of SGC7901 cells in a dose-and time-dependent manner. The proliferation inhibition rate of the combination group was significantly higher than that of the single-agent groups, with statistically significant differences compared to the negative control group($P<0.05$).After 48 hours of treatment with rAd-p53,DDP,or their combination, the apoptosis rates were 36.94%±0.78%,28.79%±2.37%,and 69.26%±0.63%,respectively,all of which were significantly higher than that of the negative control group(16.72%±1.54%,$P<0.05$).Both rAd-p53 and DDP alone, as well as their combination, upregulated the expression of KAI1/CD82 protein, with the combination group showing the most significant increase. Conclusion Both rAd-p53 and DDP alone, as well as their combination, can inhibit the growth of gastric cancer SGC7901 cells and induce apoptosis. The combination of the two drugs enhances the inhibitory effect on gastric cancer cells. rAd-p53 may induce apoptosis in SGC7901 cells by upregulating the expression of KAI1/CD82, thereby enhancing the antitumor effect of DDP.

Keywords: Recombinant human P53, Adenovirus injection, SGC7901, KAI1/CD82

1. Introduction

Gastric cancer is one of the most common malignant tumors threatening human health worldwide. The majority of cases are diagnosed at an advanced stage, resulting in poor clinical treatment outcomes. Currently, there is a scarcity of research on gene therapy targeting specific biological targets related to gastric cancer; hence, the search for new treatment strategies for gastric cancer is of utmost urgency. Cisplatin is a commonly used chemotherapeutic drug for gastrointestinal tumors in clinical practice. However, its pharmacological effects often require high doses, which are associated with significant toxic side effects. Many patients develop resistance to the drug, making it impossible to enhance its therapeutic efficacy by further increasing the dosage.

The human p53 gene plays a crucial role in the progression and evolution of malignant tumors and exists in two forms: wild-type and mutant. Mutant p53 can promote malignant cellular transformation. Studies have confirmed that the mutation rate of p53 in gastric cancer is as high as 32%. The absence or mutation of p53 is closely related to the occurrence, development, and prognosis of gastric cancer [1-2]. Recombinant Human Adenovirus p53 Injection (rAd-p53)-Gendicine is the first gene therapy drug approved for cancer treatment worldwide. It is composed of the normal human tumor suppressor gene p53 and a modified type 5 adenovirus gene. The adenovirus acts as a carrier, delivering the therapeutic gene p53 into target cells to exert its antitumor effects [3].

KAI1/CD82 is a specific tumor suppressor gene that has garnered considerable attention in domestic and international research in recent years. It was initially discovered during studies on prostate cancer [4]. Research has shown that KAI1/CD82 can inhibit tumor invasion and metastasis through multiple pathways [5]. However, the expression and correlation of KAI1/CD82 and p53 in malignant tumors have not been

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consistent in studies worldwide. Therefore, this study aims to investigate the effects of rAd-p53 and cisplatin alone or in combination on the proliferation, apoptosis, and KAI1/CD82 protein expression of gastric cancer SGC7901 cells. This research seeks to provide more theoretical evidence for the treatment of gastric cancer with rAd-p53 and to offer new insights for the development of targeted therapies for gastric cancer.

2. Materials and Methods

2.1. Materials

The human gastric cancer SGC7901 cell line was preserved by the Central Laboratory of Haikou People's Hospital. Recombinant human p53 adenovirus injection was purchased from Shenzhen Sibiono Gene Tech Co., Ltd. Cisplatin was obtained from Ge jiu Pharmaceutical Co., Ltd., Yunnan. The CCK-8 kit was a product of Do Jin do Laboratories, Japan. The rabbit anti-human KAI1/CD82 monoclonal antibody was from Beijing Boasen Biological Technology Co., Ltd. The Annexin V-FITC/PI apoptosis detection kit was from Biovision, Inc. RPMI 1640 medium was from Gibco, a brand of Thermo Fisher Scientific, USA. Trypsin and DMSO were products of Sigma-Aldrich, USA.

2.2. Methods

2.2.1. Cell culture

Cells were routinely cultured in a medium containing 10%fetal bovine serum at 37℃with 5%CO_2.When the cells covered the bottom of the flask, they were digested with 0.25%trypsin and passaged. Cells in the logarithmic growth phase were used for the experiments.

2.2.2. Experimental Grouping

The experiment was divided into the following groups

rAd-p53 Group: Concentrations of 5×10^8 v p /ml,5×10^9 v p/ml, and 5×10^{10} v p/ml.

DDP Group: Concentrations of 2.5 mg/L,5 mg/L, and 7.5 mg/L.

Combination Group: Concentrations of 5×10^8 v p/ml+2.5 mg/L,5×10^9 v p/ml+5 mg/L, and 7.5 mg/L+5×10^{10} v p/ml.

Negative Control Group: No treatment was applied.

2.2.3. Detection of gastric cancer cell proliferation inhibition by CCK-8 assay

SGC7901 cells in the logarithmic growth phase were seeded into a 96-well culture plate at a volume of 100 μ L per well. After incubation for 24 hours to allow cell adherence, the supernatant was discarded. According to the experimental grouping described in Section 2.2.2, different concentrations of rAd-p53, DDP, and their combination were added to the wells. Each group had six replicates, and the experiment was repeated on two plates. After incubation for 24,48, and 72 hours, the supernatant was discarded again, and 10 μ L of CCK-8 solution was added to each well. The plates were then incubated for an additional 4 hours. The absorbance at 450 nm was measured using a microplate reader, and the inhibition rate was calculated as follows: Inhibition rate= (1-OD value of treated group/OD value of blank group) ×100%.

2.2.4. Detection of cell apoptosis by flow cytometry

SGC7901 cells in the logarithmic growth phase were seeded into a 6-well plate at a density of 2×10^5 cells/m L. Negative control, DDP 7.5 mg/L, rAd-p53 5×10^{10} v p/ml, and DDP 7.5 mg/L+rAd-p53 5×10^{10} v p/ml groups were set up. After 48 hours of intervention, the cells were collected, digested with trypsin, and centrifuged at 1000 rpm for 5 minutes at 4°C.The supernatant was discarded, and the cells were washed twice with PBS. The cells were then resuspended in 500 μ L of Binding Buffer, to which 10 μ L of Annexin V-FITC and 5 μ L of propidium iodide (PI)were added. The mixture was gently vortexed and incubated in the dark at room temperature for 5 minutes before being analyzed by flow cytometry.

2.2.5. Detection of protein expression in sgc7901 cells by immunocytochemistry (SP Method)

SGC7901 cells in the logarithmic growth phase were seeded into a 6-well plate with 6 cover slips per well at a density of 2×10^5 cells/mL, with 2 mL per well. The cells were cultured in a 37℃incubator with

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5%CO_2.After 24 hours of cell adherence, the culture medium was removed, and the cells were treated with drugs. The experimental groups were set as follows: negative control group, DDP 7.5 mg/L group, rAd-p53 5×10^{10} v p/ml group, and DDP 7.5 mg/L+rAd-p53 5×10^{10} v p/ml group. Each group had three replicates. Each well was treated with 2 mL of culture medium containing rAd-p53, DDP, or their combination. After 48 hours of incubation, the cover slips were removed, washed with PBS, fixed with 10%neutral formaldehyde for 20 minutes, and then air-dried. Three cover slips from each group were selected for staining.

2.3. Statistical analysis

Statistical analysis was performed using SPSS 16.0. Data were expressed as mean± SD. Comparisons between groups were made using one-way ANOVA, with $P<0.05$ considered statistically significant.

3. Results

3.1. Effects of rAd-p53 and DDP alone or in combination on the growth of SGC7901 cells

The growth of SGC7901 cells was inhibited to varying degrees after treatment with different concentrations of rAd-p53, DDP, and their combination for 24,48, and 72 hours. The inhibition rates increased with the concentration of the drugs and the duration of treatment. The inhibition rates of the combination group were higher than those of the single-agent groups, with statistically significant differences($P<0.05$). See Tables 1,2, and 3 for details.

Table 1 Inhibitory Effects of Different Concentrations of rAd-p53 on SGC7901 Cells at Different Time Points($\bar{x}\pm s$,n=6)

Group	24h		48h		72h	
	OD Value	Inh. Rate (%)	OD Value	Inh. Rate (%)	OD Value	Inh. Rate (%)
A	1.247±0.027	0	1.253±0.025	0	1.243±0.026	0
B	0.987±0.023*▲	20.85	0.892±0.018*▲	28.81	0.846±0.026*▲	31.93
C	0.813±0.018*▲	34.80	0.785±0.021*▲	37.35	0.713±0.024*▲	42.64
D	0.712±0.012*▲	42.90	0.684±0.023*▲	45.41	0.618±0.025*▲	50.28

Table 2 Inhibitory Effects of Different Concentrations of DDP on SGC7901 Cells at Different Time Intervals($\bar{x}\pm s$,n=6)

Group	24h		48h		72h	
	OD Value	Inh. Rate (%)	OD Value	Inh. Rate (%)	OD Value	Inh. Rate (%)
A	1.237±0.027	0	1.223±0.025	0	1.243±0.026	0
B	1.113±0.023*▲	10.02	1.059±0.028*▲	13.40	1.006±0.026▲	19.06
C	1.013±0.028*▲	18.10	0.901±0.021*▲	26.32	0.863±0.024*▲	30.57
D	0.812±0.032*▲	34.35	0.764±0.023*▲	37.53	0.678±0.025*▲	45.45

Table 3 Inhibitory Effects of rAd-p53 Combined with DDP on SGC7901 Cells at Different Time Intervals($\bar{x}\pm s$,n=6)

Group	24h		48h		72h	
	OD Value	Inh. Rate (%)	OD Value	Inh. Rate (%)	OD Value	Inh. Rate (%)
A	1.271±0.017	0	1.263±0.025	0	1.273±0.026	0
B	0.887±0.013*▲	30.21	0.792±0.018*▲	37.29	0.645±0.026*▲	49.33

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C	0.713±0.018*▲	43.90	0.685±0.021*▲	45.76	0.521±0.024*▲	59.07
D	0.512±0.012*▲	59.92	0.484±0.013*▲	61.67	0.362±0.025*▲	71.56

3.2. Apoptosis in SGC7901 cells by rAd-p53/DDP alone or combined after 48h

Flow cytometry results showed that after 48 hours of treatment with DDP at 7.5 mg/L,rAd-p53 at 5×10^{10} v p/ml, and their combination, the apoptosis rates in SGC7901 gastric cancer cells were 28.79%±2.37%,36.94%±0.78%,and 69.26%±0.63%respectively.These rates were significantly higher than that of the negative control group(16.72%±1.54%)($P<0.05$).

3.3. Effects of rAd-p53/DDP on KAI1/CD82 expression in SGC7901 cells after 48h

Immunohistochemistry results showed that after 48 hours of treatment with DDP at 7.5 mg/L, rAd-p53 at 5×10^{10} v p/ml, and their combination, the expression rate of KAI1/CD82 protein increased. The positive cells were stained in a brownish-yellow color. Compared to the negative control group, the differences were statistically significant($P<0.05$). The increase in expression rate was more pronounced in the combination group (52.43±3.42) (Table 4).

4. Discussion

The human p53 gene is located on the short arm of chromosome 17(17P13.1), with a full length of 16-20 kb, comprising 11 exons and 10 introns. It exists in two forms: wild-type and mutant. The wild-type p53 is known as the "molecular police" and has the following functions: ① inhibiting cell proliferation as a cell cycle regulatory protein; ② binding with viral or cellular proteins to suppress cancer; ③ monitoring damage and inducing apoptosis; ④ inducing cell differentiation; ⑤ influencing the expression of other genes [6-7].

Table 4 Positive Expression Rate of KAI1/CD82 in SGC7901 Cells After 48 Hours of Treatment with rAd-p53 and DDP($\bar{x} \pm s$,n=3)

Group	KAI1/CD82Positive Expression Rate (%)
Negative Control Group	24.60±3.73
DDP7.5mg/L Group	33.30±2.45*
rAd-p53 Group 5×10^{10}	35.50±2.27*
DDP7.5mg/L+rAd-p535×10^{10}	52.43±3.42*

Mutant p53 acts as an oncogene, promoting malignant cellular transformation. The absence, mutation, and inactivation of the p53 protein play a significant role in the progression and evolution of malignant tumors. It not only directly affects tumor cells but also regulates other oncogenes or tumor suppressor genes to influence the occurrence and development of tumors [8]. Therefore, introducing the wild-type p53 gene to restore the mutated p53 within cells has become a novel strategy for cancer treatment [9]. Studies have confirmed that 60%of gastric cancers harbor p53 mutations. The mutation and deletion of the p53 gene are not only related to the onset and prognosis of gastric cancer but also closely associated with the chemosensitivity of gastric cancer cells[10].

Recombinant human adenovirus p53 injection(rAd-p53)—Gendicine is a therapeutic agent that leverages an adenovirus as a vector to deliver the p53 tumor-suppressor gene into target cells to exert antitumor effects. KAI1/CD82, a tumor-suppressor gene that has garnered significant research attention in recent years, is involved in reactions between cells and between cells and the extracellular matrix. These interactions play a crucial role in tumor invasion and metastasis. Studies have confirmed that KAI1/CD82 can inhibit tumor cell growth and metastasis by regulating cell adhesion, suppressing tumor cell motility and migration, participating in immune responses, enhancing antitumor immunity, and engaging in intracellular signaling pathways[11–12].It has been demonstrated that KAI1/CD82 is a target gene of p53,with p53 being involved in the regulation of KAI1/CD82 expression in malignant

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tumors[13].However, some studies have reached opposite conclusions. Therefore, in this study, we used the gastric cancer cell line SGC7901 as the target to investigate the effects of rAd-p53 in combination with cisplatin on the proliferation inhibition, apoptosis induction, and KAI1/CD82 protein expression in gastric cancer cells. The results showed that both rAd-p53 and cisplatin alone, as well as their combination, could inhibit the growth of gastric cancer cells, with the inhibition rate increasing gradually with the increase in drug concentration and the extension of action time, that is, in a dose-and time-dependent manner, and the combination had a stronger inhibitory effect. Flow cytometry revealed that the apoptosis rate increased with the increase in drug concentration after treatment with rAd-p53 and cisplatin, with statistically significant differences compared with the negative control group($P<0.05$), and the combination group showed a more pronounced effect than the single-agent groups. Meanwhile, through immunohistochemical experiments, we found that the expression of the KAI1/CD82 protein was upregulated after treatment with rAd-p53 alone and in combination with cisplatin, and the upregulation was more evident in the combination group. Thus, we speculate that rAd-p53 can enhance the chemotherapeutic effect of cisplatin on gastric cancer cells. The specific mechanism may be related to the re-expression of wild-type p53,which further upregulates the expression of KAI1/CD82.This is consistent with previous studies that have shown that the loss of p53 function is one of the main reasons for the reduced or absent expression of KAI1/CD82 in advanced tumors[14].Therefore, the results of this study provide a theoretical basis for the combination of chemotherapy and gene therapy for gastric cancer and also offer a new target for the development of targeted gene therapy for gastric cancer.

5. Conclusion

This study has thoroughly investigated the effects of recombinant human adenovirus p53 injection(rAd-p53) in combination with cisplatin (DDP)on the growth inhibition, apoptosis induction, and KAI1/CD82 protein expression in gastric cancer SGC7901 cells. The results demonstrate that both rAd-p53 and DDP alone significantly inhibit the growth of gastric cancer cells and induce apoptosis in a dose-and time-dependent manner. More importantly, the combination of the two drugs markedly enhances the inhibitory and apoptotic effects on gastric cancer cells, with significantly higher proliferation inhibition and apoptosis rates compared to single-agent treatments. Moreover, the combination treatment significantly upregulates the expression of the KAI1/CD82 protein, which may be associated with the re-expression of wild-type p53.This finding not only provides a more solid theoretical basis for the application of rAd-p53 in gastric cancer treatment but also offers new insights and targets for the strategy of combining chemotherapy with gene therapy for gastric cancer. Future research could further explore the molecular mechanisms of the combined use of rAd-p53 and DDP, as well as validate the efficacy and safety of their combined therapy in in vivo models, in the hope of bringing more effective treatment options to gastric cancer patients.

6. References

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