Expression levels of artemis in taxane-based chemotherapy

Introduction

Although decreasing in most countries, gastric cancer remains one of the most leading causes of cancer death in the world with poor prognosis. Patients with recurrent or unresectable gastric cancer usually have poor outcomes, with 5-year mortality of more than 95% [1]. Diffuse types ofgastric cancer are strongly suggested by World Health Organization (WHO) classification in 2000, including poorly differentiated squamous carcinoma and well-differentiated adenocarcinoma [2]. Patients are always assigned to individual subtypes based on the above definitions for different targeting treatments. In the treatment ofgastric cancer, chemotherapy results in a significant survival difference compared to the other care, and can relieve the cancer-related symptoms [3]. Cytotoxic agents are considered to be an effectivechemotherapy regimen in clinical practice, such astaxane [3]. Taxane consist ofpaclitaxel and docetaxel, is an active antitumor agent in treating of a variety of carcinomas, including gastric cancer. However, a number of patients with gastric cancer treated with taxane do not sensitive to the therapy [4]. Therefore, it is important to establishcriteria to predict the chemosensitivity for application of chemotherapy and prevention of unnecessary side effects.

Artemis protein is a phosphoprotein that has been proved to play a pivotal role in both V(D)J recombination and double-strand breaks (DSBs) repair via the nonhomologous end-joining (NHEJ) pathway [5, 6], and in the regulation of cell cycle checkpoints [7, 8]. Defects inArtemis gene are found to be extremely sensitive to radiation and cannot complete V(D)J recombination which leads to immunodeficiency [9]. Artemis function in cellular response to

radiation is evident in arresting the cell cycle following Artemis decreasing [10, 11]. However, the relationship evidence between Artemis and chemotherapy is deficiency up to now.

To gain insight into the functions of Artemis in gastric cancer patients treated with chemotherapy taxane, we retrospectively observe the expression levels of Artemis in SGC-7901 cells of 110 patients who treated with taxane-based chemotherapy (TC) and non-taxane-based chemotherapy (NTC). The results will establish new criteria to predict thechemosensitivity in chemotherapy of gastric cancer patients.

Materials and methods

Patients and clinical specimens

For retrospective analysis, we recruited 110patients withgastric cancer who receivedradical surgery at the First Affiliated Hospital of Zhengzhou University andZhengzhou People’s Hospital (Zhengzhou, Henan, China) between January 2003 and June 2008. These patients were administered with postoperative concurrent radiotherapy other than neoadjuvant chemotherapy and radiotherapy, and were pathologically classified based on the 7 th edition of the AJCC (American Joint Committee on Cancer) TNM (tumor-node-metastasis).

This study was approved by the local Ethics Committee before initiation. Informed consent was received fromall the patients.

Immunohistochemistry

The 4 μm thicknesses paraffin sections collected from the paraffin blocks of gastric tissues in each patient were sliced for DAB (Diaminobenzidine, obtained from Beijing Zhongshan Biotechnology Co. Ltd. Beijing, China) staining and the expression of Artemis protein was determined by immunohistochemistrySP (streptavidin-perosidase) method. The sections were first dewaxed and treated with 68â„ ƒ for 20 min, and they were then incubated in 3% H_2O_2, blocked with serum and incubated with primary antibody anti-Artemis at room temperature for 2 h. Primary antiserum was detected after incubation with a secondary antibody goat anti-rabbit IgG (QINAGEN biotechnology, Netherlands, Germany). Positive cells were visualized by DAB staining. After staining, 5 high-power fields (200 ×) were randomly selected in each section for observation under microscope (Olympus BX53). The percentage of Artemis expression cells was counted as follows: cases with < 25% cells were graded asnegative (–), 25%-70% cells were graded as positive (+), and > 75% cells were considered as strong positive (++).

Cell cycle analysis

SGC-7901 cells (Shanghai Bogoo Biotechnology Co. Ltd., Shanghai, China) in logarithmic growth phase were suspended inRPMI-1640 (a type of leukocyte culture medium previously developed at Roswell Park Memorial Institute) medium(Life Technologies, Inc., Paisley, Scotland) with cell density of 2×10^6 cell/mL. A volume containing 1×10^7 cells was used and treated with 5mL10 mg/mL taxane. After standing for 24 h, cells were trypsinized with trypsin (Beijing Dingguochangsheng Biotechnology Ltd. Beijing, China) and

permeabilized with TritonX-100 and stained with 1 mL 100 µg/mL propidium iodide in PBS (phosphate buffer solution). Flowcytometric data were obtained using a FACScan flow cytometer (Becton Dickinson, San Jose, USA).

Western blotting analysis

After treatment with different concentrations (5 mg/mL, 10 mg/mL, 15 mg/mL, 20 mg/mL, 30 mg/mL, 40 mg/mL) of taxane for 16 h, or treatment with 10 mg/mL taxane for different time (0 h, 4 h, 8 h, 16 h, 24 h, 36 h), SGC-7901 cells were washed 3 times with PBS, centrifuged to remove the supernatant. Then they were isolated by 100µLlysis buffer in ice for 3 min. Total protein extracts were separated by 10% SDS-PAGE (standard sodium dodecyl sulfate polyacrylamide gel electrophoresis) and transferred onto a nitrocellulose membrane and were blocked for 1 h. Membranes were incubated with the primary antibody of Artemis at dilution of 1: 1000 at 4â„ƒovernight. Blots were washed and incubated for 1 h with horse reddish peroxidase linked goat anti-rabbitIgG at 30â„ƒ. Color was developed using DAB chromogen. β-actin served as a loading control.

Statistical analysis

Statistical analysis was performed by SPSS 11. 5 software (SPSS, Chicago, IL, USA). The measurement data were expressed as mean ± SD and analyzed by Kruskal-Wallis test, Mann-Whitney test and ANOVA test. Survival curves were constructed for the outcomes of survival rate and disease-free survival rate in total patients, TC and NTC treated patients by Kaplan-Meier survival method. There is significantly statistical difference when p <0. 05.

Results

Clinical and pathologic characteristics of all patients

The 110 patients were divided into two groups according to their chemotherapies: taxane based chemotherapy (TC) group and non-taxane based chemotherapy (NTC) group. The TC group consisted of a subgroup of 60 patients who receivedtaxane-based chemotherapy (paclitaxel/docetaxel + cisplatin) and the NTC group constitute of 50 patients who underwent chemotherapy without taxane (such as epirubicin + 5-fluorouracil + cisplatin, oxaliplatin + 5-fluorouracil, and oxaliplatin + capecitabine). As shown in Table 1, 24 patients(22%) had superficial (pT1) and 42 patients (38%) had deep (pT3) depth of tumor invasion. Most of the patients (78, 71%) had metastases to regional lymph nodes. Patient characteristics in ages, TNM stages, pathological types and tumor grading were well balanced between the treatment groups.

Relationship between Artemis expression and clinical pathological characters in patients

The relationship betweenArtemis expression and clinical pathological characters in the gastric cancer tumor tissues was detected by immunohistochemistry after different chemotherapies (Table 2). The Artemis expression significantly related with node staging ($p = 0.003$), metastasis staging ($p = 0.034$) and pathological type ($p = 0.000$). However, there was no expressive difference in ages, tumor staging, tumor grading and chemotherapies.

Relationship between Artemis expression and survival rate and disease-free survival rate

Analysis of time-to-event variables ofsurvival rate and disease-free survival rate were performed with the Kaplan-Meiersurvival curves (Fig. 1). Three years survival estimates of all patients(20 patients lost to follow up) showed no significant difference existed between the expression of Artemis protein and overall survival rate. There was no relationship in both survival rate and disease-free survival rate between patients with positive Artemis expression and negative Artemis expression (Fig. 1A-B, $p > 0.05$), while the survival rate in positive Artemis expression patients (19%) were significant low than that in negative Artemis expression patients (30%) (Fig. 1C, $p < 0.05$).

Effects of taxane chemotherapies on SGC-7901 cell cycles

Treatment with taxane at 10 mg/mL for 12 h did not significantly change the numbers of SGC-7901 cell in G0/G1 phase (Fig. 2B), indicating the cell cycle arrested in G1 phase. After taxane treatment for 24 h (Fig. 2C), the cells were accumulated in the S phase, and were arrested in G3 phase after 48 h (Fig. 2D).

Effect of taxane chemotherapies on Artemis expression in SGC-7901 cells

Treatment of SGC-7901 cells with different concentrations for 16 h changed the expression level of Artemis proteins with a dose dependent manner (Fig. 3A). In addition, expression level of Artemis was significantly up-regulated 8 h after treated with 10 mg/mL taxane, and was down-regulated 24 h later (Fig. 3B).

Discussion

Artemis has multiple effects in human cells through involving in DNA repair, such as in apoptotic DNA fragmentation [12] and in regulation of cell cycle checkpoints [13, 14]. In this report, we investigated the relationship between the Artemis expression and clinical pathological characteristics in 110 gastric cancer patients treated with chemotherapies oftaxol and non-taxol regimens, and compared the Artemis expression in survival rate and disease-free survival rates between the two treated groups. Though we found the positive (+, ++) and negative (-) expression was not associated with the chemotherapy regimens, patients with positive Artemis expression had less survival rate than negative expression patients after treated with taxol, indicating that higher expression Artemis levels may result in a poor chemotherapy effects. We then examined the effects oftaxol chemotherapy to SGC-7901 cells and identified G1 and G3 cell cycle arrest after treatment. Furthermore, the intracellular Artemis levels were regulated in adosage-dependent manner andpresented a transient increase after taxol treatment. All these results suggest that Artemis may involve in renovation of taxol chemotherapy induced cell cycle arrest.

To the best of our knowledge, the relationship between the radiosensitivity genes Artemis and taxane chemotherapy have not been well depicted, while both of them were reported inducing cell block by a sequence of regulated reactions. Taxane has been clinically used in treatment ofgastric cancer for its active antitumor effect by permanently prevent proliferation of severe damage in tumor cells, such as checkpoint arrest at G1 [15] and G2/M phase

[16]. Correspondingly, chemotherapy with taxane caused G1 and G2/M arrest was well proved in this study by flow cytometry cycle analysis.

In addition to proliferation prevention, additional time for DNA repair is another potential impact of checkpoint arrest [17]. Clinical data in our study showed the expression levels ofendogenous Artemis protein was significantly associated with node staging, metastasis staging and diffuse types, indicating thatArtemis expression involved in the gastric cancer cell development after chemotherapy of taxol. Furthermore, the Artemis levels in SGC-7901 cells increased sharply at 8 h after treated with 10 mg/L taxane, and maintained a high level for the nest 16 h. While during this period, these cells were stopped growth in G1 phase for checkpoint arrest. Therefore, we assumed that, theArtemis were involved in regulating cell cycle progression by several pathway. Since the positive expression of Artemis resulting in poor chemotherapy effect according to our clinical data, we believed that the function of Artemis was to reduce the damage caused by cell-cycle-specific chemotherapy clinical data. Indeed, the important role of Artemis protein in cell cycle checkpoints has been described in previous studies [18, 19]. Artemis-defective cells are considered to be the checkpoint proficient and maintain a prolonged G2/M arrest after homologous recombination [17], resulting in recovery of cells from DNA damage via regulation of related cyclin proteins expression [7, 20]. A recent finding defined the Artemis as an effector, regulating G1 phase cell cycle progression in mammalian cells [21]. It was suggested that Artemis is capable of removing radiation-induced phosphoglycolates [22], it may function as removing taxol-induced modified DNA as well, and involved in resolution of hairpin DNA structures [23].

In conclusion, patients with positive expression of Artemis protein result in poor taxol chemotherapeutic efficacy ongastric cancer. The up-regulated Artemis in gastric cancer cells may involve in DNA repair to reduce the damage caused by taxol. Other than radiotherapy, the high expression levels of Artemis can weaken the chemosensitivity in treated patients withtaxol. Therefore, inhibition of Artemis may enhance the efficacy of taxol therapy. However, the relationship of Artemis and taxol chemotherapy should be detected in further studies in order to uncover the regulated mechanisms and guide for the clinical chemotherapy treatment in gastric cancer patients.