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APE1 overexpression is associated with cisplatin resistance in non-small cell lung cancer and targeted inhibition of APE1 enhances the activity of cisplatin in A549 cells

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ABSTRACT

Purpose: Apurinic/apyrimidinic endonuclease (APE1), a bifunctional AP endonuclease/redox factor, is important in DNA repair and redox signaling, may be associated with chemoresistance. In this study, we first investigated APE1 expression and its correlation with cisplatin resistance and prognosis in non-small cell lung cancer (NSCLC) patients. Then, we investigated the effect of chimeric adenoviral vector Ad5/F35 carrying human APE1 siRNA (Ad5/F35-APE1 siRNA) on the sensitivity of cisplatin in A549 human lung adenocarcinoma cells.

Methods: Tumor specimens from 103 patients with operable NSCLC were obtained from 1999 to 2001. Among these patients, 72 patients have been treated with at least three cycles of cisplatin-based chemotherapy. APE1 protein expression was examined by immunohistochemistry and Western blot on the tumor samples and a cultured A549 cell line, respectively. Cell survival and apoptosis were determined by MTT and TUNEL, respectively.

Results: 83.3% (20/24) cisplatin-resistant tumors showed high APE1 expression levels, while 8.3% (4/48) cisplatin-sensitive tumors showed high APE1 expression levels ($p < 0.01$). Univariate analysis indicated that overall survival and disease-free survival were significantly better in NSCLC patients with low vs those with high APE1 expression levels ($p < 0.01$). Treatment with cisplatin resulted in a dose-dependent increase in APE1 protein expression in A549 cells, and Ad5/F35-APE1 siRNA effectively inhibited APE1 expression. Ad5/F35-APE1 siRNA significantly enhanced sensitivity of A549 cells to cisplatin, associated with increased cell apoptosis.

Conclusions: Our results indicate that APE1 is a new promising target for the combination of cisplatin-based chemotherapy in NCSLC patients.

1. Introduction

Lung cancer, one of the most common malignancies in the world, is the leading cause of cancer death worldwide [1]. Non-small cell lung cancer (NSCLC) accounts for 80–85% of all lung cancer cases [2]. Despite a great effort to improve the survival of patients with NSCLC, a satisfactory level has not been achieved because the majority of patients present at an advanced, unresectable stage and their tumors exhibit an inherent resistance to chemo- and radiotherapy. Recent study has suggested that cisplatin-based doublet regimens are associated with a slightly better survival compared to non-platinum-based doublet regimens [3]. Unfortunately, the outcome of cisplatin therapy on NSCLC seems to have reached a plateau. Therefore, the biological mechanisms of cisplatin action need to be understood in order to overcome the treatment plateau on NSCLC. The molecular target of platinum action is the cellular DNA, which hampers DNA replication and transcription, resulting in cell death. Chemoresistance of tumor cells is still a major limitation to its clinical use. The molecular mechanisms that underlie this chemoresistance are largely unknown. Possible mechanisms of acquired resistance to platinum include reduced intracellular accumulation of platinum, enhanced drug inactivation by metallothionein and glutathione, increased repair activity of DNA damage, and formation of cisplatin-DNA adducts, etc. [4].

DNA-repair systems, as the molecular basis of defending against environmental damage to cellular DNA, play an important role in protecting the genomic stabilization and integrity. However, an elevated DNA repair capacity in tumor cells leads to drug resistance and severely limits the efficacy of cisplatin. The human

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apurinic/apyrimidinic endonuclease (APE1), is an essential enzyme in the base excision repair (BER) pathway which is responsible for the repair of DNA caused by oxidative and alkylation damage and thus protects cells against the toxic effects of endogenous and exogenous agents including chemotherapeutic agents [5]. A key step in BER is the processing of an AP site intermediate by an AP endonuclease. APE1 is the major AP endonuclease in human cells and accounts for 95% of the total AP endonuclease activity [6–9].

In addition to its DNA repair functions, APE1 is also a multifunctional protein that participates in other crucial cellular processes, including the response to oxidative stress and regulation of transcription factors, including HIF-1alpha, p53, NF-κB, CREB, and AP-1. The transcription factors are associated with chemoresistance [10,11]. Moreover, several studies demonstrated that APE1 was overexpressed in several human tumors and increased APE1 expression had been shown to be associated with poor prognosis [12,13]. Koukourakis et al. [13] reported that nuclear expression of APE1 in head and neck cancer was associated with resistance to cisplatin-based neoadjuvant therapy and poor outcome. In the present study, we first investigated the expression level of APE1 protein and its correlation with sensitivity and clinical outcomes of cisplatin-based therapy among NSCLC patients. Then, we investigated the effect of adenoviral vector Ad5/F35 carrying human APE1 siRNA on the sensitivity of cisplatin in human NSCLC cells.

2. Materials and methods

2.1. Patients and clinical specimens

A total of 103 patients from 1999 to 2001 were included in the present study. All the patients were from the Daping Hospital of Third Military Medical University, China. The local ethics committee approved the study. The median patient age at diagnosis was 57 years (range, 30–75 years). The histological classification of the resected tumors was based on the World Health Organization criteria. The post-operative disease staging was performed according to the revised International Staging System for Lung Cancer; the stages range from I to III. Patient characteristics are detailed in Table 1. The control group was the normal lung tissues of 36 patients.

All the patients have undergone radical surgery. No chemotherapy or radiotherapy was given to patients before surgery. Eighty-five patients received chemotherapy or radiotherapy after surgery. Among them, 72 patients received more than three cycles of cisplatin-based chemotherapy (Table 2).

Table 1
The relationship of clinicopathologic factors and APE1 protein expression.

	Number	APE1 low expression	APE1 high expression	*p* value
Age				
<60	60	17	43	>0.05
≥60	43	10	33	
Sex				
Male	75	26	49	>0.05
Female	28	11	17	
Histologic type				
Scc	62	17	45	>0.05
Ad	41	10	31	
TNM stage				
I + II	79	22	57	>0.05
III	24	5	19	
Nodal status				
N0	68	15	53	>0.05
N1-2	35	12	23	

Scc: squamous cell carcinoma; Ad: adenocarcinoma.

Table 2
The therapeutic status for NSCLC patients after surgery.

Stage	Number	Therapy after surgery			
		No therapy	Chemotherapy		Radiotherapy
			1–2 Cycles	>2 Cycles	
I	20	18	0	0	2
II	59	0	10	48	26
III	24	0	0	24	20
Total	103	18	10	72	48

2.2. Immunohistochemistry and APE1 Scoring

The expressions of APE1 protein was analyzed using immunohistochemistry. Sections from paraffin-embedded tumors were incubated overnight with mouse anti-human APE1 monoclonal antibody (Novus, Littleton, CO, USA) at a 1:2000 dilution, then incubated with goat anti-mouse secondary antibody (Pierce, Rockford, IL, USA). Antigen–antibody complexes were visualized by incubation with 3,3′-diaminobenzidine (DAB) substrate and counterstained with diluted Harris hematoxylin. Tissues were scored for: (1) percentage of cell staining and (2) intensity of staining (low, moderate, or high). To be defined as low expression, the tissue needed to meet weak staining and positive cell percentage less than 50% or moderate staining and positive percentage less than 25%.

2.3. Cell line and recombinant adenovirus vectors

A549 (human lung adenocarcinoma) cells were purchased from American Type Culture Collection (Manassas, VA, USA) and grown in RPMI 1640 (HyClone, Logan, UT, USA) supplemented with 10% fetal bovine serum, 100 units/mL penicillin and 100 μg/mL streptomycin. The cells were maintained in a humidified incubator at 37 °C in an atmosphere of 5% CO_2 and 95% air. Recombinant adenovirus vector Ad5/F35-APE1 siRNA and Ad5/F35-EGFP were constructed as described previously [14].

2.4. Cell survival assays

A549 cells were plated in 96-well plates at 4000 cells/well. One day later, cells were infected with Ad5/F35-APE1 siRNA or Ad5/F35-EGFP at a MOI of 20 for 90 min and were then washed to remove the adenoviruses. Following 48 h culture, cells were treated with cisplatin (Sigma, St Louis, MO, USA) at various concentrations (0, 0.03, 0.3, 3, and 30 μg/mL) for 72 h. Then, 20 μL MTT (5 g/L) (Sigma) was added to each well and incubated for an additional 4 h, and culture media were discarded followed by addition of 0.15 mL DMSO (Sigma) and vibration for 10 min. The absorbance was measured at 490 nm using a model 550 microplate reader. Percent absorbance relative to control was plotted as a linear function of drug concentration.

2.5. TUNEL assay for apoptosis

Apoptosis was measured by TUNEL assay (Roche, Indianapolis, IN, USA). In briefly, coverslips covered with cells were rinsed in PBS and fixed with 4% paraform at room temperature for 60 min, blocked with 0.3% methanol hydrogen peroxide at room temperature for 30 min, and then treated with 0.3% Trion X-100 at 37 °C for 30 min. The coverslips were placed in 37 °C water for bath, labelled with 50 μL of TUNEL reagent for 60 min, reacted to 50 μL of peroxidase-conjugated converter-POD at 37 °C for 30 min, and finally subjected to DAB coloration and hematoxylin.