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Expression of hENTl and ERCC1 genes in tumor tissues non-small cell lung cancer

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ABSTRACT

Objective: To investigate the expression of hENTl and ERCC1 genes in tumor tissues non-small cell lung cancer (NSCLC). **Methods:** Fresh non-small lung cancer specimens were transplanted into nude mice. Twenty mice were randomized into two groups: experimental group receiving gemcitabine plus cisplatin and control group receiving 0.9% physiological saline. The expressions of hENTl and ERCC1 mRNA in tumor tissue were detected by real-time fluorescent quantitative PCR. The volume of tumor, the weight of nude mice and tumor volume were respectively measured and calculated 2-3 times per week. Tissue samples were collected from NSCLC mice treated with gemcitabine plus carboplatin. **Results:** The histological examination showed that many tumor cells were well preserved in nude mice. The rate of transplanted tumor cells was 86.7%. The concomitant treatment study showed that the rate of TV, RTV, T/C in GEM + DDP group was the lowest. LBP + DOC, DDP + DOC obviously influenced the body weight. Compared with NS group, DDP group, GEM group, the survival period and the level of hENTl of DDP+GEM group increased obviously, the level of ERCC1 decreased significantly (P<0.05). **Conclusions:** The expression of hENT1 and ERCC1 genes in tumor tissues were closely correlated with the response to chemotherapy and prognosis of patients with NSCLC treated with gemcitabine plus cisplatin.

1. Introduction

Non-small cell lung cancer (NSCLC) is one of the malignant tumors with the highest incidence and mortality. Chemotherapy is the main treatment, but different individuals have different chemotherapy response and prognosis. The follow-up showed that the success or failure of the initial chemotherapy play an extremely important role to the effective rate and prolong survival time. Human equilibrative nucleoside transporter 1 (hENT1) is the important carrier which transport gemcitabine into the cell, and excision repair cross complementing gene l (ERCC1) is extremely important DNA repair factors. This study established subcutaneous planting in 30 nude mice, selected mice with successful implanted cancer cells and then carried out anti-tumor experiment combined with chemotherapy, and also analyzed the relations between the hENT1 and ERCC1 mRNA expression levels and the efficacy of gemcitabine chemotherapy, provide a basis for analyzing individual chemosensitivity.

2. Materials and methods

2.1. Experimental animals and agents

Sixty nude mice were selected, 4 to 8 week old, weighing

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20-25 g, provided by XX University Experimental Animal Center. Animals were kept in the isolation box of the standard laboratory. Lewis lung cancer cell line (LLC) was purchased from American Type Culture collection Stock (ATCC). GEM, DDP was purchased from Simcere Company. Total RNA extraction reagent Trizol-A in tissue were purchased from Omega Company. cDNA synthesis kit were purchased from Takara, SYBR Green Ⅰ fluorescence quantitation kit were also purchased from TaKaRa, pGM-T vector kit were purchased from Shanghai Sangon Company. PCR instrument was purchased from the British Techne Company, gel imaging system was purchased from the Sigma Company.

2.2. Preparation and selection of samples

The NCI-H460 cells were inoculated into RPMI-1640 culture medium with 10% FBS, cultured in 37 ℃ insulation incubator. Cells were suspended when they were grown to about 50%-60%. After ether anesthesia, 0.5 mL NCI-H460 prepared cell suspension was inoculated subcutaneously in mice.

2.3. Animal grouping and treatment programs

The tumor formation standard was 200-300 mm, 52 mice with successful inoculation; the success rate was 86.7%. There are 4 experimental groups, each group with 12 mice of successful inoculation. DDP group received DDP (2.5 mg/kg) 2 times/week injection in tail vein for 2 weeks; GEM group received GEM (50 mg/kg) 2 times/week intraperitoneal injection for 2 weeks; The clinical medicine and course of treatment in GEM + DDP group were the same as the GEM group and DDP group; Normal saline (NS) served as the control group.

2.4. Detection index

The length and width of the tumor were measured with a vernier caliper every 2 d. the Tumor volume was record and calculated as follows: [TV (mm^3) = 0.52×L×W]. Relative tumor volume (RTV) = 15 days TV/1 Day TV. Relative tumor proliferation rate (T/C) = experimental groups RTV/control group RTV.

2.5. Primer design and synthesis

The sequence of hENTl, ERCC1 and β-actin were as follow:
hENTl upstream primer: 5′-TGTTTCCAGCCGTGACT-3′, downstream primer: 5′-CAGGCCACATGAATACAG-3′;
ERCC1 upstream primer: 5′-CTGGGAATTTGGCGACGTAA-3′, downstream primer: 5′-ATGGATGTAGTCTGGGTGCAG-3′;
β-actin upstream primer: 5′-GCGAGAAGATGACCCAGATC-3, downstream primer 5′-GGATAGCAACGCCTGGATAG-3′.

2.6. Real-time quantitative fluorescent PCR

Two mice were sacrificed in each group 1 d after the treatment, and the tumor tissues were peeled and then received hENTl, ERCC1 testing. The plasmid standard of the hENTl, ERCC1 and housekeeping gene (β-actin) were diluted into 6 concentration groups with geometric proportion, and then real-time fluorescence quantitative PCR was carried out.
hENTl reaction conditions: 95 ℃ 60 s 1 cycle, 95 ℃ 15 s, 61 ℃ 60 s 40 cycles, 95 ℃ 60 s, 60 ℃ 30 s, 95 ℃ 30 s 1 cycles.
ERCC1 reaction conditions: 95 ℃ 60 s 1 cycle, 95 ℃ 20 s, 60 ℃ 60 s 40 cycles, 95 ℃ 60 s, 60 ℃ 30 s, 95 ℃ 30 s 1 cycles.
PCR products were identified by gel eletrophoresis, the mRNA absorbance values of hENTl, ERCC1 mRNA were standardized by internal reference β-actin optical density and the relative content of the hENTl, ERCC1 mRNA expression were obtained.

2.7. Survival time evaluation

The rest mice were used for survival observation. The time of inoculated with LLC cells was the starting point of observation, and the death of the mice in 4 groups was the end point.

2.8. Statistical analysis

All of the data were analyzed by SPSS 14. 0 statistics software, and the measurement data were expressed as mean ±SD values. One-Way ANOVA and *t*-test were applied in the comparison between groups. Log-rank test was used for survival analysis. P <0.05 was considered as statistical significance.

3. Results

3.1. Comparison of the tumor volume

After 1 d of the treatment, tumor volume of GEM + DDP group was (1376.5±189.6) mm3, while tumor volume of NS group, DDP group and the GEM group were (3 275.0±472.7), (2 643.8±342.7), (2 436.6±334.2) mm^3, respectively.

Compared with NS group,the tumor volume of GEM group, DDP group, GEM + DDP group were significantly reduced (P<0. 05). Compared with the GEM group and the DDP group, the tumor volume of GEM + DDP group was significantly reduced (P<0.05). The TV, RTV and T/C (%) of the DDP group were similar to the GEM group. There was no significant difference (P>0.05) (Table 1).

Table 1

Comparison of the tumor volume in each group.

Groups	TV(mm3)	RTV	T/C(%)
NS	3 275.0±472.7	7.5±2.0	100.0
DDP	2 643.8±342.7*	4.7±1.4*	64.3*
GEM	2 436.6±334.2*	4.5±1.3*	62.4*
GEM+DDP	1 376.5±189.6*△	3.2±1.0*△δ	45.8*△δ

Note: Compared with NS group, * P<0. 05; compared with DDP group, △P<0. 05; compared with GEM, δP<0. 05.

3.2. Survival time

In NS group, the mice all died after 52 d of LLC inoculation; In DDP group, there were 6 survived after 52 d of LLC inoculation (60.0%), but the mice all died after 59 d.

In GEM group, there were 6 survived after 52 d of LLC inoculation (60.0%), but the mice all died after 60 d. In GEM+DDP group, there were 8 survived after 56 d of LLC inoculation (60.0%), but the mice all died after 78 d. There were no obvious differences in the survival time between the DDP group or between the GEM group and NS group (P> 0.05), while the time of GEM + ES group was significantly longer (P<0.05); compared with the GEM group and ES group, the GEM + ES group had significantly longer survival period (P<0.05).

3.3. Comparison of hENTl of the tumor tissue

The hENTl in NS group, ES group, GEM group and GEM + ES group were (11.6±1.6), (23.5±2.2), (25.9±2.3), (45.5±5.4) pg/mL, respectively. Compared with NS group, the hENTl in the GEM group, DDP group and GEM + DDP group were significantly higher (P<0. 05). Compared with the other three groups, the hENTl of tumor tissue in the GEM + ES group was significantly higher (all P<0.05).

3.4. Comparison of ERCC1 of the tumor tissue

The ERCC1 expression of tumor tissues in the NS group, ES group, GEM group and GEM+ES group were (121.8±8.2), (75.3 ±6.7), (72.4±6.6), (37.4±4.6) pg/mL, respectively. Compared with NS group, the ERCC1 in GEM group, DDP group, GEM + DDP group were decreased significantly (P<0. 05). Compared with the NS group, the ERCC1 expressions of GEM group, DDP group, GEM + DDP group were significantly decreased (all P<0.05).

4. Discussion

Lung cancer is the main causes of death worldwide, which is divided into small cell lung cancer and non-small cell lung cancer, while non-small cell lung cancer accounts for 80%[1,2]. The main treatments of non-small cell lung cancer were surgery, chemotherapy, radiotherapy and molecular targeted therapy at this stage. The study of Schiller[3] showed that the side effects of platinum-based joint program vary greatly. However, there was no significant difference in clinical efficacy. Gemcitabine has obvious advantages in treating PFS patients.

Gemcitabine is a anticarcinogen which is a difluoro nucleoside antimetabolite. It can damage the cell replication, which is a water-soluble analogues of the deoxy cytidine. It is a substitute of inhibitory substrate to ribonucleotide reductase enzyme. This enzyme play an important role in the generation of the required deoxynucleotides during the process of DNA synthesis and repair. Therefore, find the related genes which can predict the efficacy of gemcitabine is become the research focus in clinical. In this study, we detected the mRNA expression of human equilibrative nucleoside transporter1(hENTl) and excision repair cross-complementing gene1(ERCC1) by real-time quantitative PCR, and explore the correlation between them and the non-small cell lung cancer and the efficacy and side effects of the gemcitabine.

As a hydrophilic substance, gemcitabine require carriers to pass freely into the cell by diffusion. While hENTl is the mainnucleoside transporter protein which participate in the gemcitabine transmembrane transport processes, and it is positively correlated to the gemcitabine anti-tumor effect. The studies of Achiwa *et al*[4] suggest that the high expression of hENTl mRNA can increase the sensitivity of gemcitabine to NSCLC cell lines. The patients with hENTl positive protein expression also benefit significantly during gemcitabine therapy, hENTl can be a predicted molecules as the gemcitabine treatment in NSCLC.

The conclusions of the study of Toffaloriol *et al*[5] are consistent with Achiwa et al. The siRNA method was used to reduce the hENTl mRNA expression levels of two lung cancer cell line A549, H1703, which can also lead to the significant increase in gemcitabine IC_{50}. Our results show that the tumor TV, RTV and T/C were significantly reduced in the GEM + DDP group, the results show that the GEM + DDP chemotherapy effect is significant. Single drug therapy can significantly inhibit the tumor size and also significantly improved survival time. hENTlmRNA has a high expression

in the GEM + DDP chemotherapy group, and it has a low expression in the NS control group, which indicates the low expression of hENTlmRNA has a poor prognosis, while in the high expression group patients who received gemcitabine chemotherapy can benefit from the treatment. So the hENTl can be considered as a predictive indicator of chemosensitivity. The results were consentaneous with the experimental results of Rongrong *et al*[6].

Some studies think that the efficacy of platinum-based chemotherapy was correlated to the ERCC1 expression[7]. The excision repairment of Cisplatin-DNA adducts were by the nucleotide excision repair pathway system. The ERCC1 gene, as an important member of protein nucleotide excision repair matrix, plays an important role during the process. The experimental results of Chen *et al*[8] show that compared to patients with high ERCC1 expression, patients with low ERCC1 expression has a longer survival time after receive the platinum-based chemotherapy. Most literature[9,10] showed that in advanced NSCLC, patients with high ERCC1 expression had a poor prognosis. The ERCC1mRNA expression levels in GEM group, DDP group, GEM + DDP group were significantly less than the control group, especially the GEM + DDP chemotherapy group. The results showed high ERCC1mRNA expression have poor prognosis, which suggested patients with low expression group were more sensitive to the gemcitabine chemotherapy drugs,and the chance of drug resistance were greater in patients with high ERCC1 expression in tumor tissue during the gemcitabine chemotherapy.

In summary, this study indicates that patients with high hENTlmRNA expression and low ERCC1mRNA expression had a better efficacy and a longer survival time of NSCLC tumor during the gemcitabine chemotherapy. For patients who receive individualized postoperative adjuvant chemotherapy, can adopt the "based on molecular biology of tumor detection" to filter the suitability of gemcitabine plus platinum chemotherapy.

Conflict of interest statement

We declare that we have no conflict of interest.

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